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Contents

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STUDY ON BEEKEEPING OF TWO QUEENS SYSTEM ON EUROPEAN HONEYBEE *APIS MELLIFERA* LINNAEUS, 1758 IN MAGWAY REGION*

Nyo Nyo Lwin[1], Thein Thein Htay [2], Myo Myo Aye[3]

Abstract

Number of cells containing on the colony structures such as open, seal, egg and empty showed very drastic changes. Seal cells were recorded that too highest condition as 33.47% as well as open cells 15.3% in 2Queen beehive 2, respectively. Especially, high condition of seal cells was recorded as 15.23% in Control2. Relation of honey production with mean weather conditions in experiments and control were recorded. Honey production was showed highly statistically significant difference as ($p < 0.001$) between double-queen and single-queen colonies. Pollen production level was no statistically significant difference as ($p > 0.05$) for double-queen colonies compared to the single-queen during the study period. Within each queen systems, production of honey and pollen were recorded statistically significant difference as ($p < 0.05$), respectively. The large weight honeys were recorded as hive number 1, 2 and 3 of single-queen and double-queen systems during the sunflower seasons. Especially, as 39.8% in hive number 3 was recorded the highest stored honey in single-queen system and as 37.3% in hive number 2 was recorded the highest weight honey in double-queen system. Within one queen system as well as two queen system, production of honey and pollen were recorded statistically significant difference as ($p < 0.05$) with ANOVA HSD test in terms of number of combs occupied by nectar and pollen. Honey production cost difference indicates that was showed higher production costs for managing single-queen colonies compared to double queen colonies as well as pollen production cost

Keywords: Honeybee, Two queens system, Life stages and bee products

Introduction

Honeybees are the main insects which help in pollination of different species of plants. Honeybees are beneficial to both agriculture plant and most wild plants as pollinator. For mankind they provided a lot in agriculture, medicine and food (Sawyer, 1981).

They are major agricultural pollinators around the world and are the keystone pollinators in tropical ecosystems. Pollination has been considered a keystone process to ecosystem function through the facilitation of both plant and animal diversity (Suwannapong *et al*., 2012).

A colony of honeybee consists of a queen, several thousand workers and in a certain season of the year- a few hundred drones. Among the members of the colony there is a division of labour and specialization in the performance of biological functions.

The queen, a true mother bee, is the only female that is completely developed sexually. In the colony, she is found in the area of the brood nest. The developmental time of the queen, 16 days, is the shortest. Workers are females that are not fully developed sexually. They do the work of the colony and maintain it in good condition. Workers have special structures and organs which are associated with the duties they perform. The adult worker emerges from the cell 21 days after the egg is laid. Drones, the males of the colony, are produced from unfertilized eggs. The body of the drone is larger than that of the worker or queen. The eyes are large and cover practically the whole head. The developmental period of drones is 24 days (Yadav *et al.*, 2017).

The presence of one queen in a colony of bees is generally considered normal. The beekeeper, however, may frequently find mother and daughter queens laying eggs in the colony.

[1] Professor/Head, Department of Biology, Yangon University of Education
[2] Associate Professor, Department of Zoology, West Yangon University
[3] Associate Professor, Department of Biology, Yangon University of Education
[*] Best Paper Award Winning Paper in Zoology(2020)

Establishing a two-queen colony is based on the harmonious existence of two queens in a colony unit.

The two-queen system has been recommended as a way to increase honey production without increasing the number of colonies operated. Bee population becomes high, due to the combined efforts of two queens, and there was grave danger of overcrowding. The two-queen colonies required regular manipulation, and serious difficulties arose when they were neglected. Super requirements increased greatly during a heavy honey flow, and it was almost impossible to give each unit enough supers (Gilbert, 1940).

Taking these facts into consideration, the present work was conducted with the following objectives.

- to investigate the beekeeping of two queens system in Pwint Phyu Township, Magway Region
- to compare the life stages and bee products between one queen and two queen systems

Materials and Methods

Study site

Beekeeping site was selected in Pwint Phyu Township, Magway Region located at latitude 20° 27'23.48" N and longitude 94°49' 29.91" E. The study area has active blooming seasons in which pollen and nectar are available for the bees (Plate. 1).

Study period

The study was carried out during October, 2017 to March, 2018.

Experimental Colony Set Up

Three normal beehive colonies were composed of one queen, about one hundred drones and about 9000 workers bee. Three sets of two-queen beehives (2QH1, 2QH2, 2QH3) were prepared by two brood chambers bottom hive which each bottom hive consist four frames and super hive which includes six seal frames as only honey collection combs (Plate. 2).

These colonies were maintained in nuclei to achieve standard hive colony size and bee colonies were transferred to moveable frame hives and managed under the same conditions and brought to the uniform strength. Three colonies were selected and allocated to the single-queen system colony management and another three selected and allocated to the two-queen system colony management. Selection of the colonies was conducted at random. These selected colonies were used and no treatment shift occurred during the study period.

Two queen system of beekeeping, each brood chamber of bottom hive was placed by one honey or pollen comb, open brood comb, two combs of seal as well as one mated queen. Especially, queen excluder was covered between bottom hive and super hive. After three days, experimental design of double queen beehive was checked to know the conditions of queen bee and inside the beehive. At the same time, three beehives with each of one queen were designated as the control experiment.

Collection of Data

Weekly data collection was made at apiary. Especially, life stages and bee products were recorded. Weather parameters such as maximum and minimum temperature and relative humidity were also recorded to relate the changes of bee products throughout the study period. Meteorological data were obtained from Department of Meteorology and Hydrology, Magway Region.

Statistical Analysis

Honey and pollen data was compared by using t-test between one and two-queen management systems. Relations of bee products and bee calendar were subjected to ANOVA- HSD test over the period data both one and two-queen management systems. Cost benefit analysis was performed on the bases of investment cost on value of amount of honey produced under each system. SPSS (Statistical Package for Social Science) version 25 was conducted.

Identification

The honeybees were identified according to the methods described by Bingham (1897).

Pwint Phyu Township, Magway Region

Plate 1 Map of study site (Source: Google Earth, 2018)

A. Sets of two queens beehives (2QH)

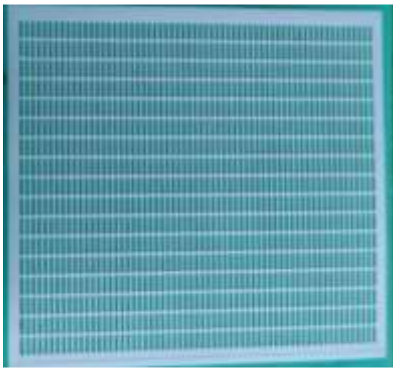


B. One queen beehives C. Apiary D. Food source plant of honeybee

Plate 2 Beekeeping of two queen system


A.Smoker


B. Bee Veil


C. Queen Excluder


D. Hive Tool


E. Digital Thermometer


F. Hygrometer

Plate 3. Equipment

Results

Comparison on the life stages of honey bee between experiments and control

Seal cells were recorded that too highest condition as 33.47% as well as open cells 15.3% in 2Queen beehive 2, respectively. Life stages of honey bee were recorded as available condition of egg, open, seal and empty in control bee hives. Especially, high condition of seal cells were recorded as 15.23% in Control2 (Fig 1 and Table 1).

Table 1 Mean of comparison on the life stages of honey bee between experiments and control

Proportion (%)	Bee Hives					
	Control1	**2QH1**	**Control2**	**2QH2**	**Control3**	**2QH3**
Egg (%)	2.49	3.13	2.16	3.07	1.4	1.95
Open (%)	8.5	14.56	7.3	15.3	5.27	12.94
Seal (%)	11.68	20.67	15.23	33.47	11.5	21.15
Empty (%)	1.5	30.39	1.3	20.02	2.01	39.42

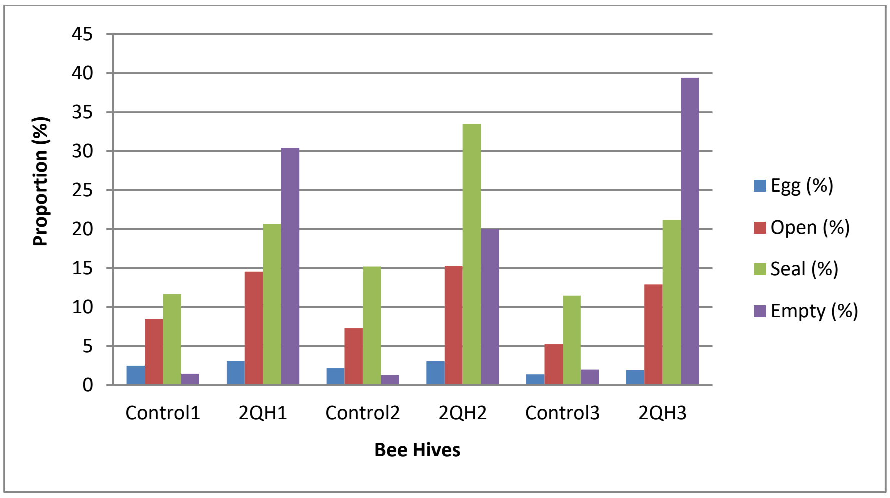


Figure 1 Mean of comparison on the life stage of honeybee between experiments and control

Relation of honey production with mean weather conditions in experiments and control

Honey production was too high weight as 23.8 kg in 2Queen hive 2 (2QH2) and 23.47kg, 25.22kg, 24.05kg in 2Queen hive 3 (2QH3) when level temperature about 30˚C were recorded in December and January. And them, pollen condition was high as 2.5kg in 2Queen hive 1(2QH1) and 2Queen hive 3 (2QH3) were also recorded in December (Fig 2,3 and Table 2, 3).

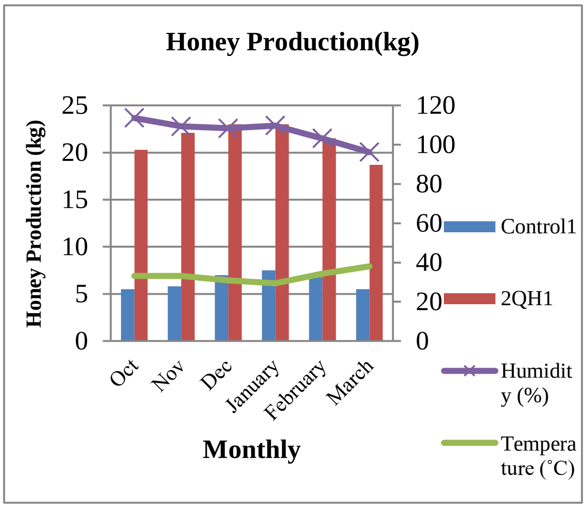
When humidity conditions between 75% to 80%, honey production was too high in both 2Queen beehive 1 and 2Queen beehive 2 as well as high condition of pollen production in these two hives (Fig 2,3 and Table 2, 3).

Table 2 Relation of Honey production with mean temperature and humidity in experiments and control

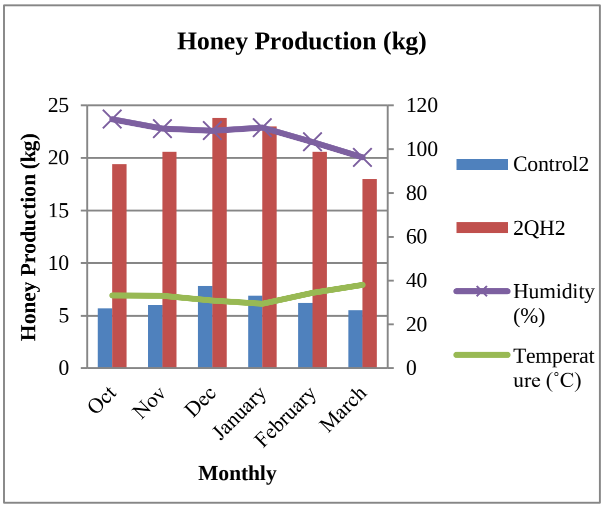
Bee Hives	**Honey Production (kg)**					
	Oct	**Nov**	**Dec**	**January**	**February**	**March**
Control1	5.5	5.8	7	7.5	6.8	5.5
2QH1	20.3	22.1	23	23	21.5	18.7
Control2	5.7	6	7.8	6.9	6.2	5.5
2QH2	19.4	20.6	23.8	23	20.6	18
Control3	6.5	7.2	9	8.8	7.8	5.4
2QH3	21.5	23.47	25.22	24.05	22	19.45
Temperature (˚C)	33.25	33.12	30.86	29.47	34.3	37.98
Humidity (%)	80.42	76.2	77.52	80.29	68.96	58.21

Table 3 Relation of Pollen production with mean temperature and humidity in experiments and control

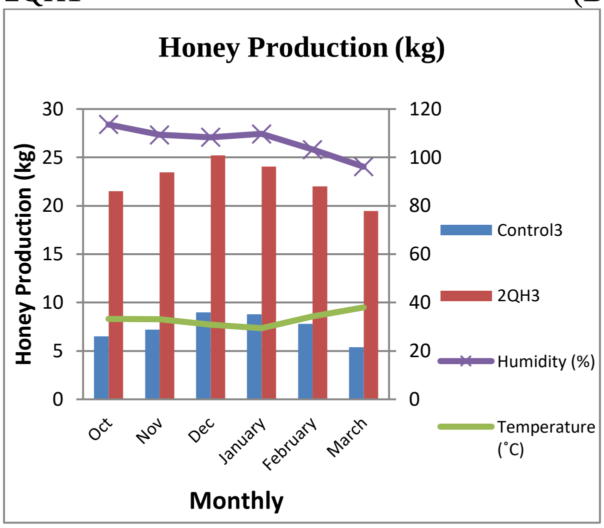
Bee Products / Bee Hives	Pollen Production (kg)					
	Oct	**Nov**	**Dec**	**January**	**February**	**March**
Control1	0.5	1.69	2	1.5	0.7	0
2QH1	1.3	1.5	2.5	1.8	1.2	0
Control2	0.5	1.5	2.1	1.5	0.5	0
2QH2	1.5	1.8	2.1	2	1.5	0
Control3	0	1.75	1.98	1.3	0.5	0
2QH3	1.2	2.3	2.5	1.6	1.7	0
Temperature (˚C)	33.25	33.12	30.86	29.47	34.3	37.98
Humidity (%)	80.42	76.2	77.52	80.29	68.96	58.21



(A) Control 1 and 2QH1

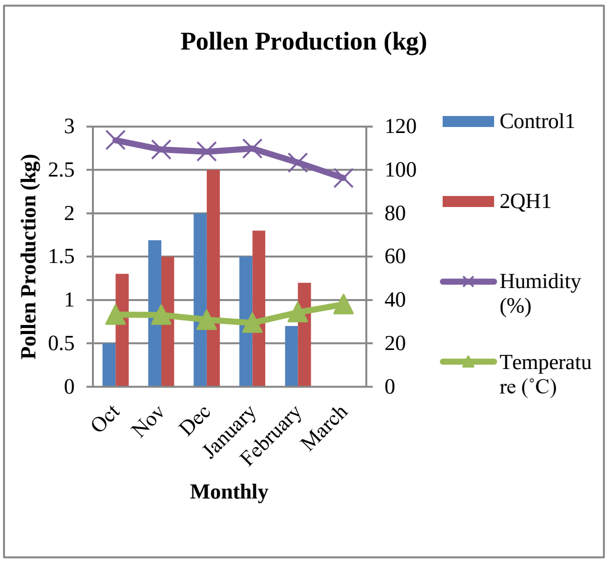


(B) Control 2 and 2QH2

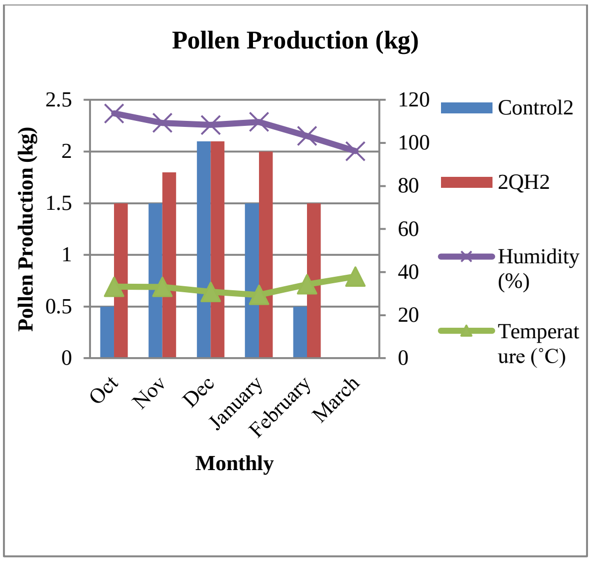


(C) Control 3 and 2QH3

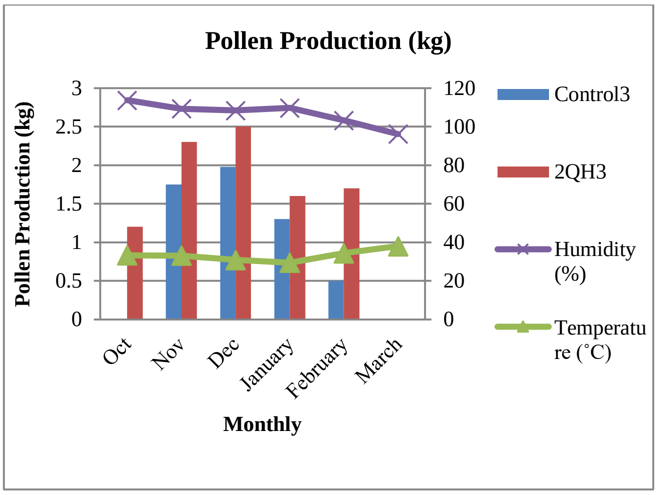
Figure 2 Relation of honey production with mean weather conditions in experiments and control



(A) Control 1 and 2QH1



(B) Control 2 and 2QH2



(C) Control 3 and 2QH3

Figure 3 Relation of pollen production with mean weather conditions in experiments and control

Comparison on the production of Honey and Pollen in Experiments and Control

Two-queen colonies produced more honey than single-queen colonies ($p < 0.001$), with t-test, with a mean of 21.65 ± 2.0 kg and a mean of 6.72 ± 1.1 kg for two-queen and one-queen colonies, respectively (Fig 4 and Table 4). The honey production level was statistically significant difference both queen systems owing to the differences in the amount of nectar available during the study period.

Two-queen colonies produced more weight of stored pollen than one-queen colonies were no statistically significant difference ($p > 0.05$), with a mean of 1.47 ± 0.78 kg and a mean of 1.00 ± 0.77 kg for two-queen colonies compared to the one-queen during the active season as shown in Fig 4 and Table 4.

Investigation on the bee products within one-queen and two-queen colonies

Within one queen system as well as two queen system, production of honey and pollen were recorded statistically significant difference as ($p < 0.05$) with ANOVA HSD test in terms of number of combs occupied by nectar and pollen.

The large weight honey was recorded as 38.1%, 38.6% and 39.8% in hive number 1, 2 and 3 of single-queen system during the sunflower seasons. Especially, as 39.8% in hive number 3 was recorded the highest stored honey. For double-queen management systems, the high weight honey was recorded that 35.8%, 37.3% and 36.3% in hive number 1, 2 and 3 during the sunflower seasons. Especially, as 37.3% in hive number 2 was recorded the highest weight honey in double-queen system.

In single-queen hives, the large weight pollen was recorded as 54.8%, 59.0% and 59.3% in hive number 1, 2 and 3 during the sunflower seasons. Especially, as 59.3% in hive number 3 was recorded the highest weight pollen. For double-queen management systems, the high weight pollen was recorded that 51.8%, 46.1% and 44.1% in hive number 1, 2 and 3 during the sunflower seasons. Especially, as 51.8% in hive number 1 was recorded the highest stored pollen in double-queen system.

T-test (Honey products of two-queen and one-queen system)

Group Statistics

	Method	N	Mean	Std. Deviation	Std. Error Mean
Honey	Q1	18	6.7167	1.13098	.26657
	Q2	18	21.6494	2.00341	.47221

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Honey	Equal variances assumed	6.073	.019	-27.538	34	.000	-14.93278	.54226	-16.03478	-13.83078
	Equal variances not assumed			-27.538	26.836	.000	-14.93278	.54226	-16.04572	-13.81984

T-test (Pollen products of two-queen and one-queen system)

Group Statistics

	Method	N	Mean	Std. Deviation	Std. Error Mean
Pollen	Q1	18	1.0011	.77228	.18203
	Q2	18	1.4722	.78351	.18468

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Pollen	Equal variances assumed	.891	.352	-1.817	34	.078	-.47111	.25930	-.99808	.05586
	Equal variances not assumed			-1.817	33.993	.078	-.47111	.25930	-.99809	.05586

Table 4 Comparison on honey and pollen products between experiments and control

Bee Hives / Bee Products	Average Production (kg)/Colony/month					
	Control1	**2QH1**	**Control2**	**2QH2**	**Control3**	**2QH3**
Honey	6.35	21.43	6.35	20.9	7.45	22.615
Pollen	1.69	3.75	1.22	2.53	1.32	3.48

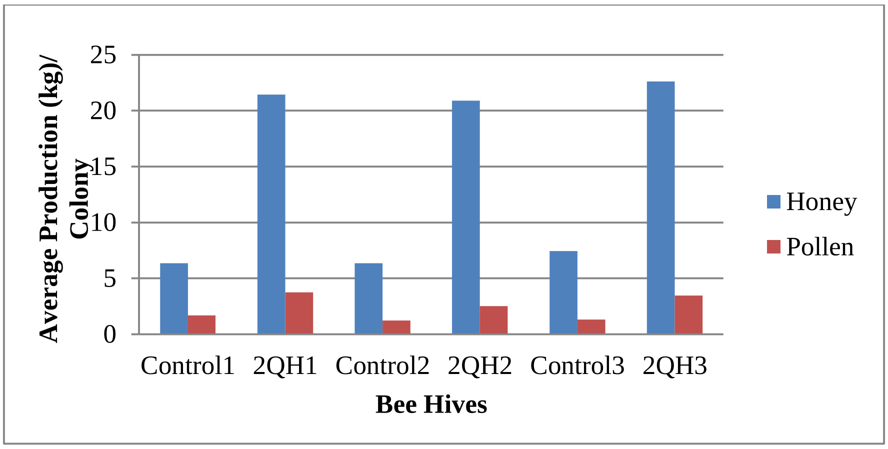


Figure 4 Comparison on the honey and pollen products between experiments and control

ANOVA HSD test in two-queen and one-queen systems

ANOVA[a]

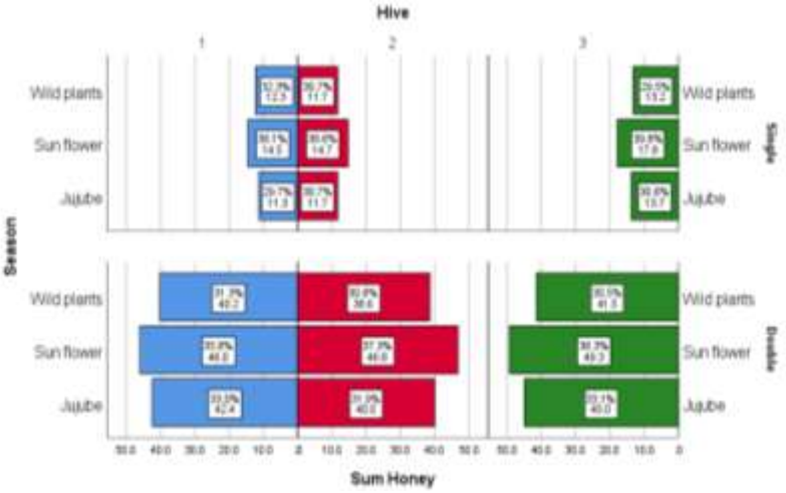
		Sum of Squares	df	Mean Square	F	Sig.
Honey	Between Groups	11.243	2	5.622	8.030	.004
	Within Groups	10.502	15	.700		
	Total	21.745	17			
Pollen	Between Groups	6.280	2	3.140	12.203	.001
	Within Groups	3.859	15	.257		
	Total	10.139	17			

a. Queen_No = Single

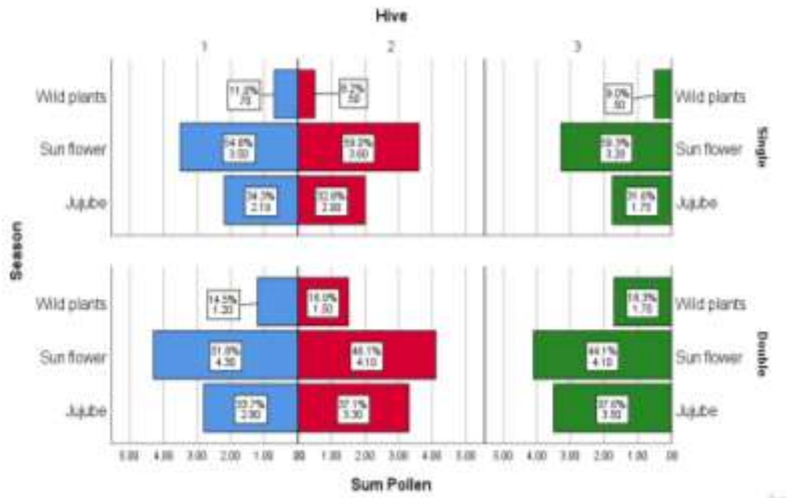
ANOVA[a]

		Sum of Squares	df	Mean Square	F	Sig.
Honey	Between Groups	41.272	2	20.636	11.481	.001
	Within Groups	26.960	15	1.797		
	Total	68.232	17			
Pollen	Between Groups	5.614	2	2.807	8.733	.003
	Within Groups	4.822	15	.321		
	Total	10.436	17			

a. Queen_No = Double



(A) Honey



(B) Pollen

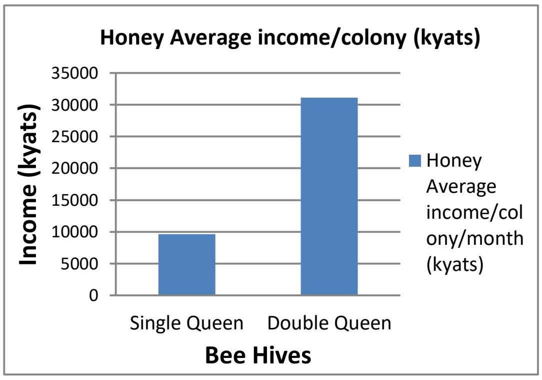
Managing income cost for double-queen and single-queen colonies

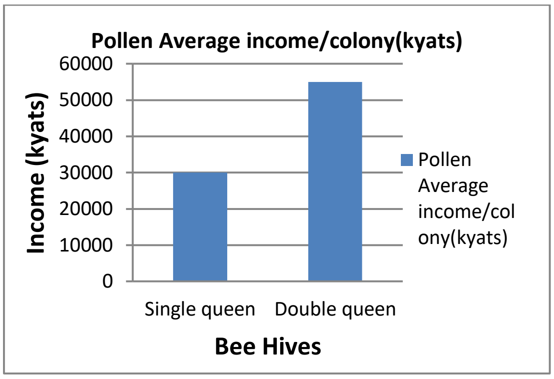
The average production cost of honey per month was over thirty thousand kyats for the double-queen colonies as well as over nine thousand kyats for the single colonies (Fig 5 and Table 5). The average production cost of pollen per month was about fifty five thousand kyats for the double-queen colonies as well as over thirty thousand kyats for the single colonies (Fig 5 and Table 5).

Honey production of two-queen system Pollen production

Table 5 Differences income cost honey and pollen from double queen and single queen colonies

Bee Hives	**Honey Average income/colony/month(kyats)**	**Pollen Average income/colony/month(kyats)**
Single queen	9660	30000
Double queen	31120	55000





(A) Honey (B) Pollen

Figure 5 Average income cost per colony per month between products of double-queen colonies and single-queen colonies

Discussion

Two-queen system offers labor advantages, lower cost per kilogram of honey produced, better quality produce, less swarm problem of bees. There is established fact about high positive correlation between bee colony population and honey yield, there is information related to how a double-queen system affects different variables of beekeeping under Magway conditions.

The two-queen system is an intensive management system designed to produce the largest possible honey crop per hive unit. The honey yield reflects the colony capacity to use the nectar supply and is affected by population, size, race or strain of bees, incidence of disease, and management (Moeller, 1976).

Honey production was showed highly statistically significant difference as ($p < 0.001$) between double-queen and single-queen colonies. The numbers of nectar and pollen combs in double-queen colonies were larger than that of single-queen colonies during the blooming seasons. Honey production potential per individual bee was greater for double-queen system colonies compared to the single-queen colonies. However, the pollen production level was no statistically significant difference as ($p > 0.05$) for double-queen colonies compared to the single-queen during the study period.

During study period, within each queen systems, production of honey and pollen were recorded statistically significant difference as ($p < 0.05$), respectively. The large weight honeys were recorded as hive number 1, 2 and 3 of single-queen and double-queen systems during the sunflower seasons. Especially, as 39.8% in hive number 3 was recorded the highest stored honey in single-queen system and as 37.3% in hive number 2 was recorded the highest weight honey in double-queen system. The result indicates that peak brood rearing activity coincides with higher honey and pollen stores, which later on are converted to maximum number of worker bees during the active honey harvesting seasons.

Honey production cost difference indicates that was showed higher production costs for managing single-queen colonies compared to double queen colonies as well as pollen production cost difference indicates that there were recorded higher production costs for managing single-queen colonies compared to double-queen colonies. From a financial analysis point of view, current study result showed that use of double queen colonies is more profitable than managing single-queen colonies in Myanmar beekeeping.

The lower production cost mainly resulted from savings from labor, beeswax, and feeding. Moeller (1976, quoted in Valle *et al*., 2004) reported that 50% more labor time is required for two-queen colonies than for one-queen colonies, indicating two-queen colonies needed less total labor per kg of honey produced. Valle *et al*. (2004) reported that only 24% more labor cost was required than what is needed for single-queen colonies.

During study period, seal cells were recorded that too highest condition as 33.47% as well as open cells 15.3% in 2Queen beehive 2, respectively. Life stages of honey bee were recorded as available condition of egg, open, seal and empty in control bee hives. Development of colony structure and food sources of double queen beehives were investigated to be depend on weather conditions especially to the proper condition of temperature and humidity.

Conclusion

The two-queen system is an intensive management system designed to produce the largest possible honey crop per hive unit if available to favourable conditions on weather and bee food sources. For the promotion of beekeeping, a beekeeper can easily get a pure honey for commercial products in small scale or large scale. The two-queen system is a constitutional management system to produce bee populations and to protect waste of larvae. Small scale beekeeping provide good livelihood suitable for rural people to eradicate poverty.

This research of breeding high quality of queen bee by two-queen system techniques that one can apply to promote the quality of Myanmar honey product. We also recommend that two-queen colonies should be studied in other potential beekeeping areas of Myanmar by involving more representative numbers of colonies for validating the system further.

As part of the research study, the proposer believes that this work will contribute to Department of Apiculture, Ministry of Agriculture, Livestock and Irrigation and local beekeepers in Myanmar. The two-queen system is a viable and insured method that bee breeders and beekeepers can rely on for that purpose. Two-queen system of beekeeping will create new job opportunity and extra income.

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INFESTATION OF SOME INSECT PESTS AND PREDATORS ON CUCURBIT PLANTS FROM KONE-THAUNG VILLAGE, SALIN ENVIRONS

Daw Maw[1], Ko Ko Naing[2]

Abstract

Infestation of some insects on cucurbit plants were observed from Kone-Thaung Village in Salin environs, during the study period from November, 2017 to June, 2018. A total of 26 insect species confined to 23 genera, 17 families and five orders were recorded. Among these 26 species recorded, 19 species were insect pest species and seven species were predators. The four species of cucurbit plants were *Lagenaria vulgaris* (bu), *Cucurbita maxima* (shwe-phayon), *Cucumis sativus* (tha-khwar) and *Citrullus vulgaris* (hpa-ye). Order Coleoptera was the most dominant agricultural insect pest species and beneficial species. Seven species are the most serious damaged on cucurbitaceous plants as agricultural pest species such as, *Xenocatantops humilis* Short-horned Grasshopper, *Arocatus melanocephalus* Elm Seed Bug, *Cletus bipunctatus* Leaf-footed Bugs *Epilachna varivestis* Mexican Bean Beetle, *Aulacophora foveicollis* Pumpkin Beetle, *A. frontalis* Black Cucurbit Beetle and *Bactrocera cucurbitae* Melon Fruit Fly were found in all study plants.

Keywords: pests, predators, cucurbit plants, Salin environs

Introduction

Insects are the most numerous and diverse group of animals. Over 790,000 species have been described, far more than for any other animal or plant group. The order Coleoptera (beetles) has 350,000 species, Diptera (flies) has 120,000 species, Lepidoptera (moths and butterflies) has 112,000 species and Hymenoptera (bees and wasps) has 108,000 species. Insects can be important pests in agriculture, forestry and homes, and can threaten human health (T.J. Lysyk, 1995).

Cucurbitaceae is of high economic value being a major source of food for man. Many species of Cucurbita (pumpkins, squashes, gourds, marrows, courgettes), Cucumis (melons, cucumbers), and Colocynthis (water melon) are cultivated for edible purposes (M. Ajuru, 2014). Watermelons, cucumber, squash, gourds, and pumpkins are cucurbits commonly grown in the area. These crop, are attacked by a variety of insects and related pests, including aphids, cucumber beetles, squash bug and squash vine borer (Ricky E. Foster, 2017).

Beneficial insects help field crop producers by reducing pests that can be economically important. Some predators, like lady beetles, feed on pest insects as both larvae and adults (Iowa Soybean Association, 2012). Insect may be helpful to humans by producing, directly or indirectly, materials of economic value, such as silk, honey, beeswax, the production of fruits, vegetables, flowers and seeds, because of pollenizing activity. Insect may be harmful to humans and causes great economic loss by damaging or destroying agricultural crops and other plants (Davidson, R. H. & W.F. Lyon, 1979).

Cucurbit crops are an important component of the processing and fresh market vegetation productivity in Salin environs. Cucumbers are consumed either raw or pickled. With the advancement of rualiziation and increase in agricultural production, the damages caused by insect pests have recently assumed serious status, which cannot be ignored. Thus, the present study with the following objectives;

- to classify and record the insect pests and predators on the cucurbit plants and
- to investigate the damage parts of the cucurbit plants

[1] Dr, Lecturer, Department of Zoology, Pakokku University
[2] Dr, Lecturer, Department of Zoology, Pakokku University

Materials and Methods

Study Area and Period

The present study area was Kone-Thaung village, Salin environs. It is situated 21° 20′ N and 95 ° 05′ E and near the Ayeyarwaddy river. This area is about 2.0km^2 (Plate 1). The present study was conducted from November, 2017 to June, 2018.

Data Collection

Specimens were randomly collected fortnightly. Specimens were caught by using insect collection net and some were picked up by hand with the aid of plastic bugs and forceps. Collected specimens were noted and color photograph taken by digital camera.

Preparation and Preservation

The collected insects were killed with mild chloroform. The collection box was used with creosote solution or sprayed insecticide so as to repel the ants and fungus infection of the specimens. And then they were put into plastic boxes.

Identification of Species

The identification method and taxonomic designation of insect species were followed after the method of according to Borror and Delong (1964), Davidson (1979), Hill (1983), Morris and Waterhouse (2001), and McDougall,S (2013).

Analysis of Data

The species composition of the insect species was analyzed following after Bisht, *et al*., (2004).

Species composition = Number of particular species / Total number of all species × 100

Plate 1 Location map of study area (Source: Google Earth)

Results

A total of 26 insect species confined to 23 genera, 17 families and five orders were identified and recorded in Cucurbit plants in Kone-Thaung Village, Salin environs.

Descriptive characters of the studied species (Plate 2)

Atractomorpha similis (Green Grass Pyrgimorph Grasshopper) is a greenish in color and weak body is tapered at the shoulders and swollen at the abdomen.

Atractomorpha sp. (Grasshoppers) is brownish in color, with well-developed tegmina and rosy wings. The grasshoppers' bodies are medium-sized, their antennae are short.

Xenocatantops humilis **(**Short-horned Grasshopper) is body was medium size, brownish. Head was small, not prominent. Antenna was relatively short, filiform. Eyes were brown, a black bar immediately behind the eye.

Trimerotropis pallidipennis (Pallid-winged Grasshopper) is a moderately-sized tan or gray insect. The center of the hind wing has a relatively narrow but conspicuous black band; the wing disk ranges from white to pale yellow, and the apical area is transparent. The hind tibia is yellow.

Arocatus melanocephalus (Elm Seed Bug), the body is dark red in color, and covered in short hairs. The head is black, antennae are also mostly black and underbelly is orange.

Dysdercus cingulatus (Red Cotton Bug) is a mainly red but has a white colour and three black spots.

Rhynocoris fuscipes (Assassin Bug) is a moderately robust elongate bug, mixture of coral red and black coloration dorsally as well as ventrally. Head is wide at the eyes, narrowed anteriorly, reddish and eyes are black and antennae are slender. Abdomen is slightly convex with corel red in color.

Bagrada hilaris (Painted Bug), the adult bug is shield-shaped, and black with white and orange markings. It is bright orange-red and turns darker as it develops, becoming black by the last instar.

Nezara viridula (Green Stink Bug), the body is bright green and shield-shaped and the eyes are usually reddish. *N. viridula* is narrow and long in the green stink bug. *Riptortus pedestris* (Broad-headed Bug) is a moderately sized elongate bug, overall dorsal color brownish, ventral pale yellow with brownish tinge. Triangular head and eyes are large. Abdomen is narrow, U-shaped black band.

Riptortus pedestris (Broad-headed Bug), the head is triangular and eyes are large and antennae long, fourth joint longest. The body is a moderately sized elongate bug, overall dorsal color brownish, ventral pale yellow with brownish tinge. Thorax slightly broad at the base and abdomen is narrow.

Riptortus linearis (Bean Bug), the head is broad, often similar in length and width to the pronotum and the scutellum and that the last antennal segments are elongated and curved.

Cletus bipunctatus (Leaf-footed Bugs) is a small elongate bug, dorsal coloration reddish brown at places, pale yellowish at other places, ventrally pale cream in color, legs of the same color as abdomen ventrally, antennae reddish brown. Head are somewhat triangular. Thorax is pronotum much broad at the humeral angles. *Cheilomenes sexmaculata* (Ladybird Beetle) is the body outline broadly oval to subrounded, dorsum moderately convex and shiny. Head with a black marking in posterior half; pronotum with a T-shaped median marking connected to a broad black band along posterior margin. Ventral side is uniformly yellow. Antenna is short and compact.

Coccinella transversalis (Transverse Ladybird Beetle) is a transverse ladybird shows little variation across its wide range. It has a black head with bright red or orange elytra boldly marked with a black band down the midline and two lateral three-lobed markings.

Cycloneda sanguinea (Unspotted Elytra) is a large ladybird beetle with red, unspotted elytra. The color ranges from orange to deep red. The white and black marks are on the head.

Epilachna varivestis (Mexican Bean Beetle) of the head is pair of prominent black eyes. Head densely punctuate and hairy. The newly emerged adult is of a straw or cream-yellow color. Shortly after emergence, eight black spots of variable size appear on each wing cover arranged in three longitudinal rows on each wing cover.

Galerita janus (False Bombardier Beetle) is a black head and black elytra. The thorax and legs are red in color.

Aulacophora foveicollis (Pumpkin Beetle) is the colour of the elytra varies from pale orange-yellow to bright orange-red to medium brown, and the abdomen is black with soft white hairs.

Aulacophora frontalis (Black Cucurbit Beetle) have the longitudinal groove on the vertex. The color of this species is yellowish brown except elytron black and shining. The head vertical area is with a longitudinal groove on each side.

Dicranolaius sp. **(**Red and Blue Beetle) is a small shiny red and metallic blue banded beetle with a dark head.

Agelastica alni (Alder Leaf Beetle) is a relatively small beetle, black or metallic blue in colour. The larvae are normally black.

Mylabris variabilis (Blister Beetle) have a cylindrical, elongate body with relatively convex, yellow-orange elytra and wide transverse black wavy stripes. Head is square shaped, with a flat forehead. Antennae are black and long with eleven segments.

Bactrocera cucurbitae (Melon Fruit Fly), the body is mostly orange-brown with a faint black T-shaped mark on the abdomen, and the clear wings have a large brown spot at the tip and a brown stripe at the hind edge.

Efferia deserti (Robber Flies), have a short conical to slender wedge-shaped, its color is usually glossy black. Abdominal coloration is usually greyish to brownish in females. Wings are clear or uniformly tinted, with tints varying from transparent brown to dark black.

Syrphus ribesii (Hoverfly) is shiny black. The eye color is bare and face is yellow. Sternites have lateral and median black marks. Lateral margins of tergites are black except at the ends of the yellow bands.

Dolichomutilla sycorax (Velvet Ant) has a pair of white spots on the second metasomal tergum and an interrupted broad white band on the third tergum.

Composition of insect species

In the present study, 26 insect species confined to 23 genera, 17 families and five orders were identified and recorded in Cucurbit plants during the study period. Among these 19 species which are confined to 16 genera, 12 families are insect pest species. Seven species distributed among seven genera and five families are predator species (Table 1, Plate 2).

In the pest species, the highest numbers 17 species on shwe-phayon, 13 species on bu, eleven species on tha-kwar and ten species on hpa-ye. In predator species, five species each on bu, shwe-phayon and tha-kwar and four species on hpa-ye were occurred in the different host plants (Fig.1, Table 2 & Table 3). In the damaged parts of the plants, attacked by insect pest species

16 species most attacked damaged part of leaves followed by 14 species of flowers, 12 species in buds, nine species in fruits and six species in stems. Infestation of some predator species on various parts of the study plants five species each on leaves and fruits, four species each on buds and flowers and three species on stems were observed from the study area (Fig.2, Table 4 & Table 5).

The most dominant order Coleoptera (10 species, 38.46%), followed by Hemiptera (eight species, 30.77 %), Orthoptera (four species, 15.38 %), Diptera (three species, 11.54 %), and Hymenoptera (one species, 3.85%) were observed (Fig. 3, Table 6). The comparison of insect pests and predators, composition of insect pest species (19 species, 73.08%), and predators (seven species, 26.92 %) were recorded (Fig. 4, Table 7).

Table 1 List of insect species recorded from Kone-Thaung Village during the study period

No	Order	Family	Scientific Name	Common Name	Pest/ Predator
1	Orthoptera	Pyrgomorphidae	*Atractomorpha similis*	Green Grass Pyrgimorph Grasshopper	Pest
			Atractomorpha sp.	Grasshoppers	Pest
		Acrididae	*Xenocatantops humilis*	Short-horned Grasshopper	Pest
			Trimerotropis pallidipennis	Pallid-winged Grasshopper	Pest
2	Hemiptera	Lygaeidae	*Arocatus melanocephalus*	Elm Seed Bug	Pest
		Pyrrhocoridae	*Dysdercus cingulatus*	Red Cotton Bug	Pest
		Reduviidae	*Rhynocoris fuscipes*	Assassin Bug	Predator
		Pentatomidae	*Bagrada hilaris*	Painted Bug	Pest
			Nezara viridula	Green Stink Bug	Pest
		Alydidae	*Riptortus pedestris*	Broad-headed Bug	Pest
			R. linearis	Bean Bug	Pest
		Coreidae	*Cletus bipunctatus*	Leaf-footed Bugs	Pest
3.	Coleoptera	Coccinellidae	*Cheilomenes sexmaculata*	Ladybird Beetle	Predator
			Coccinella transversalis	Transverse Ladybird Beetle	Predator
			Cycloneda sanguinea	Unspotted Elytra	Predator
			Epilachna varivestis	Mexican Bean Beetle	Pest
		Carabidae	*Galerita janus*	False Bombardier Beetle	Predator
		Chrysomelidae	*Aulacophora foveicollis*	Pumpkin Beetle	Pest
			A. frontalis	Black Cucurbit Beetle	Pest
		Melyridae	*Dicranolaius* sp.	Red and Blue Beetle	Pest
			Agelastica alni	Alder Leaf Beetle	Pest
		Meloidae	*Mylabris variabilis*	Blister Beetle	Pest
4.	Diptera	Tephritidae	*Bactrocera cucurbitae*	Melon Fruit Fly	Pest
		Asilidae	*Efferia deserti*	Robber Flies	Predator
		Syrphidae	*Syrphus ribesii*	Hoverfly	Predator
5.	Hymenoptera	Mutillidae	*Dolichomutilla sycorax*	Velvet Ant	Pest

Table 2 Occurrence of some insect pest species in different cucurbit plants in the study area

No	Scientific Name	Common Name	Bu	Shwe-phayon	Tha-khwar	Hpa-ye
1	*Atractomorpha similis*	Green Grass Pyrgimorph Grasshopper	+	+	-	+
2	*Atractomorpha* sp.	Grasshoppers	-	+	-	-
3	*Xenocatantops humilis*	Short-horned Grasshopper	+	+	+	+
4	*Trimerotropis pallidipennis*	Pallid-winged Grasshopper	+	+	-	+
5	*Arocatus melanocephalus*	Elm Seed Bug	+	+	+	+
6	*Dysdercus cingulatus*	Red Cotton Bug	+	-	-	-
7	*Bagrada hilaris*	Painted Bug	-	+	+	-
8	*Nezara viridula*	Green Stink Bug	+	+	-	-
9	*Riptortus pedestris*	Broad-headed Bug	-	+	+	-
10	*R. linearis*	Bean Bug	+	+	-	_
11	*Cletus bipunctatus*	Leaf-footed Bugs	+	+	+	+
12	*Epilachna varivestis*	Mexican Bean Beetle	+	+	+	+
13	*Aulacophora foveicollis*	Pumpkin Beetle	+	+	+	+
14	*A. frontalis*	Black Cucurbit Beetle	+	+	+	+
15	*Dicranolaius* sp.	Red and Blue Beetle	-	+	+	-
16	*Agelastica alni*	Alder Leaf Beetle	+	+	-	-
17	*Mylabris variabilis*	Blister Beetle	-	-	-	+
18	*Bactrocera cucurbitae*	Melon Fruit Fly	+	+	+	+
19	*Dolichomutilla sycorax*	Velvet Ant	-	+	+	-
		Total	**13**	**17**	**11**	**10**

Table 3 Occurrence of some predator species in different cucurbit plants in the study area

No	Scientific Name	Common Name	Bu	Shwe-phayon	Tha-khwar	Hpa-ye
1	*Rhynocoris fuscipes*	Assassin Bug	+	+	+	+
2	*Cheilomenes sexmaculata*	Ladybird Beetle	+	+	+	-
3	*Coccinella transversalis*	Transverse Ladybird Beetle	+	+	+	-
4	*Cycloneda sanguinea*	Unspotted Elytra	-	-	+	+
5	*Galerita janus*	False Bombardier Beetle	-	+	-	+
6	*Efferia deserti*	Robber Flies	+	-	-	+
7	*Syrphus ribesii*	Hoverfly	+	+	+	-
		Total	**5**	**5**	**5**	**4**

Table 4 Damage parts of some cucurbit plants by some insect pest species from the study area

No	Scientific Name	Common Name	Leaf	Stem	Fruit	Bud	Flower
1	*Atractomorpha similis*	Green Grass Pyrgimorph Grasshopper	√	-	-	√	√
2	*Atractomorpha* sp.	Grasshoppers	√	-	-	√	√
3	*Xenocatantops humilis*	Short-horned Grasshopper	√	-	√	√	√
4	*Trimerotropis pallidipennis*	Pallid-winged Grasshopper	√	-	-	√	√
5	*Arocatus melanocephalus*	Elm Seed Bug	√	√	-	√	√
6	*Dysdercus cingulatus*	Red Cotton Bug	√	-	-	-	-
7	*Bagrada hilaris*	Painted Bug	√	-	-	-	-
8	*Nezara viridula*	Green Stink Bug	√	-	√	√	-
9	*Riptortus pedestris*	Broad-headed Bug	-	-	-	√	√
10	*R. linearis*	Bean Bug	-	-	-	√	√
11	*Cletus bipunctatus*	Leaf-footed Bugs	√	√	-	√	√
12	*Epilachna varivestis*	Mexican Bean Beetle	√	-	√	√	√
13	*Aulacophora foveicollis*	Pumpkin Beetle	√	√	√	√	√
14	*A. frontalis*	Black Cucurbit Beetle	√	√	√	√	√
15	*Dicranolaius* sp.	Red and Blue Beetle	√	-	√	-	√
16	*Agelastica alni*	Alder Leaf Beetle	√	√	-	-	-
17	*Mylabris variabilis*	Blister Beetle	√	-	√	-	-
18	*Bactrocera cucurbitae*	Melon Fruit Fly	-	√	√	-	√
19	*Dolichomutilla sycorax*	Velvet Ant	√	-	√	-	√
		Total	**16**	**6**	**9**	**12**	**14**

Table 5 Infestation of some predator species on various parts of the study plants from the study area

No	Scientific Name	Common Name	Leaf	Stem	Fruit	Bud	Flower
1	*Rhynocoris fuscipes*	Assassin Bug	√	-	-	√	√
2	*Cheilomenes sexmaculata*	Ladybird Beetle	√	√	√	-	-
3	*Coccinella transversalis*	Transverse Ladybird Beetle	√	√	√	-	-
4	*Cycloneda sanguinea*	Unspotted Elytra	√	-	-	√	√
5	*Galerita janus*	False Bombardier Beetle	√	-	√	√	-
6	*Efferia deserti*	Robber Flies	-	√	√	-	√
7	*Syrphus ribesii*	Hoverfly	-	-	√	√	√
		Total	5	3	5	4	4

Table 6 The percentage composition of insect species in different orders

No	Order	Number of species	Percentage %
1	Orthoptera	4	15.38 %
2	Hemiptera	8	30.77 %
3	Coleoptera	10	38.46%
5	Diptera	3	11.54 %
6	Hymenoptera	1	3.85%

Table 7 The percentage composition of insect pests and predators recorded from the study area

No	Pest/Predator	Number of species	Percentage %
1	Pest	19	73.08%
2	Predator	7	26.92%
	Total		100

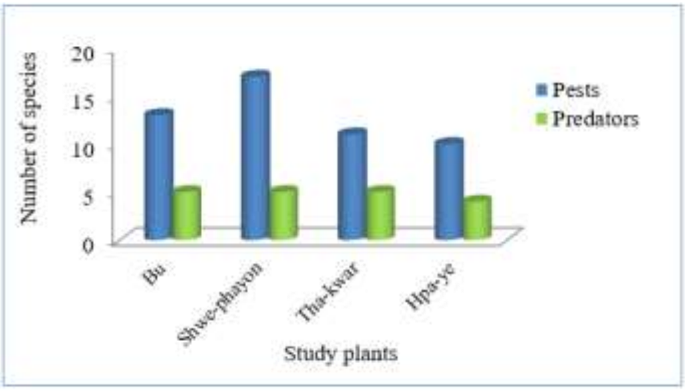


Figure 1 Comparison of some insect pests and predators in different cucurbit plants

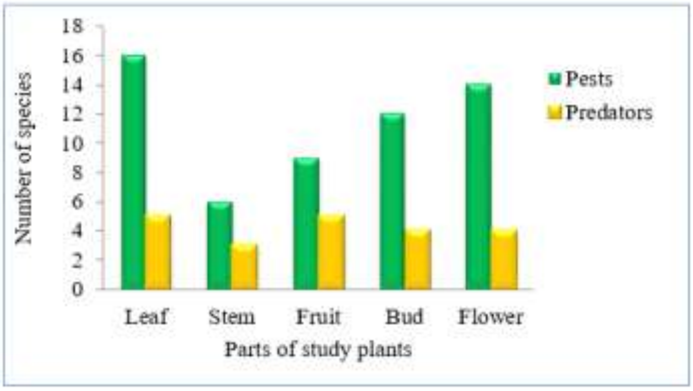


Figure 2 Infestation of some insect pests and predators on different parts of cucurbit plants

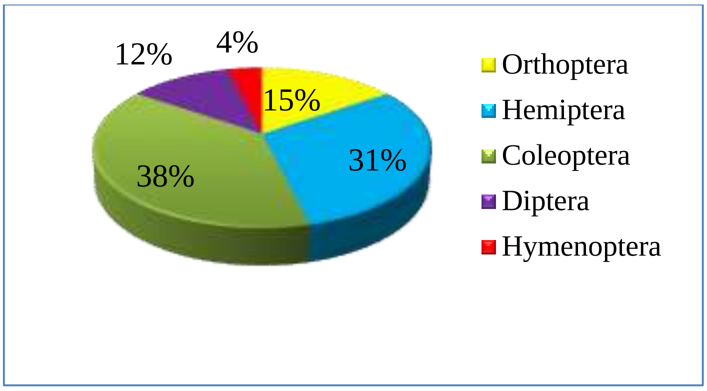


Figure 3 Percentage composition of insect species in different orders

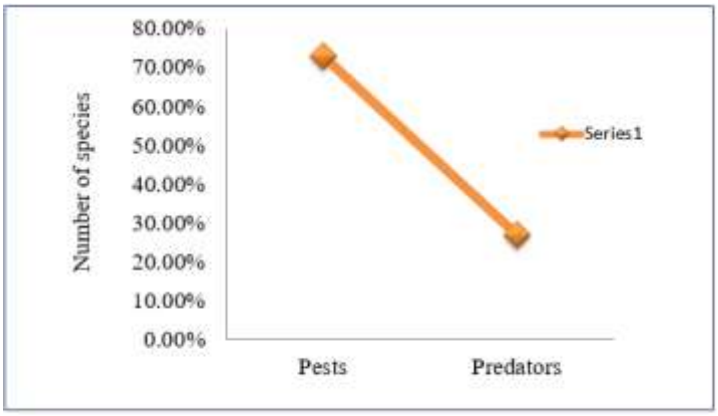


Figure 4 Percentage composition of insect pests and predators in the study area

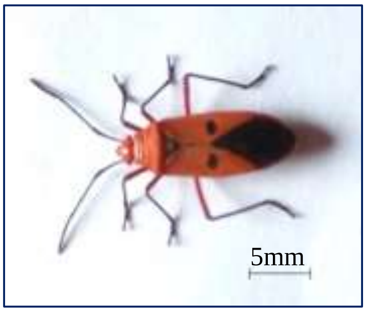
(A) *Atractomorpha similis*

(B) *Atractomorpha* sp.

(C) *Xenocatantops humilis*

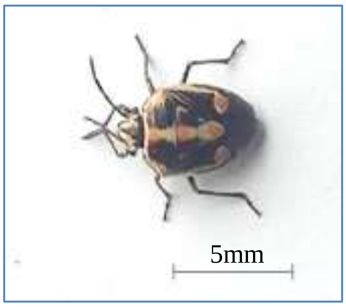
(D) *Trimerotropis pallidipennis*

(E) *Arocatus melanocephalus*



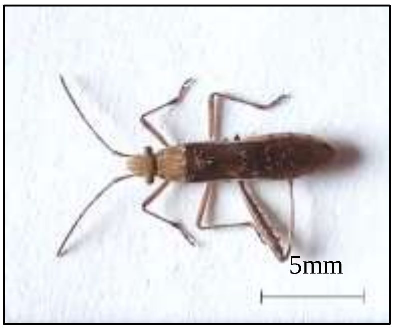
(F) *Dysdercus cingulatus*

(G) *Rhynocoris fuscipes*



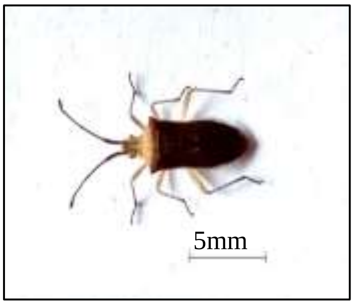
(H) *Bagrada hilaris*

(I) *Nezara viridula*



(J) *Riptortus pedestris*

(K) *Riptortus linearis*



(L) *Cletus bipunctatus*

(M) *Cheilomenes sexmaculata*

(N) *Coccinella transversalis*

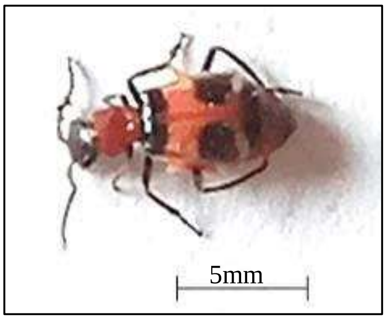
(O) *Cycloneda sanguinea*

(P) *Epilachna varivestis*

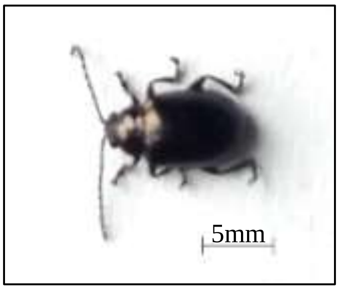
(Q) *Galerita janus*

(R) *Aulacophora foveicollis*

(S) *Aulacophora frontalis*



(T) *Dicranolaius* sp.



(U) *Agelastica alni*

(V) *Mylabris variabilis*

(W) *Bactrocera cucurbitae*

(X) *Efferia deserti*

(Y) *Syrphus ribesii*

(Z) *Dolichomutilla sycorax*

Plate 2 Insect species recorded from the study area

(A) *Lagenaria vulgaris* (bu)

(B) *Cucurbita maxima* (shwe-phayon)

(C) *Cucumis sativus* L. (tha-khwar)

(D) *Citrullus vulgaris* (hpa-ye)

Plate 3 Cucurbitaceous plants from the study area

(A) Damage parts of bu

(B) Damage parts of shwe-phayon

(C) Damage parts of tha-khwar

(D) Damage parts of hpa-ye

Plate 4 Cucurbitaceous plants damaged by some insect pest

Discussion

Watermelons, cucumber, gourds, and pumpkins are cucurbits commonly grown in the tropical areas. These crop, are attacked by a variety of insects. A total of 26 insect species confined to 23 genera, 17 families and five orders were recorded in Cucurbit plants. Among these, 19 species (73.08%) are pest species and seven species (26.92%) are predators. From the 26 species, two species could be identified up to genus level, such as *Atractomorpha* sp. and *Dicranolaius* sp. The remaining type of insects was identified down to species level.

Hemiptera true bugs represent the largest order of insects. Most bugs are plant feeders, which they pierce to suck the sap. Other bugs are predators, piercing prey. Many species are agricultural pests while others are beneficial stated that Bertone, M., (2013). In the present study, eight bug species were occurred in order Hemiptera. Among these, seven species were pest species and one species is as predator such as *Rhynocoris fuscipes* Assassin Bug was observed.

Many species of order Orthoptera were crop pest while order Diptera included numerous species of predators stated that Hill (1983). From the study area, all species are as pest in these Orthoptera as well as all species are as predators in Diptera were observed. This results the same with the Hill's report.

Bertone M., (2013) stated that order Hemiptera with 90,000 species true bugs represent the largest order of insects as well as Coleoptera are the largest group of organisms on Earth, making up over 25% of all animal species. With the results, the most dominant order Coleoptera (10 species, 38.46%), followed by Hemiptera (eight species, 30.77 %), Orthoptera (four species, 15.38 %), Diptera (three species, 11.54 %), and Hymenoptera (one species, 3.85%) were observed.

Gerald Brust (2009) mentioned a large variety of cucurbits most have a few very important pests in common such as cucumber beetles, squash bugs, mites and aphids. Some pests are more specific such as squash vine borer, which attacks pumpkins and squash, but rarely watermelon, cucumber. In the study, the insect pest species, 17 species on shwe-phayon, 13 species on bu, eleven species on tha-kwar and ten species on hpa-ye were occurred in the different host plants. Among these, seven species are the most serious damaged on cucurbitaceous plants as agricultural pest species such as, *Xenocatantops humilis* Short-horned Grasshopper, *Arocatus melanocephalus* Elm Seed Bug, *Cletus bipunctatus* Leaf-footed Bugs *Epilachna varivestis* Mexican Bean Beetles, *Aulacophora foveicollis* Pumpkin Beetle, *A. frontalis* Black Cucurbit Beetle and *Bactrocera cucurbitae* Melon Fruit Fly attacked in all study plants.

Hill (1983) described that pest damage is probably most conveniently considered according to the part of the plant body attacked, and in the following account it is studied under headings; damaged leaves, flowers and buds, fruit and stems. In the present study, the damaged parts of the plants, attacked by insect pest species 16 species most attacked damaged part of leaves followed by 14 species of flowers, 12 species in buds, nine species in fruits and six species in stems were occurred. Shepard, *et al.* (1987) stated that the natural balance between insect pests and their natural enemies is often disrupted by indiscriminate use of chemical insecticides. Although insecticides are needed in some cases, they must be used judiciously to save these vulnerable natural control agents. In the present observation, seven predator species infest on cucurbits as beneficial insect. The beneficial species often control insect pests, especially in the places use of pesticides is avoided.

Spotted beetles are distributed from East Asia to South Asia and Australia. They are polyphagous, and feed predominantly on cucurbits, tomato, potato, and kidney bean as well as eggplant. These beetles are considered to be one of the most serious groups of pests damaging eggplant stated that David (2001). During the study period, three spotted beetles such as *Menochilus sexmaculatus* Six-spotted Zigzag Ladybird, *Coccinella transversalis* Transverse

Ladybird Beetle *Epilachna varivestis* Mexican Bean Beetles were occurred. Of these three spotted beetles, two species are predators and one species is a pest. *Epilachna varivestis* Mexican Bean Beetles is a quite serious pest of many crops found in all study plants. Both adults and larvae feed on the leaves and fruits of cucurbits.

Metha and Sandhu (1989) stated that Pumpkin beetles are the major pest of cucurbit plants. In the present study, two types of Pumpkin beetles such as *Aulacophora foveicollis* Pumpkin Beetle and *A. frontalis* Black Cucurbit Beetle which can cause serious damage to cucurbit. Dhillon *et al.*, (2005) reported that *Bactrocera cucurbitae* Melon Fruit Fly is distributed widely and is a major pest of cucurbitaceous vegetables. From the results, this species attacked by all study plants and serious damaged parts of the cucurbit plants.

In conclusion, Cucurbitaceae is of high economic value being a major source of food for man. Many species of Cucurbit plants as Bu, Shwe- phayon, Tha-khwar and Hpa-ye are cultivated for edible purposes. This is an attempt to compile on the different uses of these plants and to recommend that increased in production of these plants will be profitable and will contribute to food security and livelihood sustainability in Salin environs.

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BASKING BEHAVIOR OF BURMESE STAR TORTOISES *GEOCHELONE PLATYNOTA* (BLYTH, 1863) IN MINZONTAUNG WILDLIFE SANCTUARY, MYANMAR

Khaing Khaing Thu[1], Su Su Naing[2], San San Oo[3]

Abstract

Basking behavior of Burmese Star Tortoises *Geochelone platynota* from Natogyi Township, Myingyan District, Mandalay Regiion in Minzontaung Wildlife Sanctuary were conducted March 2019 to February 2020. The observed number was total of 122 star tortoises including 54 males and 68 females. The basking duration of each turtle was an average of 25.69 minutes per day. The female was more basking time than the males, 26.27 min per day and 25.00 min per day respectively. The seasonal variation of the basking time was observed, the lowest time was in rainy season and highest in winter season in both sexes. The female basking time was the lowest in rainy season (24.00 min), the highest was in winter season (40.80 min) and moderate in the summer season (24.49 min). For the male, the lowest in rainy season, (22.71 min) the highest was in winter season, (33.29 min) and moderate in the summer season (23.17 min). In this research 33.91% of total observed female number were coming out and 38.49 **%** of total observed male number were coming out for basking each day. The percent of basking tortoises was also correlated with cloudy conditions, highest percentage was ranked depending basking activity as under 1) partly sunny, 2) sunny, 3), mostly sunny partly cloudy, 4) cloudy, 5), mostly cloudy 6)Thunderstorm , 7) rain and 8) shower . During the study period the highest temperature is 41 °C and the lowest temperature is 18°C. The basking activity is predominantly utilized to meet physiological needs (i.e., reproductive effort, metabolism, digestion, growth) via thermoregulation.

Keywords: Basking, *Geochelone platynota,* temperature, ectothermic

Introduction

Turtles, like other reptiles, are ectothermic, and their body temperature is largely dependent on that of their environment. Some turtles can maintain a fairly precise and constant body temperature if a heat source is available (Cossins and Bowler Auth, 1975; Crawford *et al*., 1983). By increasing their body temperatures, there is a corresponding increase in the rate of physiological processes (Crawford *et al.*, 1983; Hammond *et a*l., 1988), such as metabolic rate (Bennet, 1982), digestion speed, and digestion efficiency (Kepenis and McManus, 1974; Paramenter, 1981). The thermoregulatory behavior of reproductively active females is important in turtles and other reptiles. For example, increases in body temperature of female turtles can result in a greater rate of follicular development (Ganzhorn and Licht, 1983; Mendonca, 1987). Basking activity is one of the most conspicuous daily behaviors exhibited by aquatic emydid turtles. Early studies suggested that the primary physiological role of basking in turtles was to regulate body temperatures, as well as condition the skin and shell (Cagle 1950; Boyer 1965; Auth 1975). Others also hypothesized that basking increases metabolic and digestion rates (Moll and Legler 1971; Parmenter 1980; Hammond et al. 1988; Avery et al. 1993), aids in vitamin K synthesis (Pritchard and Greenhood 1968), increases follicular and egg production in female turtles during the nesting season (Vogt 1980; Krawchuk and Brooks 1998), allows turtles to rest (Boyer 1965; Waters 1974), fights infection by ‘‘behavioral fever’’ (Monagas and Gatten 1983), and rids turtles of ectoparasites (Cagle 1950; Neill and Allen 1954; Shealy 1976; Vogt 1979).Moll and Legler (1971) stated that basking is likely initiated by a single impulse, which is either triggered by an external factor or

[1] PhD Candidate, Department of Zoology, University of Yangon
[2] Associate Professor, Department of Zoology, Dawei University
[3] Professor, Department of Zoology, University of Yangon

internal physiological need, while other secondary benefits are thereafter gained. The present research is carried out with the following objectives are:

- To investigate the basking time duration
- To number of male and female tortoises
- To study monthly and seasonal variation of the basking
- To correlate the basking behavior with the weather parameters

Materials and Methods

Study site and study period

Minzontaung Wildlife Sanctuary is a protected area of Myanmar. It is located in the Natogyi Township, Myingyan District, Mandalay Region. It occupies an area of 22.6 square kilometres (8.7 sq mi) and was established in 1998-99. The study was undertaken between March 2019 to February 2020.

Study method

The total numbers of 122 tortoises and the numbers of males are 54 and females are 68. Observer location was chosen based upon preliminary observation of turtle behavior, and focused on turtle basking behavior. Data collection was conducted from 7:00 am to 5:00 pm at every day and seven days of every month. During each observation period, detailed information on basking behavior was recorded. Information of basking behavior included about numbers of tortoises, sexes, numbers of male and female, starting and ending of individual basking time. These data were noted hourly. Basking behavior was studied in the captive farm of Minzontaung Wildlife Sanctuary.

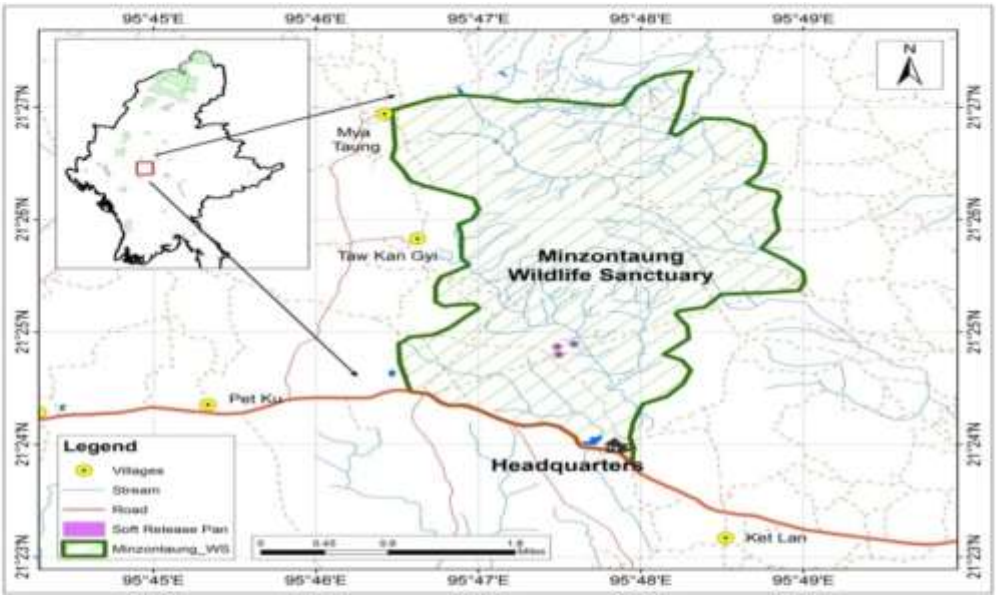

Figure 1 Map of the study area (**Source:** Minzontaung wildlife Sanctuary)

Plate 1 Captive farm in Minzontaung Wildlife Sanctuary

Plate 2 Marking system in the study site

A. Group of basking male and female Star tortoises

B. Colonial basking type of Burmese Star tortoise

C. Some male and female Star tortoises staying in the shade at afternoon

D. Some male and female Star tortoises staying in the shade at afternoon

Plate 3 Basking behavior of *Geochelone platynota*

Results

In Burmese star tortoise, time duration of basking in both sexes was fond to varies in different season, the highest in winter season (37.04 min per day), the lowest in rainy season (23.35 min per day), while in summer season, it was little more than that of rainy (23.83 min per day). The basking time of male and female was found differences. The mean of basking time is 28.07 min per day), while the mean of male basking time is (26.39 min per day) and the mean of female basking time is (29.76 min per day) (Table 1) and (Figure 2).

Table 1 Seasonal variation of basking time duration (min) of male and female tortoises (March 2019 to February 2020)

Sex	Season			Mean (min)
	Summer (min)	Rainy (min)	Winter (min)	
Male	23.71 ± 2.30	22.71± 2.59	33.29± 4.34	26.39 ± 3.09
Female	24.49± 4.67	24.00± 1.72	40.80± 2.82	29.76 ± 3.07
Mean	23.83 ± 3.4	23.35 ± 2.15	37.04 ± 3.58	28.07 ± 3.08

Monthly variation of basking time duration of male and female tortoises was found varies in different, the lowest basking time duration was occurred in September (19.99 min per day in male and 22.38 min per day in female). The highest basking time occurred in December (31.51 min per day in male and 33.54 min per day in female) (Table 2) and (Figure 3).

Table 2 Monthly variation of basking time duration (min) of male and female tortoise (March 2019 to February 2020)

Monthly	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mean
Male (min)	22.72	22.87	21.93	24.15	21.76	21.14	19.99	26.53	30.59	31.51	28.57	27.22	25.00
SD	± 1.60	± 2.87	± 2.16	± 3.01	± 1.52	± 2.12	± 2.86	± 3.48	± 4.51	± 4.88	± 4.53	± 3.61	± 1.53
Female (min)	22.98	25.10	23.74	25.55	23.09	22.72	22.38	28.75	30.94	33.54	29.19	28.67	26.27
SD	± 1.60	± 1.75	± 1.62	± 1.78	± 1.61	± 1.64	± 1.56	± 2.01	± 2.16	± 2.34	± 3.98	± 2.07	± 1.83

Not all observed tortoises were coming out to bask under the sun light, some are still hide in the shading places. Hence, the number of basking tortoises vary in different months. In all month, the basking time of females are higher than males. The highest percentage of basking was occurred in December (50.51 % in male and 52.08 %in female) (Table 3) and (Figure 4).

Table 3 Monthly mean and percent of basking numbers of male and female tortoises (March 2019 to February 2020)

Month	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mean
Male no.	28.14	22.12	20.42	17.85	18.43	14.28	8.00	21.14	25.28	27.28	23.42	23.12	20.79
Male no. in %	52.00	41.00	37.81	33.05	34.12	26.44	14.81	39.14	46.81	50.51	43.37	42.81	38.49
Female no.	25.42	23.42	21.38	19.28	18.57	15.42	8.85	27.71	34.42	35.42	24.28	22.71	21.05
Female in %	37.38	34.44	31.29	28.35	27.30	22.67	13.01	40.75	50.61	52.08	35.70	33.39	33.91
Total %	44.69	32.21	34.55	30.70	30.71	24.55	13.91	39.94	48.71	51.29	39.53	38.10	36.20

Emergent time of basking tortoises coming out to bask under the sun light was at 6:00 am in the morning and end at 4:00 pm in the afternoon. Mean basking duration was seen to peak early in the morning and gradually decreases in the afternoon of the day. For both males and females, basking number (i.e., raw basking data) showed a clear bimodal pattern with peaks around 8:00hrs first time and 12:00 hours second time across all seasons, but there were differences on basking numbers among the three seasons. The highest basking number occurred in December and the lowest basking number occurred in September. There were 918 turtles basked during summer (March, April, May), 1184 turtles basked during rainy season (June, July, August, September, October) and 1547 turtles basked during the winter months (November, December, January, February) (Table 4) and (Figure 5).

Table 4 Basking numbers of star tortoise with time line of the star tortoises (March 2019 to February 2020)

Month	6:00 am	7:00 am	8:00 am	9:00 am	10:00 am	11:00 am	12:00 am	1:00 pm	2:00 pm	3:00 pm	4:00 pm	Total
Mar	9	20	38	45	44	58	50	31	11	6		323
Apr	7	14	34	45	50	48	45	19	14	7		283
May	6	20	38	45	44	54	50	32	18	5		312
Jun	2	14	32	40	42	47	40	28	12	8		265
Jul	0	11	19	38	42	45	40	28	11	9		243
Aug	0	10	26	31	35	41	37	24	1	2		207
Sep	0	13	13	15	19	17	15	12	9	6		119
Oct	4	34	42	47	49	56	43	38	22	10	5	350
Nov	12	42	50	57	67	70	52	39	30	20	2	441
Dec	21	45	48	61	65	67	50	40	28	22	1	448
Jan	16	35	40	38	42	46	40	32	28	23		343
Feb	19	40	42	40	43	38	40	35	30	18		315

Highest percentage of basking tortoises were ranked during basking activity as under 1) partly sunny, 2) sunny, 3) mostly sunny partly cloudy, 4) cloudy, 5), mostly cloudy 6) Thunder storm, 7) rain and 8) shower. During the study period the highest temperature is 41 °C and the lowest temperature is 18°C. Weather conditions and basking duration were directly correlated. Tortoises were never almost observed to bask when it was raining. They do more basking in sunny days (Table5) and (Figure 6).

Table 5 Monthly range of Temperature (°C) and cloudy condition of the sky at study area (March 2019 to February 2020)

Weather conditions	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Freq.**
Range of Temp.	26 – 38	27 – 39	27 – 41	27 – 41	27 – 41	26 – 35	27 – 36	23 - 32	18 – 32	18 – 31	18 – 34	19 – 34	-
Av. Temp	32.00	33.00	34.00	34.00	34.00	30.50	33.00	27.50	25.00	24.50	27.00	26.50	-
Partly Sunny											√		1
sunny	√					√	√	√	√		√	√	7
Mostly Sunny		√	√			√			√	√	√		6
Cloudy				√	√	√	√	√					5
Mostly Cloudy	√	√	√	√		√							5
Weather conditions	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Freq.
Thunder storm				√	√	√	√						4
Rain					√			√					2
Shower					√		√		√				3
Freq.	2	2	2	3	4	5	4	3	3	1	3	1	33

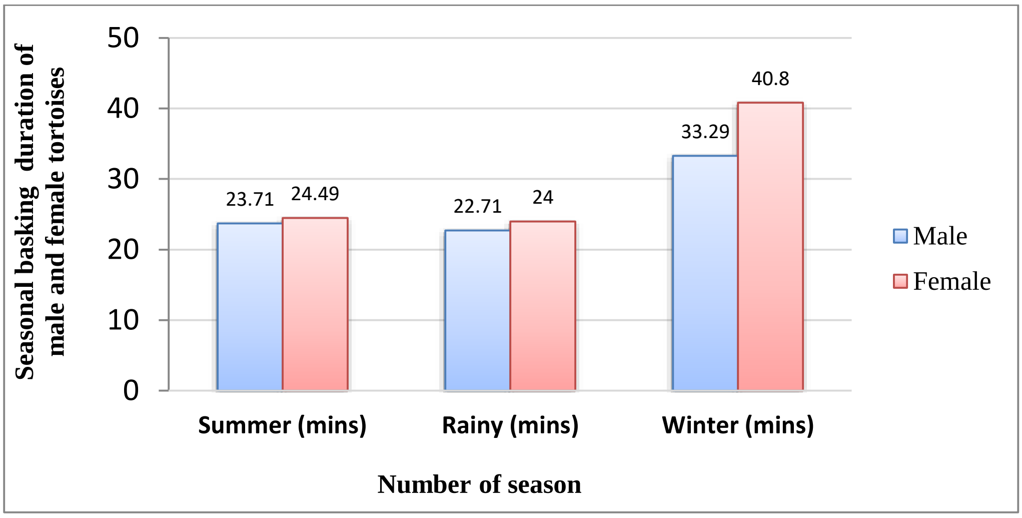

Figure 2 Comparison between seasonal basking duration of male and female tortoises

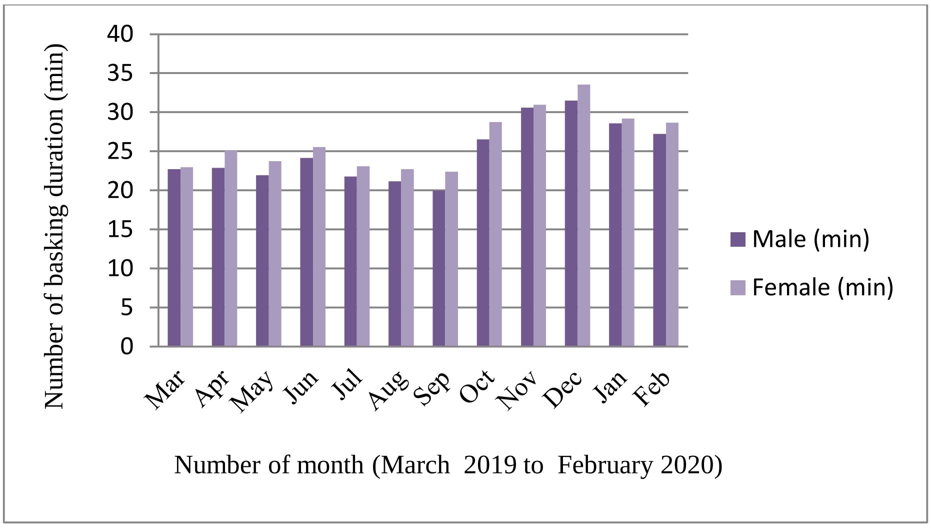

Figure 3 Monthly mean basking duration of male and female tortoises

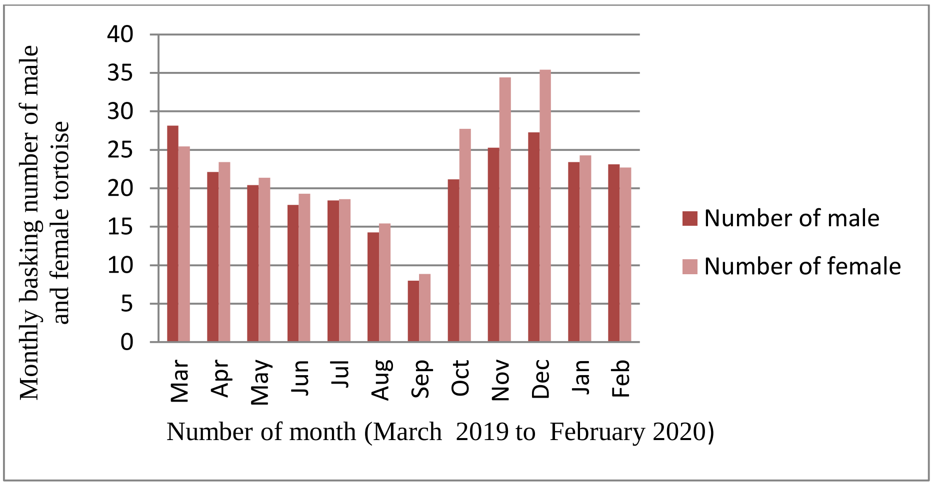

Figure 4 Monthly basking number of male and female tortoises

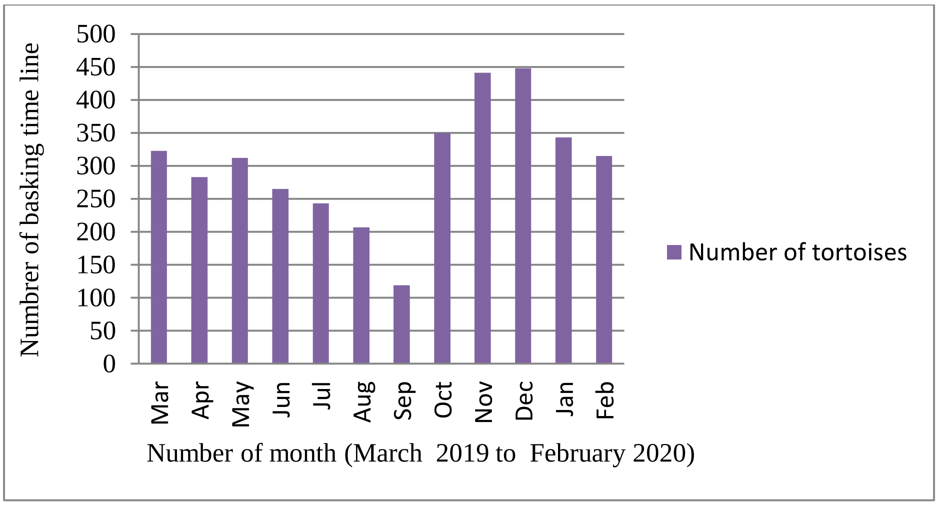

Figure 5 Monthly basking time line of *Geochelone platynota*

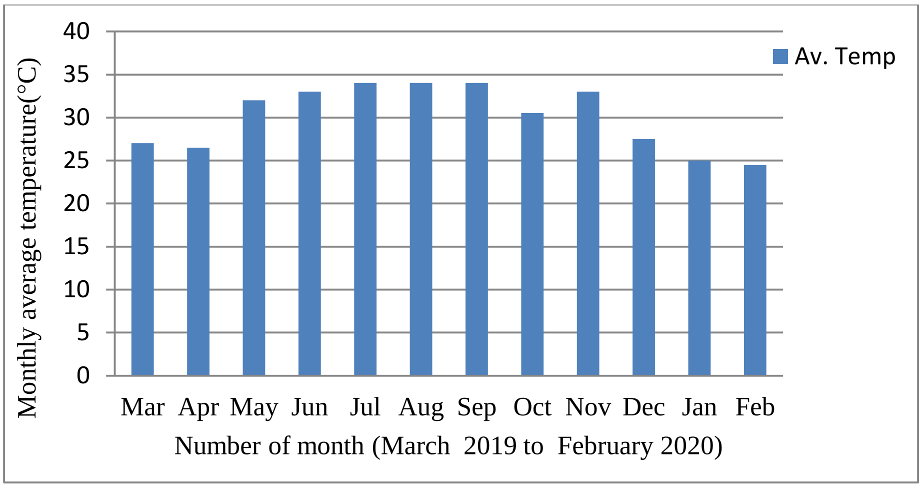

Figure 6 Monthly average temperature in the study area

Discussion

The number of basking turtles significantly differed among months with more turtles basking from October through December than in other months. The number of males and females basking also differed between months with a significantly higher percentage of females basking than males during October-December, which coincides with the breeding season. For both males and females, basking numbers showed a clear bimodal pattern with peaks around 10:00 am –13:00 pm across all seasons, but there were differences of numbers among the three seasons. The basking behavior of *Geochelone platynota* had not yet recorded in Myanmar. Thus this research suggests to relate the concerning of egg laying, hatchling and sex differentiation.

The longest duration of males and females were observed in the winter season. In viviparous lizards, females that increase basking duration during periods when energy is being allocated to developing embryos may be capable of devoting more stored energy to reproduction (Wapstra, 2000). The rate at which energy is allocated to developing follicles may be especially important for females in populations occupying the northern limit of their species' range, because the amount of thermal energy that they experience during the short active season may set strict limits on the amount of harvested energy that can be allocated to egg production (Rollinson and Brooks, 2007).

Therefore, during the winter, *G. platynota* basking is predominantly utilized to meet physiological needs (i.e., reproductive effort, metabolism, digestion, growth) via thermoregulation. According to the present research, the nesting season of *Geochelone platynota* is September to February. The numbers of basking turtles were observed to increase when close to nesting time. During that time, the winter season, when temperature is cold, more basking turtles were encountered because of turtles raised their body temperature by basking.

Conclusion

The basking time duration of Star Tortoise was for (28.07 min per day) and female was more bask (29.76 min per day) than that of the male (26.39 min per day). The mean basking time duration was found to differ significantly in seasonally (23.83 min in summer), (23.35 min in rainy) and (37.04 min in winter). According to the monthly variation, more turtles were basking from October through December than in other months. The peak of the basking tortoises occurred during the winter season. They do more basking in sunny days. Turtles like the sunlight to increase fat mobilization and reproduction.

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NESTING BEHAVIOR AND NEST STRUCTURE OF *APIS FLOREA* (FABRICIUS, 1787) FROM ALEL-THAUNG AND THA-HTAY-KONE VILLAGES IN YESAGYO ENVIRONS

Ko Ko Naing[1], Daw Maw[2]

Abstract

Nesting behavior and nest structure of *Apis florea* (Fabricius, 1787) was conducted from Alel-Thaung and Tha-Htay-Kone Villages in Yesagyo environs from December, 2018 to August, 2019. A total of 150 colonies was used for nesting by *Apis florea*. They used 31 different plant species which belong to 20 families and the man-made structures. *Tamaridus indica*, *Mangifera indica* and *Ziziphus jujuba* were most preferred nesting plants. The *Apis florea* nest occupied on the different height of the plants, the highest number of colonies was found in 3-6m height (57 colonies), followed by 0-3m (40 colonies), 6-9m (29 colonies), 9-12m (16 colonies), and 12m above (eight colonies). The measurement of comb length 18.95±7.5cm, ranging from 4 to 30 cm; comb width 16.85±5.74 cm, ranging from 2 to 26 cm and comb thickness 1.95±0.22 cm, ranging from 1 to 2 cm (n=20) were recorded. The number of honey storage cells, 36.3±6.64 (n=20), worker cells, 63.1±7.61cells (n=20), drone cells, 27.75±4.58 (n=8) and queen cells 5.71±3.69 cells (n=7) recorded in two centimeter square were observed in the study area.

Keywords: Nesting behavior, nest structure, *Apis florea*, Yesagyo environs

Introduction

Honeybees play an important ecological role as pollinators of many plant species, and their products are the basis for a multi-million dollar commercial industry around the world. They are major agricultural pollinators around the world and are keystone pollinators in tropical ecosystems. Pollination has been considered a keystone process to ecosystem function through the facilitation of both plant and animal diversity (Suwannapong. *et.al*, 2014).

The dwarf honeybee, *Apis florea* (Hymenoptera: Apidae) is a single-combed, open- nesting small bees. It is a wild species, not readily managed by man but is highly important as a pollinator of crops. It builds small sized single comb that attached to a twig of small trees or dense bushes in tropical forests, scrubby/bushy vegetation as also in farming areas, especially orchards (Basavarajappa, 2008).

The little bee *A. florea* builds a single vertical comb nest, which is constructed around the stem of a bush, branches of bushes, hedges, trees or a dried thick stick in the shaded places. The nesting location of *A. florea* is unique, not easily accessible to animals including mankind that could help avoid animals including human interferences and vehicular traffic. Accordingly, *A. florea* builds its colony at interior side away from the road. *A. florea* avails various plant species including human-built structures for nesting under shady places on the twigs/branches. Shady places help protect the colony members from bright light, strong winds and inclement weather conditions (Vaudo, *et. al.*, 2012).

The single comb of the *Apis florea* nest contains cells of four sizes. The large storage cells for the honey are very deep and constructed in such a manner that the comb bulges out on either side and at the top. The small worker cells are located below the honey storage cells. The considerably larger drone cells are mostly found in the lower part of the comb. The pear shape queen cells, which are the largest of the cells, are located near the bottom. These can be observed when a colony loses its queen, and are emergency queen-cells (Ruttner, 1988).

[1] Dr, Lecturer, Department of Zoology, Pakokku University
[2] Dr, Lecturer, Department of Zoology, Pakokku University

Apis florea choose different elevations in trees and shrubby plants were found suitable for the construction of colonies. The combs were found at the peripheral and middle regions of thickly interspersed canopy in trees and shrubs. Bee flora or bee plant, are the plants from which bees collect pollen and nectar. Honeybees forage on a variety of plant species to collect nectar and pollen, including agricultural crops and native plants. Yesagyo environs are the largest areas on a variety of agricultural crops and native plants for preferring by *Apis florea.* So, the present study was conducted with the following objectives;

- to investigate the nesting behavior and nest height above the ground of *Apis florea*
- to observe different type of cells in the combs by *Apis florea* in this study areas

Materials and Methods

Study area and period

The study area was conducted the Alel-Thaung Village (Site I) is situated 21° 29' 46″ N, 95° 14′ 29″ E. This area is about 4.8km. Tha-Htay-Kone Village (Site II) is situated 21° 30′ 13″ N, 95° 14′ 34″ E, and the areas is about 3.2km. The two study areas are situated on the west of the Chindwin River in Yesagyo environs. The study period lasted from December, 2018 to August, 2019.

Data collection

All the wild colonies were observed self-observation with the help of native people. The observations were made twice a month. The number of wild colonies was recorded in large trees, small trees, shrubs, man-made structures, grasses and ornamental plants. The collected combs from the fields were put into the plastic bags.

Measurements of comb

The height of the nests above the ground was measured to the nearest meter by using a bamboo pole. The length, width and thickness of honey combs were measured with Vernier caliper. The number of worker cells, drone cells, honey storage cell and queen cells in two centimeter square of each comb were counted and recorded.

Identification

The honeybees were identified according to Bingham (1897), Suwannapong (2011) and Noah Wilson-Rich (2014). The name of plants was identified by Kress *et.al*, (2003).

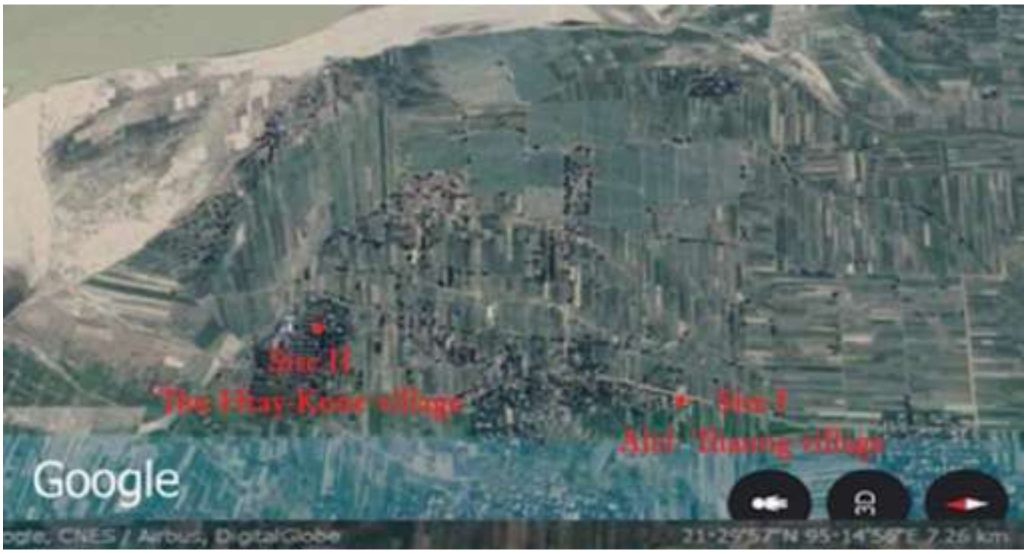


Figure 1 Location map of study area (Source: Google Earth)

Results

Descriptive account on the species studied

Phylum	- Arthropoda
Class	- Insecta
Order	- Hymenoptera
Family	- Apidae
Genus	- *Apis*
Species	- *A. florea* (Fabricius, 1787)
Common name	- Dwarf honey bee
Local name	- Yin-pya

There are three kinds of bees in a colony: the worker, drone and queen. Workers do not lay eggs, but they carry on all the other duties in the colony, including comb building, brood care, and colony protection. The worker brood cells are made on the flat surface in the middle of the comb (Plate 1 A & Plate 2 C). Drones have no pollen baskets or specialized glands. Drone cells were found on the lower part of the comb (Plate 1B & Plate 2 D). Queen is the largest bee in the colony, and has a long, pointed abdomen. Queen cells are found at the bottom edge of the comb and are quite large (Plate 1 C & Plate 2 E).

Nesting habit of the *Apis florea*

Apis florea colonies built a small comb nest, and the comb is constructed around the stem of a bush, branches of bushes, hedges, trees or a dried thick stick in the shaded places. *A. florea* avails various plant species including human-built structures for nesting under shady places on the twigs (Table 1)**.** A variety of 31 plants belonging to 20 families utilized as nesting plants and two human built structures for nest construction were recorded in the study area. The highest number of 26 nesting plants in Site I, followed by 16 nesting plants in Site II was observed. Human built structure as yard and pagoda were observed in both study sites during the study period (Table 2, Fig. 1).

Nest height above the ground of the *Apis florea*

Apis florea constructed their nests at height ranging from 0-3m to 12m above the ground. The plant height with the highest number of colonies was found in 3-6m (30 colonies), followed by 0-3m (27 colonies), 6-9m (20 colonies), 9-12m (nine colonies) and 12m above (seven colonies) in Site I (Table 3, Fig. 2). In Site II, plant height with the highest number of colonies was found in 3-6m (27 colonies), followed by 0-3m (13 colonies), 6-9m (nine colonies) and 9-12m (seven colonies), and 12m above (one colonies) (Table 4, Fig. 2,).

Nest structure of the *Apis florea*

The top the nest encircles the strong stem is thick, but as the comb is built further down, it becomes thin in depth, although as broad as the upper portion. In the same comb, brood is present in the lower section and the honey is found in the upper section. The honeycomb forms the interior structure of the hive, is made up of six-sided cells, and is made of beeswax. The comb cells of honeybee found in hexagonal in shape except the honey storage cells, these cells are asymmetrical hexagonal shape (Plate 2 A).

Comb measurements of the *Apis florea*

The highest colonies were 32, colonies in December and lowest six, colonies in July during in months (Table 5). The measurement of comb length 18.95±7.5cm, ranging from 4 to 30 cm; comb width 16.85±5.74 cm, ranging from 2 to 26 cm; comb thickness 1.95±0.22 cm, ranging from 1 to 2 cm (n=20) (Table 6).The number of honey storage cells, 36.3±6.64 (n=20), worker cells, 63.1±7.61cells (n=20), drone cells, 27.75±4.58 (n=8) was recorded in two centimeter square. The number of queen cells was 5.71±3.69 cells (n=7) were recorded in the study areas (Table 7, Fig 3, Plate 2).

Table 1 Nesting host plants of *Apis florea* in study areas

No	Family	Scientific Name	Common Name	Local Name	Plant type	No,of colonies
1	Rhamnaceae	*Ziziphus jujuba*	Jujube	Zee	Tree	30
2	Meliaceae	*Azadirachta indica*	Neem	Tama	Tree	10
3	Caesalpiniaceae	*Tamarindus indica*	Tamarind	Magyi	Tree	6
4		*Cassia siamea*	Siamese cassia	Mezali	Tree	10
5		*Bauhinia purpurea*	Swedaw-ni	Swe-daw	Tree	1
6		*Acrocarpus fraxinifolius*	Pink cedar	Ye-tama	Tree	5
7	Anacardiaceae	*Mangifera indica*	Mango	Thayet	Tree	18
8	Annonaceae	*Annona squamosa*	Custard apple	Awzar	Small Tree	2
9		*Annona muricata*	Sour-Sop	Duyin-awza	Small Tree	1
10	Arecaceae	*Borassus flabellifer*	Plamyra palm	Htan	Tree	2
11	Gramineae	*Bambusa sp.*	Bamboo	Nil	Grass	3
12	Mimosaceae	*Albizia lebbek*	Zo-fek	Kokko	Tree	15
13		*Leucaena leucocephala*	Aweya	Baw-zagaing	Tree	9
14		*Pithecellobium dulce*	Tayok-magyi	Thinbaw-magyi	Tree	2
15		*Acacia concinna*	Kin-mon-gyin	Kin-mon-gyin	Climber	1
16	Myrtaceae	*Psidium guajava*	Guava	Malaka	Small Tree	2
17		*Eugenia jambolana*	Malay Apple	Thabye	Tree	1
18	Rutaceae	*Citrus medica*	Lemon	Shauk	Shrub	2
19		*Feronia elephantum*	Wood Apple	Thee	Tree	2
20	Hypericaceae	*Garcinia mangostana*	Mangosteen	Mingut	Small tree	1
21		*Calophyllum inophyllum*	Pannay Tree	Pon-nyet	Tree	1
22	Rubiaceae	*Ixora coccinea*	Flame Tree	Pon-na-yeik	Shrub	2
23	Moraceae	*Morus indica*	Indian Mulberry	Posa	Small Tree	1
24	Boraginaceae	*Cordia myxa*	Taung-thanut	Thanat	Tree	2
25	Euphorbiaceae	*Emblica officinalis*	India Gooseberry	Zeebyu	Tree	1
26	Amaranthaceae	*Amaranthus blitoides*	Hin-nu-nwe	Hin-nu-nwe	Herb	1
27	Meliasnaceae	*Swietenia macrophylla*	Mahogany	Mahogany	Tree	2
28	Musaceae	*Musa sapientum*	Banana	Hnget-pyaw	Herb	5
29	Papilionaceae	*Dolichos lablab*	Lablab Bean	Pe-pazun	Climber	5
30	Solanaceae	*Solanum melongena*	Egg-Plant	Khayan	Shrub	1
31		*Capsicum frutescens*	Chilli	Nga-yok	Shrub	4

Table 2 The nesting plants used by *Apis florea* in each study sites

Sr no	Scientific name	Common name	Site I	Site II
1	*Azadirachta indica*	Neem	√	√
2	*Acrocarpus fraxinifolius*	Pink cedar	-	√
3	*Annona squamosa*	Custard apple	√	-
4	*Albizia lebbek*	Zo-fek	√	√
5	*Acacia concinna*	Kin-mon-gyin	√	-
6	*Amaranthus blitoides*	Hin-nu-nwe	√	-
7	*Annona muricata*	Sour-Sop	-	√
8	*Bauhinia purpurea*	Swedaw-ni	-	√
9	*Borassus flabellifer*	Plamyra palm	√	√
10	*Bambusa sp.*	Bamboo	√	√
11	*Cassia siamea*	Siamese cassia	√	√
12	*Citrus medica*	Lemon	√	-

Sr no	Scientific name	Common name	Site I	Site II
13	*Calophyllum inophyllum*	Pannay Tree	√	-
14	*Cordia myxa*	Taung-thanut	√	√
15	*Capsicum frutescens*	Chilli	√	-
16	*Dolichos lablab*	Lablab Bean	√	-
17	*Emblica officinalis*	India Gooseberry	-	√
18	*Eugenia jambolana*	Malay Apple	√	-
19	*Feronia elephantum*	Wood Apple	√	-
20	*Garcinia mangostana*	Mangosteen	√	-
21	*Ixora eoccinea*	Flame Tree	√	√
22	*Leucaena leucocephala*	Aweya	√	√
23	*Mangifera indica*	Mango	√	√
24	*Morus indica*	Indian Mulberry	√	-
25	*Musa sapientum*	Banana	√	-
26	*Pithecellobium dulce*	Tayok-magyi	√	-
27	*Psidium guajava*	Guava	√	-
28	*Swietenia macrophylla*	Mahogany	-	√
29	*Solanum melongena*	Egg-Plant	√	-
30	*Tamarindus indica*	Tamarind	√	√
31	*Ziziphus jujuba*	Jujube	√	√
	Human-built Structure	-	√	√
		Total	**26**	**16**

Table 3 Occurrence of *Apis florea* colonies in different plant height in study Site I

Sr no.	Scientific name	Common name	Plant height					
			0-3m	3-6m	6-9m	9-12m	12m above	Total
1	*Azadirachta indica*	Neem	2	3	1	-	-	6
2	*Annona squamosa*	Custard apple	-	2	-	-	-	2
3	*Albizia lebbek*	Zo-fek	-	-	3	2	5	10
4	*Acacia concinna*	Kin-mon-chin	1	-	-	-	-	1
5	*Amaranthus blitoides*	Hin-nu-nwe	1	-	-	-	-	1
6	*Borassus flabellifer*	Plamyra palm	-	-	1	-	-	1
7	*Bambusa sp.*	Bamboo	-	-	2	-	-	2
8	*Cassia siamea*	Siamese cassia	1	-	2	2	-	5
9	*Citrus medica*	Lemon	2	-	-	-	-	2
10	*Calophyllum inophyllum*	Pannay Tree	-	1	-	-	-	1
11	*Capsicum frutescens*	Chilli	4	-	-	-	-	4
12	*Cordia myxa*	Taung-thanut	-	-	1	-	-	1
13	*Dolichos lablab*	Lablab Bean	5	-	-	-	-	5
14	*Eugenia jambolana*	Malay Apple	1	-	-	-	-	1
15	*Feronia elephantum*	Wood Apple	-	2	-	-	-	2
16	*Garcinia mangostana*	Mangosteen	-	1	-	-	-	1
17	*Ixora eoccinea*	Flame Tree	1	-	-	-	-	1
18	*Leucaena leucocephala*	Aweya	-	3	2	-	-	5
19	*Mangifera indica*	Mango	3	5	2	3	-	13
20	*Morus indica*	Indian Mulberry	1	-	-	-	-	1
21	*Musa sapientum*	Banana	3	2	-	-	-	5
22	*Pithecellobium dulce*	Tayok-magyi	-	2	-	-	-	2
23	*Psidium guajava*	Guava	-	2	-	-	-	2
24	*Solanum melongena*	Egg-Plant	1	-	-	-	-	1
25	*Tamarindus indica*	Tamarind	-	1	2	-	-	3
26	*Ziziphus jujuba*	Jujube	-	6	4	2	2	14
	Human-built Structure		1	-		-	-	1
		Total	**27**	**30**	**20**	**9**	**7**	**93**

Table 4 Occurrence of *Apis florea* colonies in different plant height in study Site II

Sr no.	Scientific name	Common name	Plant height					
			0-3m	**3-6m**	**6-9m**	**9-12m**	**12m above**	**Total**
1	*Azadirachta indica*	Neem	1	2	1	-	-	4
2	*Acrocarpus fraxinifolius*	Pink cedar	2	3	-	-	-	5
3	*Albizia lebbek*	Zo-fek	-	-	2	2	1	5
4	*Annona muricata*	Sour-Sop	1	-	-	-	-	1
5	*Bauhinia purpurea*	Swedaw-ni	-	-	1	-	-	1
6	*Borassus flabellifer*	Plamyra palm	-	-	-	1	-	1
7	*Bambusa sp.*	Bamboo	-	-	1	-	-	1
8	*Cassia siamea*	Siamese cassia	-	2	1	1	-	4
9	*Cordia myxa*	Taung-thanut	-	-	1	-	-	1
10	*Emblica officinalis*	India Gooseberry	-	1	-	-	-	1
11	*Ixora eoccinea*	Flame Tree	1	-	-	-	-	1
12	*Leucaena leucocephala*	Aweya	2	2	-	-	-	4
13	*Mangifera indica*	Mango	2	3	-	3	-	8
14	*Swietenia macrophylla*	Mahogany	-	2	-	-	-	2
15	*Tamarindus indica*	Tamarind	-	3	-	-	-	3
16	*Ziziphus jujuba*	Jujube	3	9	2	-	-	14
	Human-built Structure		1	-	-	-	-	1
		Total	**13**	**27**	**9**	**7**	**1**	**57**

Table 5 Occurrence of *Apis florea* colonies on different nesting plants during various months

Sr no.	Scientific name	Dec	Jan	Feb	March	April	May	June	July	Aug	Total	colonies (%)
1	*Ziziphus jujuba*	6	5	5	6	1	2	1	1	3	30	20.0
2	*Azadirachta indica*	2	1	2	2	1	1	1	-	-	10	6.7
3	*Tamarindus indica*	-	1	1	-	1	-	1	1	1	6	4.0
4	*Cassia siamea*	2	2	1	-	-	1	2	1	1	10	6.7
5	*Bauhinia purpurea*	-	1	-	-	-	-	-	-	-	1	0.7
6	*Acrocarpus fraxinifolius*	2	1	2	-	-	-	-		-	5	3.3
7	*Mangifera indica .*	5	3	2	3	2	1	-	-	2	18	12.0
8	*Annona squamosa*	-	-	1	-	1	-	-	-	-	2	1.3
9	*Annona muricata*	-	-	-	1	-	-	-	-	-	1	0.7
10	*Borassus flabellifer*	-	1	-	1	-	-	-	-	-	2	1.3
11	*Bambusa sp.*	1	-	1	1	-	-	-	-	-	3	2.0
12	*Albizia lebbek*	4	1	1	2	2	2	1	1	1	15	10.0
13	*Leucaena leucocephala*	1	1	2	2	2	1	-	1	-	9	6.0
14	*Pithecellobium dulce*	-	-	1	-	1	-	-	-	-	2	1.3
15	*Acacia concinna*	-	-	1	-	-	-	-	-	-	1	0.7
16	Psidium guajava	-	1	-	1	-	-	-	-	-	2	1.3
17	*Eugenia jambolana*	-	-	-	1	-	-	-	-	-	1	0.7
18	*Citrus medica*	1	-	1	-	-	-	-	-	-	2	1.3
19	*Feronia elephantum*	-	-	1	-	1	-	-	-	-	2	1.3
20	*Garcinia mangostana*	-	1	-	-	-	-	-	-	-	1	0.7
21	*Calophyllum inophyllum*	-	1	-	-	-	-	-	-	-	1	0.7
22	*Ixora eoccinea*	1	-	-	1	-	-	-	-	-	2	1.3
23	*Morus indica*	-	-	-1	-	-	-	-	-	-	1	0.7
24	*Cordia myxa*	1	-	-	-	-	1	-	-	-	2	1.3
25	*Emblica officinalis*	-	-	-	-	-	1	-	-	-	1	0.7
26	*Amaranthus blitoides*	1	-	-	-	-	-	-	-	-	1	0.7
27	*Swietenia macrophylla*	-	-	-	-	-	-	2	-	-	2	1.3
28	*Musa sapientum*	1	-	-	1	1	-	-	1	1	5	3.3
29	*Dolichos lablab*	1	2	1	1	-	-	-	-	2	5	3.3
30	*Solanum melongena*	-	-	-	1	-	-	-	-	-	1	0.7
31	*Capsicum frutescens.*	2	-	1	1	-	-	-	-	-	4	2.7
	Human-built Structure	1	-	-	-	-	-	1	-	-	2	1.3
	Total	**32**	**21**	**23**	**25**	**13**	**10**	**9**	**6**	**11**	**150**	

Table 6 Measurements of some honey combs of *Apis florea* in the study areas

Sr no.	Measurement of honey comb (cm) (n=20)		
	Length	Width	Thickness
1	26.00	19.00	2.00
2	25.00	23.00	2.00
3	17.00	26.00	2.00
4	25.00	17.00	2.00
5	14.00	12.00	2.00
6	9.00	7.00	2.00
7	10.00	12.00	2.00
8	17.00	18.00	2.00
9	16.00	18.00	2.00
10	4.00	2.00	1.00
11	22.00	20.00	2.00
12	14.00	13.00	2.00
13	22.00	20.00	2.00
14	30.00	24.00	2.00
15	20.00	18.00	2.00
16	16.00	18.00	2.00
17	30.00	20.00	2.00
18	14.00	12.00	2.00
19	22.00	20.00	2.00
20	26.00	18.00	2.00
Mean ± SD	18.95 ± 7.5	16.85 ± 5.74	1.95 ± 0.22

Table 7 Numbers of honey storage cells, worker cell, drone cell, and queen cells of *Apis florea*

Sr no.	No; of honey storage cells (2cm^2) (n=20)	No; of worker cells (2 cm^2) (n=20)	No; of drone cells (2 cm^2) (n=8)	No; of queen cells (n=7)
1	29.00	58.00	22.00	0.00
2	33.00	66.00	25.00	0.00
3	39.00	61.00	0.00	0.00
4	42.00	59.00	0.00	0.00
5	36.00	67.00	0.00	0.00
6	27.00	57.00	0.00	0.00
7	38.00	66.00	0.00	0.00
8	42.00	61.00	0.00	0.00
9	37.00	62.00	0.00	5.00
10	29.00	51.00	0.00	0.00
11	30.00	70.00	30.00	1.00
12	38.00	56.00	0.00	0.00
13	48.00	60.00	24.00	12.00
14	54.00	82.00	33.00	7.00
15	30.00	58.00	32.00	8.00
16	32.00	56.00	0.00	0.00
17	34.00	67.00	26.00	2.00
18	38.00	66.00	0.00	0.00
19	34.00	80.00	30.00	5.00
20	36.00	59.00	0.00	0.00
Mean ± SD	36.3 ± 6.64	63.10 ± 7.61	27.75 ± 4.58	5.71 ± 3.69

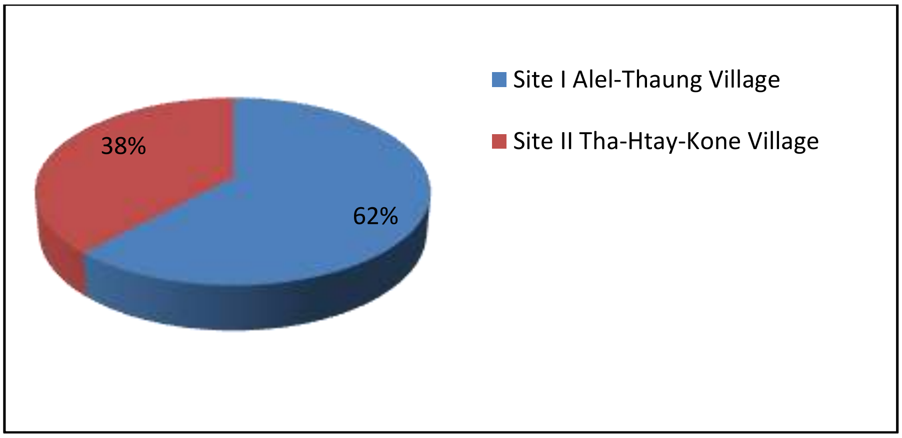


Figure 1 Percentage composition of nesting host plants by *Apis florea* in the study sites

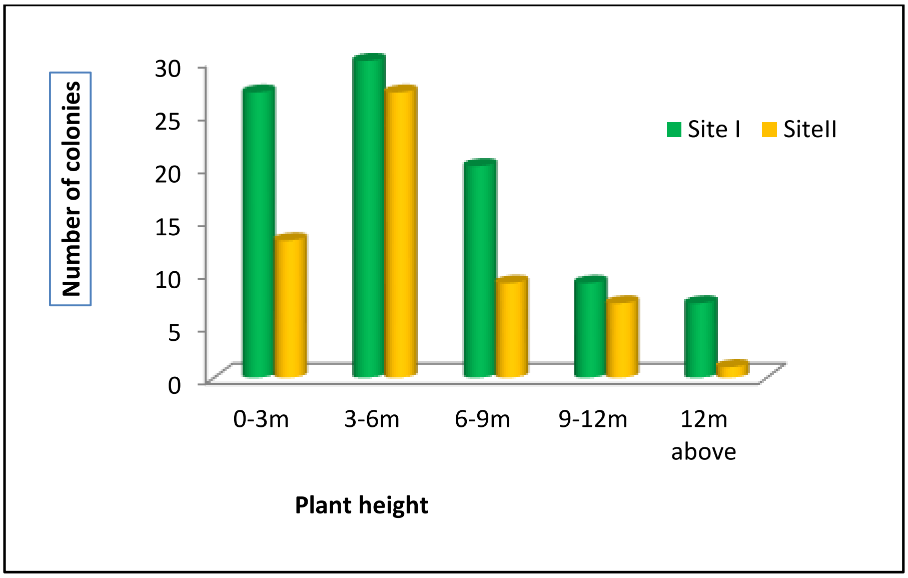


Figure 2 Comparison of *Apis florea* colonies in different plant height in the study areas

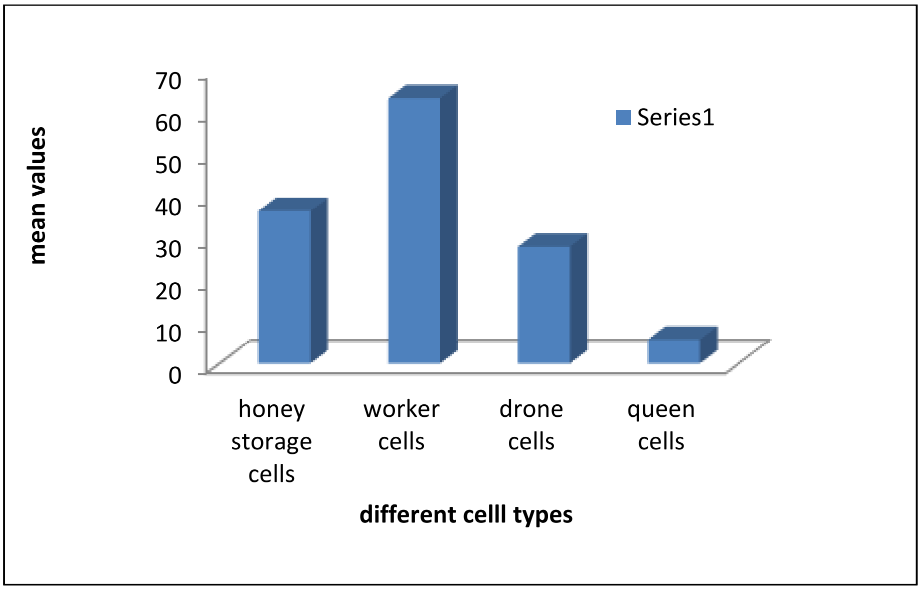
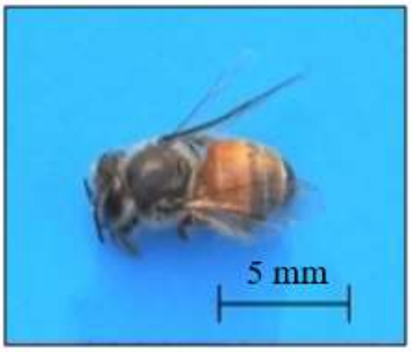


Figure 3 Mean value of different cell types in *Apis florea*



(A) Worker

(B) Drone

(C) Queen

Plate 1 Three castes of *Apis florea*

(A) Structure of honey comb

(B) Honey storage cells

(C) Worker cells

(D) Drone cells

(E) Queen cells

Plate 2 Different cell types of *Apis florea*

Discussion

A. florea prefers to live under wild conditions, not readily managed by man. It builds small sized single comb surrounded the plant twig/branch from which the comb was suspended. A total of 150 colonies and 31 plant species belonging to 20 families and two human built structures at different place for nesting were recorded from Yesagyo environs.

A. florea nests are attached to a wide variety of plants. Nests of *A. florea* occur in wooded areas, urban settings, areas with intensive agricultural activity as well as in the savanna stated by Franssen (1932). In the study, *Apis florea* preferred to nest on various plants of Caesalpiniaceae, Mimosaceae families with the highest (four species, 12.90% in each), followed by Annonaceae, Myrtaceae, Rutaceae, Hypericaceae and Solanaceae (two species, 6.45% in each). The remaining families with one species each, (3.23%) were recorded.

Zewdu *et al.* (2017) stated that concerning preference of plant species to the nest were found on dried and alive *Ziziphus* spp. *A. florea* in the assessed localities prefers small branches of *Zizphus* for their nesting. In the present study, the highest colonies on *Ziziphus jujube* (Zee) (30 colonies, 20.00%), followed by *Mangifera indica* (Thayet) (18 colonies, 12.00%), *Albizia lebbek* (Kokko) (15 colonies, 10.00%), *Azadirachta indica* (Tama), *Cassia siamea* (Mezali) (ten colonies, 6.67% in each), *Leucaena leucocephala* (Baw-zagaing) (nine colonies, 6.00%) and *Musa sapientum* (Hnget-pyaw) (five colonies, 3.33%) were observed.

The present data showed the highest number of 26 nesting plants (61.90%) in Site I. It can be assumed that Site I including agricultural crops and native plants are pollinated by honeybee. Of these, several plant species are visited by honeybees such as corn, sunflower, mango, cucurbit plants and jujube. While the lower number of 16 varieties of nesting plants (38.10%) was occurred in Site II. It can be assumed that this area is less suitable habitats as foraging nesting plants preferring for the honeybee.

According to Narayanaswamy and Basavarajappa (2013), *A. florea* preferred more in lower elevations (i.e., ground level to upto 15ft height) compared to higher elevations. However, *A. florea* didn't prefer much higher elevations (i.e., 15.1ft onwards) for nesting. In the present study, the highest number of colonies was found in 3-6m (30 colonies, 20.00%), followed by 0-3m (27colonies, 18.00%), 6-9m (20 colonies, 13.33%), 9-12m (nine colonies, 6.00%) and 12m above (seven colonies, 4.66%) in Site I. In Site II the highest number of colonies was found in 3-6m (27 colonies, 18.00%), followed by 0-3m (13 colonies, 8.67%), 6-9m (nine colonies, 6.00%), 9-12m (seven colonies, 4.66%) and 12m above (one colonies, 0.67%).

Khin Su Myat (2015) observed that the mean value of length and width of honey comb were 18.33±4.36cm and 19.43±4.68cm respectively. In the present data, the mean value of length, width and thickness of honey comb were 18.95±7.5cm, 16.58±5.74cm and 1.95±0.22cm respectively. May Yu Maw (2016) observed that the mean value of number of honey storage cells, worker cells, drone cells and queen cells were 68.20±9.14cm, 114.05±4.55cm, 45.44±2.99cm and 6.33±1.15cm respectively per three centimeter square. In the present data the mean value of number of honey storage cells, worker cells, drone cells and queen cells were 36.3±6.64cm, 63.10±7.61cm, 27.75±4.58cm and 5.71±3.69cm cells respectively per two centimeter square. Worker and drone brood are reared in a hexagonal cells, queen development takes place in cells shaped somewhat like peanuts. Swarm queen cells are built along the lower edge of the comb, often in large numbers: as many as 20 cells of various ages may be seen in a colony stated by (Michener, 2000). In the present study, queen cells of 1- 12 range in each colony were observed.

Narayanaswamy and Basavarajappa (2013), stated that total 139 normal colonies were recorded from January to June and the highest (36) normal colonies were recorded during February, followed by March (31) and April (29 colonies). However, in May and June the normal colonies were less (10 each) were occurred during different months at Manasagangotri campus. In the present study, total 150 colonies were recorded from December to August and highest (32, colonies) were recorded during December, followed by March (25, colonies), February (23, colonies), January (21, colonies), April (13, colonies), August (11, colonies), May (ten, colonies), June (nine, colonies), and July (six, colonies). Thus, the colonies mostly depended on the proportion of floral abundance and favorable weather condition.

It can be concluded that honey bees produce honey. Without the work of honey bees our agricultural crops would suffer huge losses, as the plants would produce considerably less fruit and seed. Honey bees are the farmer's favorite insects. Therefore, the honey bee's commercial value comes from the role it plays in the pollination of crops as well as when enough bees are present in a forest, they provide a better pollination that leads to improved regeneration of trees and conservation of the forest's biodiversity. At present, conservation of native honeybee species and preserve natural bee habitat is of primary importance in Yesagyo environs.

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NUTRITIONAL VALUES AND CHEMICAL CONSTITUENTS OF QUILLS OF *HYSTRIX BRACHYURA* (LINNAEUS, 1758) USED IN TRADITIONAL MEDICINE IN MYANMAR

Thein Gi Naing[1] and Khin Mya Mya[2]

Abstract

Quills of *Hystrix brachyura* which are commonly used in Myanmar Traditional Medicinal system were selected for chemical and pharmacological investigation. Powder of quills of *Hystrix brachyura* were tested for the presence of macroelements, microelements, toxic elements and proximate analysis were also determined. Chemical analysis revealed the presence of calcium 2.75±0.4%, magnesium 0.34±0%, in the *Hystrix brachyura* respectively. Proximate analysis showed that *Hystrix brachyura* contained moisture 7.92±0.28%, ash 0.4±0.02%, water soluble ash 99.8±0%, acid insoluble ash 0.4±0%, protein 92.65±2.53%, fat 0.22±0.20% and fiber 1.14±0.04% respectively. Proximate analysis showed protein content in the highest amount in quills of *Hystrix brachyura* and chemical analysis showed calcium in high quantity. The content of arsenic was found to contain below levels according to the WHO standard. Cadmium, lead and mercury were not found in these samples. Moreover, mineral and element contents were also examined with reasonable concentration in these animal parts. This finding indicated that these animal parts may be used safety in the traditional medicine. However, as these parts come of natural living assets, there is a need to consider synthetic materials as substitute since in the long run, might impose a threat to these living assets.

Keywords: *Microelements, Macroelements, Toxic Elements and Proximate Analysis*

Introduction

Most of Myanmar traditional drugs are derived from sources of plants and animals. Wild and domestic animals and their by-products (e.g., hooves, skins, bones, feathers, milk and tusks) are important ingredients in the preparation of curative, protective and preventive medicine in Myanmar Traditional Medicine. According to the World Health Organization (1993), about 80% of the world people rely primarily on animal and plant-based medicines. About 20 % of Myanmar traditional medicine is based on animal-derived substances. Therefore these crude animal's products were selected for chemical and pharmacological investigation.

In Myanmar, quill of *Hystrix brachyura* also called Hpju Zu: which are commonly used in Myanmar Traditional Medicine formulation (TMF). Ash form of porcupines' quills is the ingredients of TMF-21(Hsi: Hsei: Phyu) and TMF-67 Pan:na chew:kja' pjau' Hsei:. Therefore in this research these crude animal's products were selected for chemical and pharmacological investigation.

The Malayan porcupine or Himalayan porcupine *Hystrix brachyura* is a species of rodent in the family Hystricidae. Three subspecies are extant in South and Southeast Asia (Woods and Kilpatrick, 2005).The Geographical distribution of Malayan porcupine ranges from Nepal through north-east India to Bangladesh, central and southern China, throughout Myanmar, Thailand, Lao PDR, Cambodia and Vietnam, through Peninsular Malaysia, to Singapore, Sumatra (Indonesia) and throughout Borneo (Azlan and Engkamat, 2006).

It is found in various types of forest habitats, as well as open areas near forests. It may stray into nearby agricultural areas. It digs into the ground and inhabits dens near rocky areas, where it lives in small groups. It has a gestation period of 110 days and a litter size of two or three. The species may give birth to two litters annually. Their habitat is terrestrial where they live in the hole

[1] Lecturer, Head of Department of Zoology, University of Traditional Medicine, Mandalay
[2] Dr, Professor (Retd), Head of Department of Zoology, University of Mandalay

of tree barks or roots. They also live in a burrow, from which a network of trails penetrate into surrounding habitat. They can be found in all forest types up to 1500m altitude (Parr, 2003).

Most porcupines are about 60-90 cm (25-36 in) long, with 20-25 cm (8-10 in) long tail. Weighing 5-16 kg (12-35 lb), they are rounded, large, and slow, and use aposematic strategy of defense. Porcupines occur in various shades of brown, gray, and white (Parker *et, al.,* 1990).

Porcupines' quills, or spines, take on various forms, depending on the species, but all are modified hairs coated with thick plates of keratin, and embedded in the skin musculature. Quills grow in varying lengths and colours, depending on the animal's age and species. Old World porcupines have quills embedded in clusters, whereas in New World porcupines, single quills are interspersed with bristles, under fur, and hair. The quills of New World porcupines are much smaller about 4 inches or 10 centimeters long and their end of each quill has a small barb. Quills are released by contact or may drop out when the porcupine shakes its body. New quills grow to replace lost ones (Attenborough, 2014).

There are some possible antibiotic properties within the quills, specifically associated with the free fatty acids coating the quills. The antibiotic properties are believed to aid a porcupine that has suffered from self-injury. Porcupines grow new quills to replace the ones they lose (David *et.al.,* 1990).

Porcupines are only occasionally eaten in Western culture, but are very popular in Southeast Asia, particularly Vietnam, where the prominent use of them as a food source has contributed to significant declines in their populations (Diana *et al.,* 2010*).*

The presence of barbs, acting like anchors, makes it more painful to remove a quill that has pierced the skin. The shape of the barbs helps makes the quills more effective both for penetrating the skin and remaining in place. The quills have inspired research for such applications as the design of hypodermic needles (Rijal *et, al.,* 2012).

Ash form of porcupines' quills was used by rulers of early Myanmar dynasties. Traditional medicine, As the In terms of Myanmar Traditional Medicine, porcupines' quills' taste is salty and cool in nature. The ash of porcupines' quills has been given analgesic and astringent property, sweet and cool in nature. It is also used in the treatment of cough, asthma, chest injuries, mouth disease, certain gastric and intestinal disorder, oliguria, hotness of urine, gonorrhea, piles, bowel disorder, blood vomiting and white discharge in Myanmar traditional medicine (Ashin Nagathein, 1975).

The medicinal fauna is largely based on wild animal’s part or whole, including some endangered species. After many years the natural sources such as animal’s species can be endangered. Synthetic materials need to be taken into consideration because the natural sources are living animals. Besides zoo therapeutic practices are being influenced on cultural aspects, the relations between humans and biodiversity. Thus in the present study, has been under taken with the following objectives:

- to identify the porcupines' quills of Myanmar that are used in Traditional Medicine
- to investigate the nutritional values and chemical constituents of porcupines' quills used in traditional medicine in Myanmar
- to assess their relevance in traditional medicinal practice

Materials and Methods

Study period

The study period lasted from July 2019 to February 2020.

Samples collection and identification

The quills of *H. brachyura* were purchased locally from Baja Hsei: zain (traditional medicine shop at Zay Cho Market, Mandalay) in July 2019. The collected specimens were brought to laboratory; Department of Zoology, University of Traditional Medicine, Mandalay and then color of specimens were immediately noted down and recorded with digital camera. These were specimens were preserved in drying place for later identification and measurement. A hand lens and dissecting microscope were used to observe these specimens. Identification was followed according to the Raha *et al*. (2015).

Samples preparation

The samples 150g quills of *H. brachyura* were first washed thoroughly with distilled water and then washed with sterile water mixed with 3c clime juice and next washed with sterile water to remove foreign matters and then sample is dried at drying condition in air oven. One hundred and fifty grams of samples were crushed into smaller pieces and then make powder by blender. The powder was sieved using a stainless steel sieve to get fine powder and then sterilized for an hour in the air oven at 105˙C and stored in bottles prior to analysis. Proximate analysis, mineral composition and pharmaceutical product were carried out on dried powder. The Proximate analysis were carried out according to the extraction is one of the procedure of Association of official analytical chemistry (A.O.A.C, 2000). Crude protein content was calculated using the Kjeldahl method 920.152 (2000). Crude fibre content was determined by the method 978.10 (2000) Fiber Cap Method. Lipid (fat) content was determined according to the Soxhlet method 960.39 (2000). Crude ash (inorganic matter) was determined according to method 942.05 (2000).

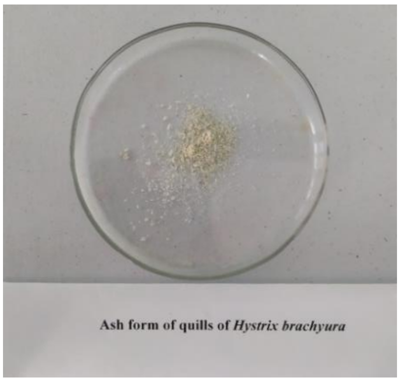
Determination of elements by energy dispersive X-ray Fluorescence Spectrophotometer(EDXRF)

The elemental analysis (macroelements, microelements, toxic elements) of the quills of *H. brachyura* powder were carried out at the SPECTRO X- Lab, at the Department of Geology, University of Mandalay and the M.G.A Petrochemical Lab Mandalay, Myanmar. The determination of elements of the powder of quills of *H. brachyura* was used by the FP- Pellets-121997ne1 method. X-ray fluorescence (XRF) play an important role in elemental analysis.An EDXRF system consists of several basic functional components: an X-ray excitation source, sample chamber, Si(Li) detector, signal processing and recording system .

Data entry and data analysis

Data entry and data analysis was used by Microsoft excel to compare element concentrations in percentages. The results were expressed as mean ± standard deviation (SD).

A. Powder form of quills *Hystrix brachyura*



B. Ash form of quills *Hystrix brachyura*

Plate 1 Preparation of powder and ash form of quills *Hystrix brachyuran*

Results

Systematic oosition of the studied species

Phylum	-	Chordata
Class	-	Mammalia
Order	-	Rodentia
Family	-	Hystricidae
Genus	-	*Hystrix*
Species	-	*H. brachyura* (Linnaeus, 1758)

1. Quills of *Hystrix brachyura*

Scientific name	-	*Hystrix brachyura*
Local name	-	Hpju Zu:
Common name	-	East Asian Porcupine

Each quill is marked with black and white bands. It can be up to 20 inches (51 centimeters) long. The quills of East Asian porcupine are smaller about 8 inches (20 centimeters) long and rigid (Plate 2).

According to the findings of this study on these samples, the highest amount of protein in quills of *Hystrix brachyura* were investigated in these samples (Table 1). The total ash, water soluble ash and acid insoluble ash involved in the powder of quills were shown in Table 2. The result for the mineral analysis indicates that calcium is the most abundant mineral present in these samples. Magnesium, sodium, chlorine, potassium and sulfur were detectable amount in these samples (Table 3). Manganese, aluminum, silicon, iron and zinc were also found in reasonable amount in the powder of quills of *Hystrix brachyura* (Table 4). The content of arsenic was found only in detectable amount and thus below level according to the WHO standard. Cadmium, mercury and lead were not found in these samples (Table 5).

Table 1 Proximate composition of the powder of quills of *Hystrix brachyura*

No	Parameters	Quantity 1 (%)	Quantity 2 (%)	Quantity 3 (%)	Quantity 4 (%)	Quantity 5 (%)	Mean ±SD (%)
1	Moisture	7.69	7.69	7.78	8.22	8.22	7.92±0.28
2	Ash	0.36	0.36	0.37	0.4	0.4	0.38±0.02
3	Carbohydrate	0	0	0	0	0	0±0.00
4	Protein	94.5	94.5	94.5	89.88	89.88	92.65±2.53
5	Fiber	1.11	1.11	1.11	1.18	1.18	1.14±0.04
6	Fat	0.37	0.37	0.37	0.00	0.00	0.22±0.20

Table 2 Total ash, Water soluble ash and Acid insoluble ash involved in the powder of quills of *Hystrix brachyura*

No.	Parameters	Quantity ((%)
1.	Total ash	0.4±0.02
2.	Water soluble	99.8±0
3.	Acid insoluble	0.4±0

Table 3 Percentage of macroelements involved in the powder of quills of *Hystrix brachyura*

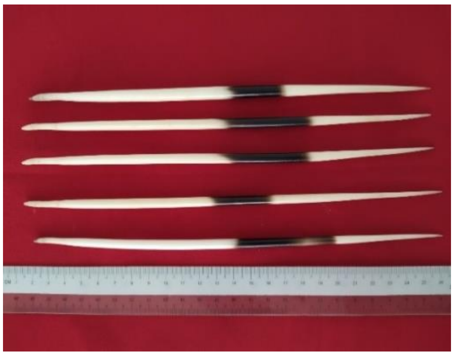
No	Elements	Quantity 1 (%)	Quantity 2 (%)	Quantity 3 (%)	Mean ±SD (%)
1.	Magnesium(Mg)	0.59	0.59	0.00	0.34±0
2.	Sodium (Na)	0.00	0.00	0.00	0.00±0
3.	Calcium (Ca)	2.98	2.98	2.29	2.75 ±0.4
4.	Chlorine (Cl)	0.00	0.00	8.36	1.54±4.83
5.	Potassium (K)	3.93	0.00	0.00	1.31±2.27
6.	Sulfur (S)	0.00	0.00	0.00	0.00±0

Table 4 Percentage of microelements involved in the powder of quills of *Hystrix brachyura*

No	Elements	Quantity 1 (%)	Quantity 2 (%)	Quantity 3 (%)	Mean ±SD (%)
1	Aluminium (Al)	0.00	0.00	0.00	0.00±0
2	Silicon (Si)	0.1	0.1	0.1	0.1±0
3	Manganese (Mn)	0.03	0.03	0.24	0.1±0.12
4	Iron (Fe)	0.002	0.002	0.22	1.33±1.99
5	Copper (Cu)	0.0003	0.0003	0.00	0.00 ±0
6	Zinc (Zn)	0.43	0.43	0.36	0.4±0.04

Table 5 Percentage of heavy metals involved in the powder of quills of *Hystrix brachyura*

No	Elements	Quantity 1 (%)	Quantity 2 (%)	Quantity 3 (%)	Mean±SD
1	Arsenic (As)	0.0008	0.0008	0.0001	0.0006±0.0004
2	Cadmium (Cd)	-	-	-	-
3	Mercury (Hg)	-	-	-	-
4	Lead (Pb)	-	-	-	-



A. Quills of *Hystrix brachyura*

B. Proximal end of quills of *Hystrix brachyura*

C. Distal end of quills of *Hystrix brachyura*

Plate 2 Quills of *Hystrix brachyura*

Discussion

The Proximate Composition of powder of quills of *Hystrix branchyura* revealed that these contained reasonable amounts of moisture, carbohydrate, fiber, protein and fat. Carbohydrate and fat contents were found to be very low in these samples. This confirms that sample is not a good source of fat. The contents of fiber were found to be considerable amount in these samples. Mixtures of soluble and insoluble fibers improve diabetic glucose control and lower serum triglycerides (Anderson, 1990). The content of protein was found to be highest in the powder of quills of *H. brachyura*. Protein provides essential amino acids, particularly important during growth and development, and it is a source of energy (Thomas *et al*., 2004). It is a reflection of total inorganic matter present in these samples and also indicates that these samples possess abundant minerals like calcium which are essential for good health (Oloyede, 2008).

The result for these minerals analysis revealed that the powder of quills of *H. brachyura* is a good source of macroelements. Especially calcium is the most abundant mineral present in these samples. The high content of calcium confirms its medicinal role in bone formation; calcium acts essential for the normal clotting of blood, by stimulating the release of thromboplastin from the blood platelets. Calcium is an activator for several key enzymes, including pancreatic lipase, acid phosphatase, cholinesterase, ATPases, and succinic dehydrogenase. Through its role in enzyme activation, calcium stimulates muscle contraction (i.e. promotes muscle tone and normal heart beat) and regulates the transmission of nerve impulses from one cell to another through its control over acetylcholine production. Calcium, in conjunction with phospholipids, plays a key role in the regulation of the permeability of cell membranes and consequently over the uptake of nutrients by the cell. Calcium is essential for the absorption of vitamin B12 from the gastro-intestinal tract (Reinhold, 1975).

Sodium and magnesium contents were examined detectable amount in these samples. Sodium is an extracellular cation involved in the regulation of plasma volume and acid- base balance, nerve and muscle contraction. Magnesium like calcium stimulates muscle and nerve irritability (contraction), regulation of intracellular acid-base balance, and it also plays an important role in carbohydrate, protein and lipid metabolism (Reinhold, 1975).

Potassium, chlorine and sulfur were found detectable amount in these samples. They serve a vital function in controlling osmotic pressures and acid-base equilibrium. Chlorine also plays a specific role in the transport of oxygen and carbon dioxide in the blood, and the maintenance of digestive juice pH. Sulfur is an essential component of several key amino acids. Sulfur involved in the detoxification of aromatic compounds within the animal body (Reinhold, 1975).

The presence of these essential minerals contributes to its medicinal values. The element concentration was expressed as part per billion (ppb). However, for the convenience of this study the results were also expressed in percentage. Aluminum, silicon, iron, zinc, manganese and copper were found in more reasonable amount in these samples. Zinc plays a vital role in lipid, protein, and carbohydrate metabolism. Iron plays crucial roles in haemopoiesis, control of infection and cell mediated immunity. Iron serves essential for oxygen and electron transport within the body (Reinhold, 1975).

Aluminum serves as antacids, astringents, buffered aspirin (Public health Statement, 2008). Silicon acts leukocyte activation, Coagulation and fibrinolysis cascades. It is not support microbiological growth (COLAS, 1995). The presence of these minerals in powder form encountered in these quills of *H. brachyura* can also be seen as a good source of health.

In the powder of quills of *H. brachyura* sample, Arsenic was found only in detectable amount and thus below level according to the WHO standard. Cadmium, lead and mercury were not found in these samples. Thus these samples have been found to be harmless to use as medicine.

The present study has shown that powder form of quills of *H. brachyura* contain high ash, considerable presence of essential minerals and protein. Therefore these minerals and protein could provide essential amino acids, source of energy particularly important during growth and development.

Therefore the ash form of quills of *H. brachyura* used in the treatment of cough, asthma, chest injuries, mouth disease, certain gastric and intestinal disorder, oliguria, hotness of urine, gonorrhea, piles, bowel disorder, blood vomiting and white discharge, menstrual disorders in Myanmar traditional medicine (Ashin Nagathein, 1975).

Parts of these animals are still used in the medicines of traditional medical practice and found to be effective and the results of chemical analysis also clearly indicated that, these parts are harmless and can be used safely and even promote the wellbeing of the uses of traditional medicine however there is still a need to convert to synthetic materials because these raw materials currently in practice come from the natural living assets and might impose a threat in future.

Conclusion

From the results obtained on chemical analysis of these animal`s parts, it could be said that these samples contain good source of minerals. The harmful contents like arsenic, lead, cadmium and mercury were not found in these samples. Proximate analysis showed that quills of *Hystrix brachyura* contains large amount of protein up to 92.65 ± 2.53. The considerable amounts of fibers were found in these samples. Thus the above findings indicated that these animal parts may be used safely in the Myanmar traditional medicine.

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SEASONAL PREVALENCE OF DENGUE VECTOR *AEDES AEGYPTI* IN SOME ENDEMIC AREAS OF MONYWA TOWNSHIP

Than Myat Soe[1], Yi Yi Mya[2], Ngwe Paw[3], Aung Kyaw Swar Oo[4]

Abstract

Aedes aegypti is recognized as dengue fever mosquito that transmits chikungunya, zika and yellow fever. Vector surveillance is a significant tool to determine factors of related dengue transmission. Entomological survey was carried out in three different endemic areas of Monywa Township, from August 2018 to July 2019. The monthly mosquito larval survey was carried out by examining all containers present in houses of both urban and rural areas. A total of 50 houses in each locality were visited on the basis of systematic sampling method during every month. The potential breeding habitats were screened for the prevalence of *Ae. aegypti* larval population. A total of 1800 houses and 10405 containers were examined for breeding sites of *Ae. aegypti* in all seasons in the whole year. Out of these, 893 houses and 1818 containers were positive. The overall house index (HI), container index (CI) and breteau index (BI) were 49.61%, 17.47% and 101% respectively. Entomological indicators (HI, CI, BI) of *Ae. aegypti* larval population were above the critical level and a dynamic population of *Ae. Aegypti* correlated with rainfall in the study. Regular water supply system and dumping sites should be supported to all study areas. Public health education, community intervention for prevention of vector breeding, information from mass media and regular entomological survey are required to create public awareness and to control mosquito transmitted diseases.

Keywords: Seasonal prevalence, Dengue, *Aedes aegypti*, larval indices, Monywa Township

Introduction

Aedes aegypti is recognized as dengue fever mosquito that transmits chikungunya, zika and yellow fever (Subahar, Lubis, & Winita, 2019). Dengue is the most important vector-borne viral disease for humans, both in terms of morbidity and mortality(Da Cruz Ferreira *et al*., 2017). The incidence of dengue extremely increases worldwide in recent decades. Many dengue cases are underreported and misclassified (Subahar *et al*., 2019). South-East Asia, the Pacific, East and West Africa, the Caribbean and the Americas are dengue endemic areas(Singh *et al*., 2015). Almost half of the world's population are at risk of infection, with major social and economic consequences. According to World Health Organisation(WHO) report, 50–100 million dengue infections occur annually, while a recent study calculated that dengue cases may be closer to 400 million (Bowman, Runge-Ranzinger, & McCall, 2014).

Dengue is classified as notifiable disease since 1964 in Myanmar. In 1970, first dengue outbreak with 1,654 cases and 91 deaths occurred in Yangon. Then, dengue spread to other States and Regions. In 2015, the highest number of dengue cases (42,913) was recorded according to all States and Regions dengue reports. In 2016, the prevalence of dengue in Myanmar was found as 10,770 dengue cases and 58 deaths. The dengue cases and deaths declined by 75% and 59%, respectively in 2016 compared to 2015. Obviously, dengue endemic has increased year by year up from 1970 to 2015 (1,654 in 1970 to 42,913 in 2015). Fortunately, the Case Fatality Rate (CFR) dramatically decreased from 5.50% in 1970 to 0.33% in 2015(Sokunna *et al*., 2017).

Chikungunya fever, an arboviral infection caused by chikungunya virus (CHIKV), is Alphavirus (family Togaviridae) disease, with *Ae. aegypti*. This virus was first isolated in Tanzania

[1] Research Officer, Medical Entomology Research Division, Department of Medical Research (Pyin Oo Lwin Branch)
[2] Dr, Director (Inspection), Department of Medical Research (Pyin Oo Lwin Branch)
[3] Research Assistant-4, Medical Entomology Research Division, Department of Medical Research (Pyin Oo Lwin Branch)
[4] Dr, Associate Professor, Department of Zoology, Sagaing University of Education

in 1953 during a large outbreak. There is rapid increasing of number of countries (over 60) reporting CHIKV outbreak since 1953 (Simo *et al*., 2019). Chikungunya is a vector-borne disease of considerable significance and prevalence in South-East Asia (SEA) Region. The disease has been reported from countries of South and East Africa, South Asia and South-East Asia. In the WHO South-East Asia Region, outbreaks have been reported from India, Indonesia, Myanmar, Sri Lanka, Thailand and Maldives (WHO, 2009).

Vector surveillance is a significant tool to determine factors of related dengue transmission. Entomology indicators are used in vector surveillance to predict the risk of a dengue outbreak. The most used indicators are house index (HI), container index (CI) and breteau index (BI) (Subahar *et*, *al.*, 2019). *Ae. aegypti* also would prefer to oviposit in a less lighting area or shaded where in that particular area the temperatures are lower compared to the area that exposed to direct sunlight. The types of containers, water quality, and conditions of water containers are necessary for breeding. The condition that would lead to mosquito infestation is such as stored water in the container for an extended period, extensive rainfall during the rainy season, and ambient relative humidity and temperature. Infestation of vectors to new geographical areas, warm and humid climate, increased population density, water storage pattern in houses, and storage of trash, for instance, recyclable materials can serve as risk factors for dengue virus infections (Madzlan *et al.*, 2016). The reproduction of *Ae. aegypti* from tropical to subtropical zones occurs all the year-round, and their abundance is associated with rainfall (Ahmed *et al*., 2007).

Therefore, the current study was investigated to examine the seasonal prevalence of dengue vector *Ae. aegypti* in some endemic areas of Monywa Township with the specific objectives; to evaluate the monthly prevalence of indices (HI, CI, BI) of *Ae. aegypti* larvae and to observe the relationship between *Ae. aegypti* larvae population (BI, HI and CI) and rainfall in different periods.

Material and Methods

Study area

Monywa Township is one of the 37 Townships of Sagaing Region. The region was severely hit by dengue in 2015 and the numbers of cases and death tolls, respectively, took their positions at the first and second places in the list of reported dengue cases. Monywa Township is situated on the eastern bank of the Chindwin River, 136 kilometres west of Mandalay and located in the central dry zone of Myanmar. The township has a population of 372,095 and is divided into 26 wards (Myanmar Government, 2015). According to the recommendation of Vector Borne Disease Control unit (VBDC) of Sagaing Region, three localities, including one urban and two rural areas were selected based on the last year dengue fever cases reported for Monywa Township. Among the localities, Myawaddy quarter, which is a part of Monywa City is an urban area and other two villages, Kamma and Kyauksitpon villages, are rural areas.

Study design and study period

The present study was performed mostly based on fieldwork and investigations were carried out from August 2018 to July 2019.

Sampling methods

The monthly survey of mosquito larvae was conducted by examining all containers present in houses of three selected localities. A total of fifty houses per locality were paid a visit every month on the basis of a systematic sampling method (Depkes, 2003). The potential breeding habitats in the selected areas, such as cement tanks, metal containers, plastic containers, iron drums, earthen pots, flower vases, discarded tires and other water-filled items were carefully examined for the presence of immature stages of *Ae. aegypti*. The presence of larvae in small breeding habitats around the dwellings was carefully searched by using flashlight and pipette. The search for bigger water containers was accomplished by dipping the net into the containers (WHO, 2003). The type of habitats, in which mosquito larvae were present and their location were recorded. The sample larvae were collected and brought to the insectary room of Medical Entomology Research Division (Pyin Oo Lwin Branch), where the larvae were reared until they become adults. The adult mosquitoes were identified according to the keys of Leopoldo M Rueda (Rueda, 2004).

Data analysis

Field data was recorded in appropriate forms, and statistical analysis was conducted using Microsoft Excel.

Larval prevalence indices

The collection of larval examination method of WHO (Depkes, 2003) was used to confirm the presence of larvae in the containers. Three larval indices such as house index (HI), container index (CI) and Breteau index (BI) were calculated as follows:

1. HI = (No. of houses positive for *Aedes* larvae) / (No. of houses inspected) × 100
2. CI = (No. of positive containers) / (No. of containers inspected) × 100, and
3. BI = (No. of positive containers) / (No. of houses inspected) × 100

Weather parameters

Monthly weather parameters, including rainfall were obtained from the Meteorological and Hydrology Department, Monywa**,** Sagaing Region.

Results

The prevalence of larval population in three different dengue endemic areas of Monywa Township was computed as house, container and breteau indices in Table 1. A total of 1800 houses and 10405 containers were examined for breeding sites of *Ae. aegypti* in all seasons in the whole year. Out of these, 893 houses and 1818 containers were positive. *Ae. aegypti* larvae were detected in 49.61 % house index (HI), 17.47 % container index (CI) and 101% breteau index (BI) as the number of indices in three study areas.

Myawaddy quarter

A total of 600 houses were visited during house to house larval survey for *Ae. aegypti* breeding in all kinds of temporary and permanent water bodies of Myawaddy quarter. Among them, 265 houses were found as the breeding sites of *Ae. aegypti*. A total of 2901 water containers were searched for *Ae. aegypti* breeding, out of which 527 were found as positive containers. The overall house index (HI), container index (CI) and breteau index (BI) were 44.17 %, 18.17 % and 87.83 % respectively. The highest number of HI, CI, BI was found in October, and the lowest number of indices was found in March (Table 2). The relationship of increasing level of indices (HI, CI and BI) with the meteorological condition, especially rainfall is shown in Figure.2. The heaviest rainfall occurring June, September and October resulted in higher HI, CI and BI in those months. The highest monthly total rainfall (172.97 mm) was recorded during October followed by June (169.16 mm) and September (137.92 mm). There was no rain in February and March which months were recorded as the lowest level of indices. The population summit (BI-224 %) in October correlated to the heaviest rainfall, followed by September (BI- 178 %) and June (BI-176 %) (Figure 1).

Table 1 Prevalence of indices of *Ae. aegypti* larvae in different localities of Monywa Township

Localities searched	Houses visited	Houses positive	Containers searched	Containers positive	HI (%)	CI (%)	BI (%)
Myawaddy quarter	600	265	2901	527	44.17	18.17	87.83
Kamma village	600	318	3322	588	53.00	17.70	98.00
Kyauksitpon village	600	310	4182	703	51.67	16.81	117.17
Total	1800	893	10405	1818	49.61	17.47	101.00

HI= house index, CI= container index, BI= breteau index

Table 2 Monthly prevalence indices of *Ae. aegypti* larvae in Myawaddy quarter, Monywa Township

Months	Houses visited	Houses positive	Containers searched	Containers positive	HI (%)	CI (%)	BI (%)
August	50	21	220	25	42	11.36	50
September	50	32	351	89	64	25.36	178
October	50	36	436	112	72	25.69	224
November	50	17	194	27	34	13.92	54
December	50	15	171	24	30	14.04	48
January	50	15	184	24	30	13.04	48
February	50	14	166	21	28	12.65	42
March	50	13	203	18	26	8.87	36
April	50	18	172	25	36	14.53	50
May	50	28	194	46	56	23.71	92
June	50	35	345	88	70	25.51	176
July	50	21	265	28	42	10.57	56
Total	600	265	2901	527	44.17	18.17	87.83

HI= house index, CI= container index, BI= breteau index

Kamma village

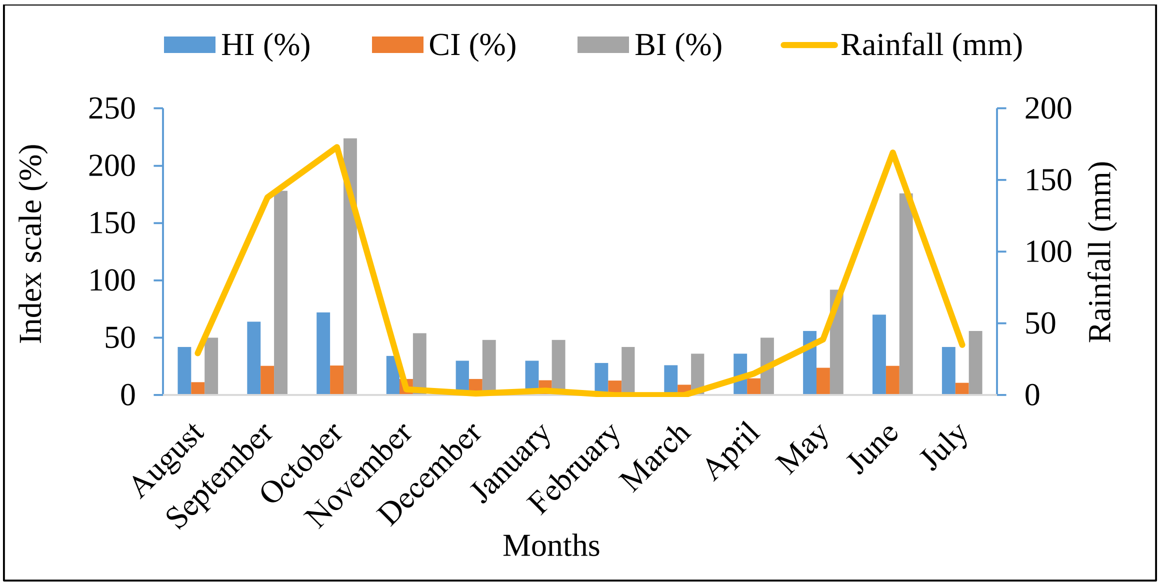
In Kamma village, 600 houses were searched for *Ae. aegypti* breeding, out of which 318 houses were found positive. A total of 588 containers was positive (containing *Ae. aegypti* larvae) out of 3322 wets (water filled) containers in the visited houses. The overall indices of *Ae. aegypti* larvae with house index (HI) 53%, container index (CI) 17.70 % and breteau index (BI) 98 % were found in the study area. In October, the highest numbers of HI, CI, and BI were 76 %, 26.53 % and 208 % respectively and the lowest numbers of indices were found in March with HI 24 %, CI 5.58 % and BI 26 % respectively (Table 3). The effectiveness of rainfall on *Ae. aegypti* population increasing was found in Kamma village. The highest of HI, CI and BI were found in October which month occurred the heaviest rainfall and followed by June and September with correlation larval indices and rainfall. The lowest of HI, CI and BI were recorded in March which month was no rain. The peak of *Ae. aegypti* larval population (BI-208 %) was found in

October with relation to the heaviest rainfall and followed by June (BI-174 %) and September (BI-156 %) (Figure 2).

Table 3 Monthly prevalence indices of *Ae. aegypti* larvae in Kamma village, Monywa Township

Months	Houses visited	Houses positive	Containers searched	Containers positive	HI (%)	CI (%)	BI (%)
August	50	30	316	55	60	17.41	110
September	50	36	432	78	72	18.06	156
October	50	38	392	104	76	26.53	208
November	50	23	231	34	46	14.72	68
December	50	16	201	26	32	12.94	52
January	50	20	217	26	40	11.98	52
February	50	13	209	17	26	8.13	34
March	50	12	233	13	24	5.58	26
April	50	25	238	42	50	17.65	84
May	50	34	264	47	68	17.80	94
June	50	37	330	87	74	26.36	174
July	50	34	259	59	68	22.78	118
Total	600	318	3322	588	53	17.70	98

HI= house index, CI= container index, BI= breteau index



HI= house index, CI= container index, BI= breteau index

Figure 1 Relationship of *Ae. aegypti* larvae population (BI, HI and CI) with rainfall in different periods of Myawaddy quarter, Monywa Township

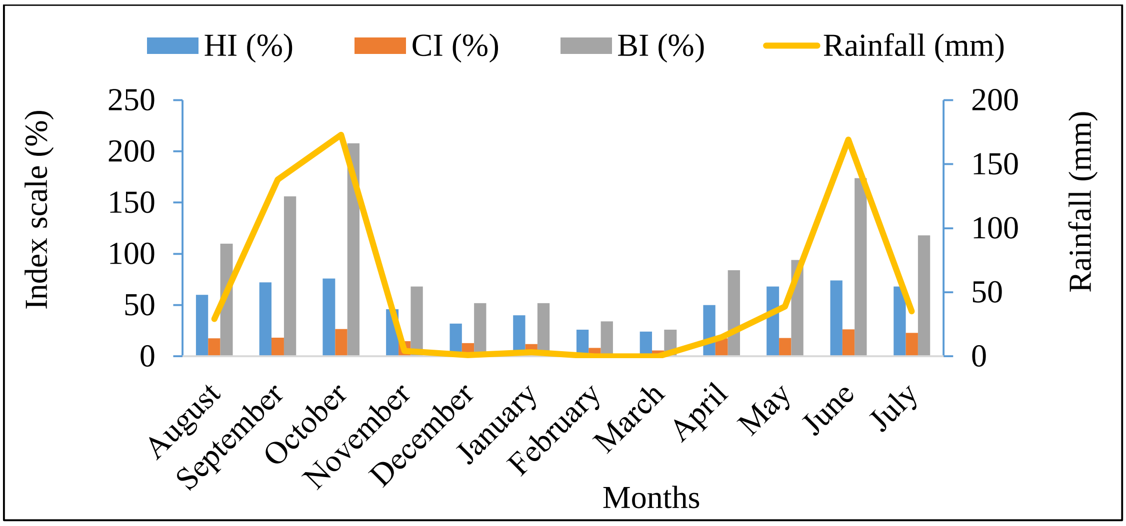
Kyauksitpon village

In total, 600 houses and 4182 water containers were inspected in Kyauksitpon village. Out of these, 310 houses were positive for *Ae. aegypti* breeding sites and 703 containers were positive for larval infection. The overall indices were 51.67 % house index (HI), 16.81 % container index (CI) and 117.17 % breteau index (BI) respectively. In monthly indices, the month of October was the highest with HI 82 %, CI 23.60 %, BI 226 % and the month of March was the lowest with HI 30 %, CI 7.77 %, and BI 48 % (Table 4). Indices of *Ae. aegypti* larval population were examined in relation to monthly rainfall, where the seasonal pattern of *Ae. aegypti* was fairly close to variations in rainfall. The maximum of breteau index (BI-226 %) was recorded in October corresponding to the heaviest rainfall and followed by June (214 %) and September (202 %) (Figure 3).

Table 4 Monthly prevalence indices of *Ae. aegypti* larvae in Kyauksitpon village, Monywa Township

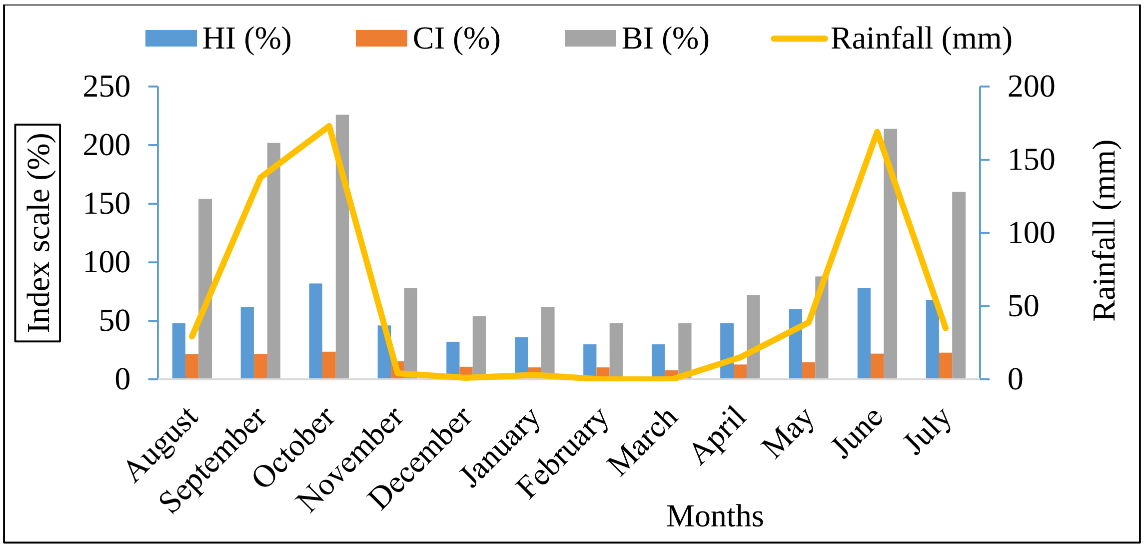
Months	Houses visited	Houses positive	Containers searched	Containers positive	HI (%)	CI (%)	BI (%)
August	50	24	355	77	48	21.70	154
September	50	31	467	101	62	21.63	202
October	50	41	580	113	82	23.60	226
November	50	23	252	39	46	15.48	78
December	50	16	251	27	32	10.76	54
January	50	18	313	31	36	10.10	62
February	50	15	235	24	30	10.21	48
March	50	15	309	24	30	7.77	48
April	50	24	283	36	48	12.72	72
May	50	30	300	44	60	14.67	88
June	50	39	485	107	78	22.06	214
July	50	34	352	80	68	22.73	160
Total	600	310	4182	703	51.67	16.81	117.17

HI= house index, CI= container index, BI= breteau index



HI= house index, CI= container index, BI= breteau index

Figure 2 Relationship of *Ae. aegypti* larvae population (BI, HI and CI) with rainfall in different periods of Kamma village, Monywa Township



HI= house index, CI= container index, BI= breteau index

Figure 3 Relationship of *Ae. aegypti* larvae population (BI, HI and CI) with rainfall in different periods of Kyauksitpon village, Monywa Township

Discussion

In Myanmar, vector borne diseases are a major public health problem. The rapid growth of population and industrial installation in urban and semi-urban areas has resulted in an alarming increase in mosquito's density. Water storage practices in the household for multipurpose use in city and rural areas provide year-round breeding opportunities for the vector. Untreated water in containers originated from pipe supply, tube wells, surface wells, ponds, and rainwater also encourage mosquito density(Maung Maung Mya *et al*., 2016).

In the present study, two types of study areas which are urban and rural were examined for *Ae. aegypti* larvae population indices. The housing index (HI) of Kamma village was the highest and followed by Kyauksitpon village and Myawaddy quarter. In the prevalence of index (CI), Myawaddy quarter was the highest and followed by Kamma village and Kyauksitpon village. However, the highest number of breteau index was found in Kyauksitpon village and followed by Kamma village and Myawaddy quarter. In all study areas, piped water supply system was not found, and the water taken from tube wells and bore wells is used for the requirement of households. Moreover, the less number of the well was found in rural areas and no coverage on the numbers of resident and house. In Kamma village, water from two houses was distributed to all houses in the village by the monthly fee.

In Myanmar, Maung Maung Mya *et al*., 2016 reported that entomology surveys in periurban areas of Mingalarywarthit and Taungnar villages, Hpa-an Township Kayin State which areas had high Dengue Haemorrhagic Fever (DHF) prevalence within the last five years. In Mingalarywarthit village, HI, CI, BI were 86.28%, 59.86 % and 172.50% respectively, whereas in Taungnar villages, HI, CI, BI were 80%, 22.06% and 206% respectively. Similarly, another study described entomological indicators with HI was 25.7%, CI was 15.5%, and BI was 48% (Thae Zar Chi Bo *et al*., 2016).

In Indonesia, the author reported 28% HI, 20.7% CI and 28.0% BI in dengue vector surveillance in Sujung village, Banten (Subahar *et al*., 2019). In India, researcher detected *Aedes* mosquitoes indices; HI, CI and BI were 43.3%, 16.4% and 146.8% respectively in 2014(Singh *et al*., 2015). The indices of *Ae. aegypti* were reported that HI, CI and BI were 14.29 %, 4.83% and 34.29% respectively in Thailand (Wongkoon *et al*., 2007). When compare the indices, the present study's entomological indicators were found higher than other research data. Differences of environmental conditions, weather conditions and times of study can effect on larval indices of *Ae. aegypti* mosquito.

World Health Organization (WHO) described that HI>5% and BI>20% for any locality are indicators of dengue- sensitive (WHO, 2003). Three levels of risk for dengue transmission: low (HI<0.1%), medium (HI 0.1%–5%), and high (HI>5%) were expressed by Pan American Health Organization(Sanchez *et al*., 2006). The National Institute of Communicable Diseases in India defined a high risk of DHF transmission when BI was ≥ 50, HI was ≥ 10, and a low risk of transmission when BI was ≤ 5, HI was ≤ 1(Wongkoon *et al*., 2007). Therefore, all larval indices of the present study are above the critical levels.

In the current study, the seasonal prevalence of *Ae. aegypti* was examined in relation to monthly rainfall. The highest indices (HI, CI, BI) were found in all study areas in October which month was the heaviest rainfall for one year. In March, rainfall was very low and the population of *Ae.aegypti*; HI, CI and BI were the lowest. The increasing of *Ae. aegypti* population was observed at above 100 mm rainfall in June, September and October. In Myawaddy quarter, seasonal prevalence and rainfall were more related than other study areas. This area had more tube wells and more vector control interventions. In Kamma village and Kyauksitpon village, the seasonal pattern of *Ae. aegypti* was moderately close to variations in rainfall. Although the less rainfall in some months, entomological indicators were to increase in these rural areas cause of water storage system. Therefore, different factors of seasonal prevalence between the urban area and rural areas were found in the present study.

In conclusion, high level indices of *Ae. aegypti* larval population were recorded in the present study and a dynamic population of *Ae. Aegypti* correlated with rainfall. The regular water supply system and dumping sites should be supported in all study areas. The elimination of discard containers and covering water storage containers should be made to reduce the vector population efficiently. Public health education, community intervention for prevention of vector breeding and information from mass media are required to create public awareness and to control mosquito transmitted diseases. A regular entomological survey is necessitated in the study areas for monitoring of *Ae. aegypti* breeding.

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REPRODUCTIVE BEHAVIORS OF INDIAN FLYING FOX, *PTEROPUS GIGANTEUS* (BRUNNICH, 1782) AT MUNICIPAL COMPOUND IN PYAY TOWNSHIP

Than Than Htay[1], Aung Aung[2], Moe Moe Aung[3]

Abstract

Reproductive behaviours of *Pteropus giganteus* was conducted at Municipal compound in Pyay Township from July, 2018 to June, 2019. A total of 469 copulations were observed consists of more than 2300 individuals of both sex. During day they take rest in roosting trees. The maximum number of pairing were observed in September.Courtship displays and copulatory behaviours were observed throughout the day at the dirunal roost. Two modes of copulatory behaviours (frontal and dorsal) were observed during copulatory of *P. giganteus*. During copulation of *P. giganteus* and also used auditory, olfactory and tactile communications during pre and post-copulation period. The bats were more active before 12:00AM and reproduced single individuals. The lactating females retained their pups with them until the latter become three months old.

Keywords: Reproductive behaviours, mating season, *Pteropus giganteus,* roosting

Introduction

Most species of mammals live in the tropics, and many breed seasonally. Mammals in seasonal environments have evolved two general strategies of reproductive timing. Seasonally breeding species with a long gestation cannot use an opportumistic strategy, since the long delay between copulation and birth would cause them, consistently to miss the optimum periods for the most difficult parts of their reproductive cycle (Heideman and Bronson, 1994).

Reproduction is an important aspect of a species biology (Bong *et al*., 1999). There have been many studies of bat reproduction in the tropics but very little has been reported for flying fox from Myanmar.

Reproduction plays an essential role to contribute survival and growth in the life cycle of vary species of living animals in the world. The fruit bats are mostly similar to many of the general reproductive characteristic found in other mammalian species. They reproduce sexually, give birth to live young and females nurse their young from one of two mammae located on each side of their thorax.

The objectives of the present study

- to study and record the sexual behaviours that normally occur at roost site
- to observe the quantify of their reproductive behaviours

Materials and Methods

Study area and site

Reproductive behaviours of *Pteropus giganteus* was conducted at Municipal compound in Pyay (N 18°49' 19.663" E 95° 12' 47.368") which was located on the Eastern bank of Ayeyarwady River, in Pyay Townshi Four roosting sites were focused to study the reproductive behaviours of *Pteropus giganteus.*

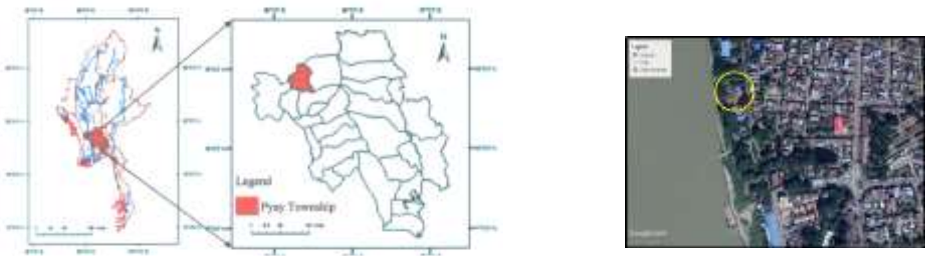
[1] Lecturer, Department of Zoology, Pyay University
[2] Dr, Lecturer, Department of Zoology, Taungoo University
[3] Dr, Associate Professor, Department of Zoology, University of Mandalay

Study period

Study period lasted from July, 2018 to June, 2019.

Data collection

Survey was carried out to study the mating behaviour of Indian flying fox. The observation was made for whole day, from 6:00 AM to 4:00 PM. After every 50 minutes study time, take a rest for 10 minutes. The behaviours were observed from vantage points through binocular. In addition, the frequency, time and duration of copulations were observed. At least 8 days per month were spent under the tree to study and record the different reproductive behaviours. The observation was made for 30 minutes in each roosting trees at the day time. The time of matig was investigated followed by the time consumed during copulation. Behaviour of both males and females were recorded during the entire course of, pre-mating, mating and post-mating behaviours.



Source from (Geography Department, Pyay University)

Figure 1 Map of the study area (Pyay Township)

A.*Ficus virems* Aitom-I

B. *Ficus virems* Aitom-II

C. *Terminalia catappa* L

D. *Albizzia lebbek* Benth

Plate 1 Four roosting site

Results

The individuals of *Pteropus giganteus* were actively involed in courtship display throughout the day. Courtship behaviours were divided into pre-copulatory phase, copulatory phase and post-copulatory phase.

Courtship and mating behaviours

The reproductive behaviours of Indian flying foxes occurred during annual mating seasons and was conducted predominantly at the day roosts. Courtship commenced early in the morning and occurred in repetitive sequences throughout the day. Copulation was always initiated by a male.

It is noticed that flying foxes mate thtroughout the year however it was recorded that the frequency was maximum during the months of September. The difference in size and colour between male and female is a symbol of sex. The males are larger than the females and their abdomen are pale in colour and tinged with gray while the females are bright buff.

Based on the observation of a number of pairing bats it was recorded that during courtship the male first approach prospective a female and relay the message by grooming the wings and body of female, and by licking the valva of the female (Plate 2). At first the female usually warn the male by vocalization and attempt to avoid the approaching male by various ways. She may then shift her roosting place however is pursued by the ardent male. Though the first attempt usually is not successful, the 2nd and 3rd attempts let the female get the message.

The male mounted from the rear and hold down the female until the final act of coitus (Plate 3,4). Courtship and coitus took approximately (30-45) mintues. females often appeared to resist copulation attempts through struggling and evasion of the male. During copulation, female simultaneously produced loud vocalizations "Ghee". After copulation, female fled to other branches and the male again followed the female to attempt copulation. Moreover, courtship and mating behaviours were more observed than normal when flying foxes were alerted by disturbances.

Monthly observation of courtship and mating behaviour of *P.giganteus* started to record in first week of July, 2018 in this study. Medium mating pair occured July, 2018, May and June, 2019 in study periods. During the study period, maximum number of 197 mating pairs was recorded in September 2018 and minimum number of two, mating pairs in March 2019(Table 1).

Maximum numbers of mating pairs were observed before 12:00 AM in the day roost in every month. (Table-2). The incident mating and incident breeding during the year 2018-2019 was shown in (Table 3).

Gestation

Pregnancy period lasted approximately six months, since young pups were encountered from March to May, after a lapse of six months from the period of maximum mating frequency throughout the colony. (Plate 5).

Parturition

Along the study period it was noticed that pregnant female gave birth to a single young synchronously between early March and May. After parturation the young was oriented in the same position like the mother and clinged to one of the two nipples. During suckling session the mother groomed her young pup occassionally before wrapping the pup with her wings in protection. (Fig.5).

Lactation

The lactation period lasted approximately 15 weeks. During this period the mother nursed her young and gave protection from the various disturbances. The pup was carried everywhere by the mother while it clings by gripping her with the claw of feet and by grasping her axillary teat in the mouth. After a few weeks, the young was left with other pups in the roost trees at night while the mother venture out to foraging site.


Plate 2 Male and Female, Male trying to stimulate the female for submission of *Pteropus giganteus*


Plate 3 Courtship behaviour, copulatory phase (abdominal) of *Pteropus giganteus* within Municipal compound


Plate 4 Courtship behaviour, copulatory phase (dorsal) post copulatory phase of *Pteropus giganteus* within Municipal compound



Plate 5 Pregnant female bat, infant attach and two months old bat

Table 1 Monthly total number of courtship and mating pairs of *Pteropus giganteus* (from July-2018 to June-2019)

Months	Courtship	Mating
July , 2018	14	12
August	53	55
September	240	197
October	153	52
November	95	31
December	71	33
January , 2019	33	21
February	79	44
March	3	2
April	14	5
May	16	6
June	17	11

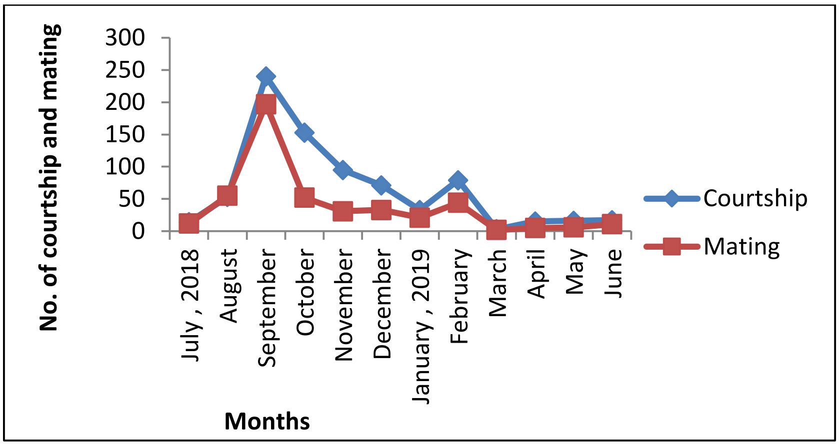


Figure 2 Monthly numbers courtship and mating pairs of *Pteropus giganteus* during study period

Table 2 Relation of paring numbers and day roosting time of *Pteropus giganteus* during study period

Numbers of paring		
Months	**Before-Noon**	**After-Noon**
July , 2018	10	2
August	47	8
September	149	48
October	47	5
November	29	2
December	33	0
January , 2019	21	0
February	39	5
March	1	1
April	3	2
May	4	2
June	8	3

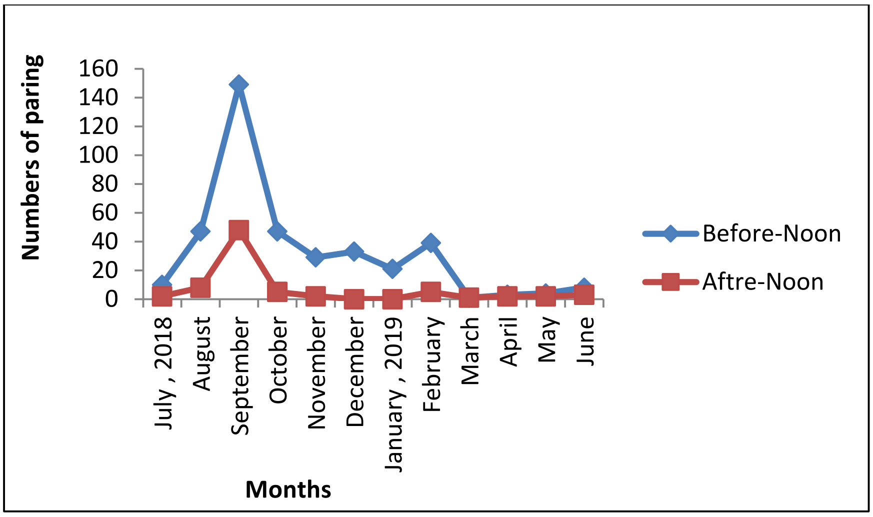


Figure 3 Relation of pairing numbers and day roosting time of *Pteropus giganteus* during study period

Table 3 Recorded frequency of incident mating and incident breeding during study period

	Frequency			
Months	**Incident mating**	**Incident Breeding**	**Mating**	**Courtship**
July , 2018	++	–	12	14
August	+++	–	55	53
September	+++	–	197	240
October	+++	–	52	153
November	+++	–	31	95
December	+++	–	33	71
January , 2019	+++	–	21	33
February	+++	–	44	79
March	+	+	2	3
April	+	++	5	14
May	++	+++	6	16
June	++	–	11	17

\+ = minimum frequency
++ = medium frequency
+++ = maximum frequency

Discussion

Courtship and mating behaviours were observed throughout the morning and then these behaviour delayed after 12:00 am. This may be due to more active in the morning than afternoon, because more mating activities were found when the bats were alerted. Moreover mating behaviours were less observed when there was very windy and rainy.

Neuweiler (2000) reported that the mating behaviour of Indian flying fox, *P.giganteus* follows the first period of sleep in the morning, a few males start attempting to copulate with females. This activity is accompanied by long and loud intimidation cries. By stimulating nearby males with these cries, the entire colony is a mass of screeching and copulating pairs. Half an hour later, the colony is again peaceful and quiet. Sein Sein Win (2006) describe that a similar event was found in her study. Males always started to couplate with females and during this time loud sounds were continuously produced. May Myo Nyunt (2007) stated that copulation usually took place in the morning with vocalization of the males throughout the colony. Some males were also found flying around the roost trees as though in search of the prospective female. In present study, female stimultaneously reproduces loud vocalization during copulation.

Sein Sein Win (2006) stated that peak mating frequency started in Septmeber. Moe Moe Aung (2006) stated that copulation occured throughout the year, but peak from September through February. May Myo Nyunt (2007) described that, it is noticed that flying foxes mate throughout the year however it was recorded that the frequency was maximum during the months of September through December.

In the present study, *P.giganteus* was observed the mating behaviour is throughout the year however it was recorded that the maximum number of mating pairs were in September during the study period.

Neuweiler (2000) stated that copulation of *P.giganteus* occured within a 30 to 40 minutes period each morming. May Myo Nyunt (2007) stated that courtship and coitus took approximately 60 miutes. In the present study, courtship and coitus took approximantely (30-45) minutes.

In present study peak frequency of mating pairs were found in September through February. Moe Moe Aung (2006) and May Myo Nyunt, 2007) reported that peak mating period occurred in September. Therefore mating period of *P. giganteus* in the study revealed uniformly the same although in different localities and regarded optimum conditions favoured for reproduction of this species in Myanmar.

Based on the peak mating period in September and the onset of parturition time in March, the gestation period of *P. giganteus* was estimated as 150-160 days. Sein Sein Win (2006) and May Myo Nyunt (2007) also reported that the pregnancy period lasted about six months.

Conclusion

A total of 469 copulation were observed duing July, 2018 to June 2019, four roosting site in Municipal compound at Pyay Township. This colony consists of more than 2300 individuals of both the sexes of *Pteropus giganteus*.

In the present study, mating pairs were recorded in every months of study period. Of these months, the maximum numbers were recordes in September 2018 and minimum in March to May 2019.

In the present study, there was 35-40min long duration in the courtship from start to end. Maximum number of mating pair were observed before 12 am than 12 pm in every months of study period.

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RELATIVE ABUNDANCE AND DIVERSITY OF INSECTS IN SOME CUCURBIT PLANTS FROM HNAW-KONE VILLAGE, PAKOKKU TOWNSHIP

Ni Ni Aye[1] ,Than Naing Oo [2] , Myint Myint Htwe [3]

Abstract

Relative abundance, species diversity, richness and evenness of insects from cucurbit plantations were investigated during May to August 2019. A total of 56 species belonging to 28 families in six orders were collected from studied plants (gourd, pumpkin, squash and watermelon). Insect population was more abundance in order Hemiptera followed by Coleoptera and Lepidoptera. The lowest population was found in order Odonata. Pumpkin plants have the highest diversity and gourd plants have lowest diversity of insects. The highest similarity value was found between pumpkin and squash plants.

Keywords: Insects, Relative abundance, Species diversity, Cucurbit plants

Introduction

Cucurbitaceae is a fairly large family containing about 100 genera and 800 species which are essentially distributed in tropical or subtropical regions and relatively few species extending into temperate climate. Cucurbitaceae are used as fruits and vegetables and most of them have economic value (Rahman, 2013). Agriculture is a major component of the Myanmar economy, contributing 42% to its GDP and involving 70% of the labour force (CIA 2006). Seventy-five percent of the population of Myanmar live in rural areas and depend on agriculture for their livelihood (Singleton 2003). Cucurbit vegetables are cultivated throughout in Myanmar.

Insect pests are major problem for productivity of cucurbit plants. Insects are important because of their diversity, ecological role, and influence on agriculture, human health, and natural resources (Berenhaum, 1995). They inhabit all habitat types and play major roles in the function and stability of terrestrial and aquatic ecosystems (Godfrays, 2002). Environmental variation contributes diversity of species in natural ecosystem and in agro ecosystems (Alencar *et al.,* 2013).

Species richness is currently the most widely used diversity measure. Relative species abundance in a community is another factor that affects diversity (Hurlbert, 1971). Species diversity is the most common representation of ecological diversity, uses mathematical indices broadly known as diversity indices, derived from combining information on richness and evenness (Hamilton, 2005).

The present study was aimed

- to study the relative abundance of insects in each order

- to investigate the diversity of insects in cucurbit plants from Hnaw-kone village.

Materials and Methods

Study Area

Hnaw-kone village is situated in Pakokku Township, Magway Region, located between North latitude 21° 22' 32.45" and East longitude 95° 09' 38.59" (Plate 1).

[1] Dr, Lecturer, Department of Zoology, Pakokku University
[2] Dr, Lecturer, Department of Zoology, Pakokku University
[3] Dr, Lecturer, Department of Zoology, Pakokku University

Plate 1 Location map of the study area (**Source:** From Google Earth, 2019)

Site I. Gourd plants

Site II. Pumpkin plants

Site III. Squash plants

Site IV. Watermelon plants

Plate 2 Study Area

Study Period

The study period was lasted from May to August 2019.

Specimen Collection, Identification and preservation

Collection of insects was randomly carried out in the early hours of the day, chosen one day in a week. The host (cucurbit) plants were selected and insects were collected by hand pickling, with forceps and hand nets (sweep net) throughout the fields. The species identification was made by the following Hill (1983) and Nair (1995). The insects were killed with mild chloroform and then preserved in insects boxes for future study.

Data analysis

(a)Relative Abundance

$$\text{Relative Abundance} = \frac{\text{Total number of individuals of a species}}{\text{Total number of individuals of species in a order}} \times 100$$

(Bisht *et al*., 2004)

(b) Diversity of insect species

Species richness, diversity and evenness of collected species were analyzed by four methods; Margalef's Index (1958), Simpson's index diversity (1949), Shannon-Wiener's index (1948),and Hill's Evenness index (1973), cited by Ludwig and Reynolds, 1988.

For Margalef's species richness Index (1958)

$$d = \frac{S - 1}{\text{Ln (N)}}$$

Where, d = Margalef's species richness index

S = Number of species

N = Total number of individuals

For Simpson's index diversity (1949),

$$D = \sum_{i=1}^{s} \frac{n_i \,(n_i - 1)}{n\,(n - 1)}$$

D = Simpson's index of diversity

n_i = number of individuals in the i th species

n = total number of individuals of all species

For Shannon-Wiener's index (1948),

$$H' = - \sum_{i=1}^{s} (\frac{n_i}{n}) \text{ Ln } (\frac{n_i}{n})$$

Where, H' = Shannon index of species diversity

S = number of species

n_i = number of individuals in the i^{th} species in the sample

n = total number of individuals of all species in the sample

Hill's Evenness index (1973)

$$E = \frac{(1/D) - 1}{e^{H'} - 1} = \frac{N2 - 1}{N1 - 1}$$

Where, E = Hill's evenness index (which approaches zero)

D = Simpson's index of diversity

H' = Shannon's index of species diversity

N_1 = number of abundant species in the sample

N_2 = number of very abundant species in the sample

(c) Sorensen's similarity index (1948)

$$S = \frac{2C}{\mathrm{A} + B}$$

C=the number of species similar to both sites

A= the number of species in the site A

B= the number of species in the site B

Meteorological data

Meteorological data such as temperature, rainfall and relative humidity were obtained from the Meteorological and Hydrological Department, Pakokku Township.

Results

A total of 5271 individuals and 56 species belonging to 28 families and six orders were identified and recorded from four areas of cucurbit plantations (Table 1). During the study period, gourd, pumpkin, squash and watermelon plants were observed for collection of insects. Highest number of species and individuals (48 and 1597) were collected from pumpkin plants. The lowest number of species (37) was recorded from gourd plants and the lowest individuals (1088) was recorded from squash plants. Large numbers of insects (>200) were found in five species, *Aphis gossypii(1009)*, *Bactrocera cucurbitae* (253), *Engytatus varians* (255), *Aulacophora foveicollis* (253), *Sphenarches anisodactylus* (279). The least numbers (under 20) of insects were recorded in *Teleogryllus commodus* (16) *Crocothemis servilia* (16) *Chrysocoris stolli* (10) *Bradymerus acutangulus* (7) *Diaphania nitidalis* (9) *Promachus* sp. (14) *Syphus* sp.(12) (Table 1).

Relative abundance of insect species in orders

In the gourd plants (site I) order Hemiptera (62.45%) was the numerically predominant order and most species rich, followed by Coleoptera (19.73%), Lepidoptera (7.94%) and Diptera (4.49%). In the pumpkin plants (site II), the highest relative abundance (54.98%) was found in order Hemiptera followed by Coleoptera (19.47%), Lepidoptera (10.77%), Orthoptera (10.77%), and Diptera (8.45%) (Table 2).

In the squash plants (site III), the results showed the similar trend with site I and Il, the highest was also found in Hemiptera (43.36%) followed by Coleoptera (19.85%) and Lepidoptera (17.56%). In the watermelon plant, Hemiptera (35.53%) was the first, Orthoptera ranked the second dominant order with the value (18.91%) followed by Lepidoptera (16.01%)(Table 2).

In general, within all study sites, Hemiptera was dominant order, Coleoptera was second dominant order followed by Lepidoptera (Table 2).

The least relative abundance values (2.94%, 2.11%, 7.65%) were found in the order Odonata from site II, III and IV. In site I, the least value (1.59%) was found in order Orthoptera. Individually, among the collected insects, *Bradymerus acutangulus and Daphania nitidalis* were the lowest relative abundance value (1.13%in each). *Aphis gossypii* had the highest relative abundance value (19.14%)(Table 2).

Diversity of insect species

In site I, the diversity indices showed that Hemiptera insects have the highest diversity with Margalef's species richness value (d=2.9377), Simpson diversity (D=0.1448) and Shannon - Wiener's diversity (H'=2.5419). Order Diptera have the highest evenness index value (E=1.1025) and Odonata have the lowest value of evenness index (E=0.4547) (Table 3).

In site II, based on the values calculated by (d= 3.0984, D=0.1324 and H'= 2.5635) showed that Hemiptera was the highest diversity, Odonata was the lowest diversity. The highest evenness value (E=0.9450) was found in order Orthoptera, the lowest evenness value (0.5467) was observed in order Hemiptera (Table 4).

In site III, (d=2.3862) and (H'=2.0971) point out the Hemiptera was the highest diversity, the highest value (D=0.1487) showed that Coleoptera was most diverse order. The highest evenness (E= 0.9780) was found in Odonata, the lowest (E= 0.5369) was found in Hemiptera (Table 5).

In site IV, the highest value (d =1.9995) showed the Hemiptera was dominant, but (D) = 0.1616 and H'=1.9908, showed that Orthoptera was most diverse, Coleoptera insects were most evenly distributed in the order with the highest value (E) = 0.9301, the lowest (E= 0.4603) was found in Hemiptera(Table 6).

Generally for all study sites, calculated diversity indices (d, D, and H') showed that the order Hemiptera was most diverse order, (E) value showed that Hemiptera was less evenly distributed order.

Within the study areas, according to Simpson diversity (D=0.055), Shannon diversity (H'=3.391) and species richness (d=6.3721) indices, diversity values showed that the site (II, pumpkin) plants have the highest diversity values. Site I, gourd plants have lowest diversity values of D=0.0694, H'=3.1893, and d=4.9459. Highest evenness value (E= 0.6279) was observed in watermelon plants (Site IV) but the evenness value was lowest in the squash plants (E = 0.5718) (Table 7).

Similarity

The highest similarity was found in squash and pumpkin plants (0.860), the lowest similarity was found in gourd and watermelon plants (0.623) (Table 8).

Weather parameter and cucurbit plants

In the present study, all cucurbit plants were sown at the onset of the rainy season. The similar weather condition was observed in the study area, there is no rainfall in June and maximum rainfall (038 mm) was found in August. The mean temperature was ranged from 26.9ºC to 32.0ºC. The highest humidity (81%) was found in August, and the lowest (53%) was found in May. Among the observed plants, pumpkin plants were more thrive than the other cucurbit plants.

Table1 The Relative abundance of insect orders in different Cucurbitaceous plants in Hnaw-kone Village

No.	Order	Name of Species	Gourd	Pumpkin	Squash	watermelon	Total	R.A(%)
1	Orthoptera	*Atractomorpha crenulata*	9	22	7	23	61	0.157
2		*Trilophidia japonica*	14	0	8	26	48	0.911
3		*Acrida acuminata*	0	7	16	39	62	1.176
4		*Acrida conica*	0	0	6	49	55	1.043
5		*Oxya japonica*	0	17	8	44	69	1.309
6		*Teleogryllus commodus*	0	0	1	15	16	0.304
7		*Gryllus* sp.	0	8	3	19	30	0.569
8	Odonata	*Orthetrum sabina*	53	25	8	53	139	2.637
9		*Orthetrum glaucum*	2	17	12	26	57	1.081
10		*Crocothemis servilia*	0	5	3	8	16	0.304

No.	Order	Name of Species	Gourd	Pumpkin	Squash	watermelon	Total	R.A(%)
11	Hemiptera	*Chrysocoris stolli*	10	0	0	0	10	0.190
12		*Eysarcoris guttiger*	26	40	12	0	78	1.479
13		*Bagrada hilaris*	22	60	13	0	95	1.801
14		*Agonoscelis nubila*	19	11	8	10	48	0.911
15		*Nezara viridula*	33	22	7	11	73	1.385
16		*Spilostethus pandurus*	38	25	25	0	88	1.670
17		*Graptostethus servus*	38	23	19	0	80	1.518
18		*Arocatus melanocephalus*	17	13	10	12	52	0. 987
19		*Creontiades pallidus*	21	17	18	0	56	0.062
20		*Engytatus varians*	44	119	92	0	255	4.838
21		*Dysdercus cingulatus*	58	26	29	0	113	2.144
22		*Cletus bipunctatus*	22	19	14	17	72	1.366
23	Hemiptera	*Coreus marginatus*	13	11	0	11	35	0.664
24		*Clavigralla gibbosa*	23	16	15	0	54	0.024
25		*Riptortus pedestris*	31	24	0	38	93	1.764
26		*Leptocorisa acuuta*	59	46	41	0	146	2.769
27		*Aphis gossypii*	314	272	216	207	1009	19.14
28		*Rhynocoris fuscipes*	36	31	7	18	92	1.745
29		*Leucinodes orbonalis*	0	23	0	16	39	0.740
30	Coleoptera	*Zelus longipes*	0	23	0	18	41	0.778
31		*Geocoris pallens*	24	14	0	14	52	0. 987
32		*Coptosoma japonicum*	34	27	11	18	90	0.707
33		*Coptosoma cribrarium*	23	16	0	14	53	0.006
34		*Phyllotreta cruciferae*	0	26	10	21	57	0.081
35		*Aulacophora foveicollis*	44	101	55	53	253	4.790
36		*Monolepta signata*	0	31	20	0	51	0.968
37		*Coccinella transversalis*	83	47	34	0	164	3.111
38		*Cheilomenes sexmaculata*	86	52	18	30	186	3.529
39		*Cycloneda polita*	36	23	35	0	94	1.784
40		*Harmonia axyridis*	37	26	23	0	86	1.632
41		*Bradymerus acutangulus*	0	5	2	0	7	1.133
42		*Gelerita janus*	0	0	19	15	34	0.645
43	Lepidoptera	*Diaphania indica*	11	8	25	5	49	0.929
44		*Diaphania nitidalis*	0	0	9	0	9	0.003
45		*Leucinodes orbonalis*	0	26	0	28	54	1.024
46		*Spoladea recurvalis*	0	9	8	8	25	0. 474
47		*Euchrysops cnejus*	34	27	16	19	96	1.821
48		*Sphenarches anisodactylus*	45	82	80	72	279	5.293
49		*Uthethesia pulchella*	14	7	6	8	35	0.664
50		*Euchromia orientalis*	11	13	47	42	113	2.143
51	Diptera	*Efferia apicalis*	0	10	10	11	31	0.588
52		*Promachus* sp.	0	6	4	4	14	0.266
53		*Syphus* sp.	0	0	0	12	12	0.227
54		*Bactocera cucurbitae*	30	102	58	63	253	4.838
55		*Liriomyza pusilla*	35	0	0	17	52	0.987
56		*Chrysosoma leucopogon*	0	17	0	23	40	0.759
			1449	1597	1088	1137	5271	
			37	48	45	40	56	

Table 2 Relative abundance of insect orders

Sr. No	order	Gourd (site I)		Pumpkin (site II)		Squash (site III)		Watermelon (site IV)	
		No. Total	RA (%)	No. Total	RA (%)	No. Total	RA (%)	No. Total	RA (%)
1	Orthroptera	23	1.59	54	3.38	49	4.50	215	18.91
2	Odonata	55	3.79	47	2.94	23	2.11	87	7.65
3	Hemiptera	905	62.45	878	54.98	537	49.36	404	35.53
4	Coleoptera	286	19.73	311	19.47	216	19.85	119	10.46
5	Lepidoptera	115	7.94	172	10.77	191	17.56	182	16.01
6	Diptera	65	4.49	135	8.45	72	6.61	130	11.43

Table 3 Diversity of insect orders in gourd plants (site I)

Diversity Indices		Orthoptera	Odonata	Hemiptera	Coleoptera	Lepidoptera	Diptera
1	d	1.0261	0.2495	2.9377	0.7072	0.8430	0.2396
2	D	0.5019	0.9286	0.1448	0.2282	0.2673	0.4952
3	H	0.6693	0.1562	2.5419	1.5337	1.4328	0.6902
4	N_1	1.952	1.1691	12.7038	4.6353	4.1904	1.9941
5	N_2	1.9921	1.0769	6.9039	4.3823	3.7414	2.0194
6	E	1.0412	0.4547	0.5045	0.9304	0.8592	1.1025

Table 4 Diversity of insect orders in pumpkin plants (site II)

Diversity Indices		Orthoptera	Odonata	Hemiptera	Coleoptera	Lepidoptera	Diptera
1	d	0.7521	0.5194	3.0984	1.2196	1.1656	0.6116
2	D	0.2907	0.4125	0.1324	0.1833	0.2828	0.5912
3	H	1.2774	0.9419	2.5635	1.8536	1.5519	0.8038
4	N_1	3.5873	2.5648	12.9811	6.3828	4.7204	2.2340
5	N_2	3.4399	2.4237	7.5498	5.4561	3.5350	1.6916
6	E	0.9430	0.9098	0.5467	0.8279	0.6814	0.5601

Table 5 Diversity of insect orders in squash plants (site III)

Diversity Indices		Orthoptera	Odonata	Hemiptera	Coleoptera	Lepidoptera	Diptera
1	d	1.5417	0.6378	2.3862	1.488	1.1423	0.4677
2	D	0.1828	0.3833	0.2068	0.1487	0.2612	0.6667
3	H	1.7428	0.9724	2.0971	1.9995	1.5689	1.6089
4	N_1	5.7133	2.6443	8.1425	7.3853	4.8014	1.8384
5	N_2	5.4696	2.6082	4.8349	6.7226	3.8281	1.500
6	E	0.9483	0.9780	0.5369	0.8962	0.7439	0.5964

Table 6 Diversity of insect orders in watermelon plants (site IV)

Diversity Indices		Orthoptera	Odonata	Hemiptera	Coleoptera	Lepidoptera	Diptera
1	d	1.1171	0.4478	1.9995	0.6277	1.1529	1.0272
2	D	0.1616	0.4627	0.2842	0.3031	0.2447	0.2944
3	H	11.9908	0.8823	1.8672	0.2447	1.6025	1.4595
4	N_1	7.3214	2.4165	6.4701	3.4718	4.9654	4.3038
5	N_2	6.1891	2.1612	3.5177	3.2993	4.0850	3.3961
6	E	0.8209	0.8198	0.4603	0.9302	0.7779	0.7253

Table 7 Species richness, diversity and evenness of insects at four study sites

No.	Name of Species	Gourd (Site I)	Pumpkin (Site II)	Squash (Site III)	Watermelon (Site IV)
1	Total number of individuals	1449	1597	1088	1137
2	Total number of species	37	48	45	40
3	d	4.9459	6.3721	6.2928	5.5428
4	D	0.0694	0.055	0.0676	0.0576
5	H'	3.1839	3.391	3.2226	3.2973
6	N_1	24.1407	29.6956	25.0930	27.0395
7	N_2	14.4032	18.1769	14.7521	17.3517
8	E	0.5792	o.5985	0.5718	0.6279

Table 8 The similarity (Sorensen) matrix of insects between four study sites

Study Sites	Gourd (Site I)	Pumpkin (Site II)	Squash (Site III)	Watermelon (Site IV)
Site II (Pumpkin)	0.776	-		
Site III(Squash)	0.729	0.860	-	
Site IV(Watermelon)	0.623	0.773	0.706	-

Discussion

The present work was conducted from May to August 2019. The total of 5271 individuals and 56 species belonging to six orders (Orthoptera, Odonata, Hemiptera, Coleoptera, Lepidoptera and Diptera) were recorded in the present study.

The highest relative abundance was found in order Hemiptera, followed by Coleoptera, Lepidoptera, Orthoptera, Diptera and Odonata. During the present study, collected 23 species were belonging to the order Hemiptera, nine species in the Coleoptera and eight species in the Lepidoptera.

On the whole, in all study sites of the present study, the relative abundance value of order Hemiptera was dominant than other insects. The calculated diversity indices revealed that the values of species richness (d), Shannon's index (H') Simpson's index (D) were also highest in the Hemiptera out of the six orders. This findings showed that the larger number of cucurbit pests (> 200) were found from *Engytatus varians, Aphis gossypii, Aulacophora fovicollis, Sphenarches anisodactylus* and *Bactocera cucurbitae* in the Hemiptera. This finding is agreed with the Borrer and Delong (1964), they reported that the Hemiptera was a large and widely distributed group of insects. Fayyaz, *et al*., (2016) conducted the survey to determine the diversity and relative abundance of insects in pumpkin plantation in Haripur District (Pakistan) and indicated the Coleoptera was the most abundance insect order. The present finding differ from Fayyaz, *et al*., 2016, this may be due to different environmental condition.

In all host plants, pumpkin plants were notability the highest in terms of diversity (d, D, H'). The least was found in the gourd plants. This may be due to host preference of some insect species. Singh *et al.,* (2000) pointed out the host suitability of insects, watermelon was the favorites host and pumpkin was median preference for red pumpkin beetle, *Aulacophora fovicollis.* The variation of diversity values among the insects indicated that the different condition (flowering and fruiting stages) of the plants influenced the abundance of insect fauna. So, the results were agreed with the finding of Singh *et al,* (2000).

The highest similarity was observed between pumpkin and squash plants. The lowest similarity was observed between gourd and watermelon plants. David *et al.*, (1994) pointed out the plants in two communities have been believed to co-evolve with their insects herbivores. Fayyaz *et al.,* (2016) observed that the Cucurbitacae vegetables; watermelon, squash, pumpkin, and round gourd constitutes an important group of vegetables having same type of insect fauna. This is close conformity with the present finding.

Conclusion

From this study, many insects were observed from four cucurbit plants. Relative abundance and all diversity indices showed that order Hemiptera was most diverse order. Based on the evenness values, the lowest value in Hemiptera indicated that insects were poorly distributed in this order. The suppression of the most abundance insects, *Engytatus varians, Aphis gossypii, Aulacophora fovicollis, Sphenarches anisodactylus* and *Bactocera cucurbitae* were important to yield the better quality fruits. Cultivators are necessary to develope the knowledge for pest management in the fields, application of the excessive insecticides destroyed the pests with the beneficial insects. So biological control measures should be used for productivity of safety qualifies food.

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ROLE OF WADING BIRDS AS PREDATORS ON THE INVASIVE GOLDEN APPLE SNAIL (GAS) *POMACEA CANALICULATOR* IN WETLAND HABITATS OF MAUBIN DISTRICT, AYEYARWADY REGION

Myo Sandar Win[1], Ah Mar Yi[2], Theingi Soe Myint[3], Kathy Khine[4], Hele Swe Po[5]

Abstract

The present research of wading birds was observed in wetland habitats where intruded by invasive golden apple snail (GAS) *Pomacea canaliculator* in Maubin District, Ayeyarwady Region. Field surveys were carried out from February, 2019 to January, 2020. Distance sampling point count method (Buckland *et.al*., 2004) was applied in this research. A total of 21 wading bird species (2985 individuals) of 15 genera belonging to seven families in three orders were recorded. Out of total, 11 wading birds were observed as predatory or on the invasive species of golden apple snail. According to their food types, snails were main food type for them. These wading birds were molluscivores. The effects of the wading bird species on snail abundance were studied by the flock sizes of wading birds. The flock sizes of wading birds were recorded in two different sites (low wading birds pressure site and high wading birds pressure site). A total of 40 flocks were recorded in two different sites (25 flocks in low wading birds pressure site and 15 flocks in high wading birds pressure site). The highest numbers of flock size were recorded in the wading birds species of *Anastomus oscitans*, (11 flocks) and *Plegadis falcinellus* (5 flocks). During the study period, the golden apple snail destroyed the paddy fields only in a single night in the study site. Moreover, it consumed large quantities of different plant species which were as habitats for some bird species. The result showed the GAS has become a pest for pre-germinated rice crops and other aquatic plants in the study area. Based on the data, the wading birds could potentially feed on golden apple snails in their feeding ground. It is proposed that the wading birds were good predators or biological control agents on the invasive golden apple snail (GAS) *Pomacea canaliculator.*

Keywords: Golden apple snail (GAS), invasive, *Pomacea canaliculator,* wading birds, wetland

Introduction

Wading birds are most commonly associated with wetlands, streams, and other aquatic habitats. Most wading birds possess long legs and toes, and long and sometimes curved bills – adaptations enabling them to live and feed in shallow-water habitats. Wading birds rely heavily on wetland habitats including inland and coastal emergent marshes and wooded swamps. Wading birds are naturally adapted to wetlands, streams, and other aquatic ecosystems in North America. Habitats used by wading birds are diverse -ranging from aquatic complexes to dry upland meadows, pastures, and crop fields. Inland freshwater ponds, lakes, streams, wetlands with emergent aquatic vegetation, coastal marshes, riparian and wooded wetlands and bogs, mangroves, and estuaries are the most common sites used for feeding and nesting (Sauer *et.al*., 1999).

The freshwater golden apple snail (GAS), *Pomacea canaliculator* is endemic to South America. Golden apple snails were introduced several times in Asia (Hayes *et al*.,2008) as a food source and for use in commercial aquaculture, but these intended uses were not commercial successful. Thus, unused specimens of *P. canaliculator* were discarded into and rapidly spread through aquatic habitats (Halwart, 1994) and their release led to them becoming a major pest in wetland agricultural systems, most particularly as a rice pest (Hickel *et al*., 2012).). Invasive golden apple snails possess many characteristics of successful invaders. They exhibit high reproductive

[1] Dr, Lecturer, Department of Zoology, University of Yangon
[2] Dr, Lecturer, Department of Zoology, University of Yangon
[3] Dr, Associate Professor, Department of Zoology, Myeik University
[4] Dr, Lecturer, Department of Zoology, Loikaw University
[5] Dr, Lecturer, Department of Zoology, Loikaw University

potential, fast growth rate, high dietary flexibility and strong resistance to a number of environmental conditions including hypoxia, high temperature and desiccation (Cowie, 2002; Estebenet and Martín, 2002; Boland *et al*., 2008). *Pomacea* (Ampullariidae) is a large genus of apple snails with more than 100 described species (Cowie and Thiengo, 2003). *Pomacea canaliculator* has become a major pest of rice in Asia and taro in Hawaii because of its voracious appetite for these semi-aquatic crops (Halwart, 1994; Naylor, 1996; Cowie, 2002; Joshi and Sebastian, 2006).

In Southeast Asia and elsewhere, chemical control of GAS using molluscicides has been favored due to the immediate results, but these chemicals are known to be detrimental to the environment and human health (Carlsson and Bronmark, 2006). Some farmers have attempted to remove or destroy the GAS egg masses deposited on vegetation as an alternative to chemical control (Joshi, 2007), whereas in South America wooden perches have been used to attract snail kites *Rosthramus sociabilis* as effective predators of the snails. In recent years, it has become clear that *P. canaliculata* could have reduced wetland biodiversity by grazing on macrophytes and by predation on benthic invertebrates, and altered wetland function by releasing nutrients into the water (Kolar and Lodge, 2001).

The role of wading bird species as predators on golden apple snail (GAS) *Pomacea canaliculator* in Ayeyarwady Region is still poorly known in the current situation. The systematically research has not been performed wading birds as predators on the invasive golden apple snail in this region. Hence, the present research was conducted to record the wading birds and their populations and flock size, to observe the food types of wading birds, to examine the role of the wading birds as predators on golden apple snails, to conduct the wading birds efficiency on snails abundance and to assess the threats by golden apple snail to wetland habitats.

Materials and Methods

Study area

Maubin District, Ayeyarwady Region lies at north latitude 16° 43' and east longitude 95° 39'. In Maubin District, there are four townships such as Maubin, Pantanaw, Nyaungdon, and Danuphyu. Ayeyarwady Region is situated in southern Myanmar (Figure 1).

Study site

Maubin Township

(North latitude 16° 41' and East longitude 95° 32')

Study period

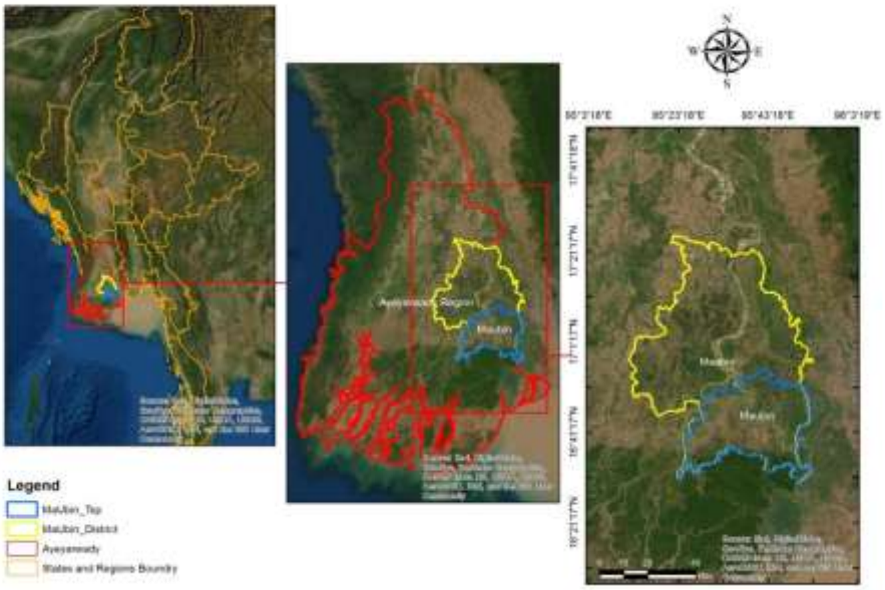
The survey was conducted from February, 2019 to January, 2020.

Field data collection

The research data were collected by the observing of the wading birds and their populations. Moreover, the distribution of golden apple snails (GAS), and varying sizes of empty shell of golden apple snail were also recorded (Plate 1). Distance sampling point transect method (Buckland *et al*., 2006) was applied for this research for recording the wading birds. Five to ten of transect lines were randomly established at study site. Transects length were between 500 m and 1000 m and at least 300 m apart each line according to the habitat types.

Data analysis

Recorded wading birds were identified by Lee *et al*., 2018 (A field guide to the Waterbirds of ASEAN). To determine the effect of the wading bird species on snails abundance by studying the flock sizes of wading birds and to assess the effect of the wading birds predation on snails varying sizes by randomly sampled live snails and preyed-on shell. The flock size of wading birds were recorded in two kinds of sites where visited by wading birds (1) low wading birds pressure sites (frequent but with low abundance, one flock = 1 – 50 individuals) (2) high wading birds pressure site (frequent and high abundance, one flock = 51+ individuals). The food types of wading birds were categorized by visual observation according to Lope *et.al*., 2003. No data collected by bird stomach content for categorize of bird food type. Snails were measured the size (height and width). The recorded snails categorized into 2 size-classes on their reproductive potential: (a) juvenile or non-reproductive (≤ 25mm heigh) and (b) adult or mature (> 25mm heigh) based on Carson *et.al*., 2004.



Source : Esri,Digital Globe, Geoeye, Earthstar Geographic,CNES/Airbus DS,USDA,USGS, AeroGRID, IGN, and the GIS user Community

Figure 1 Map of the study site

Results

Recorded wading birds in the wetland habitats

The survey was conducted in wetland habitats in Maubin Township, Maubin District, Ayeyarwady Region. During the survey period, a total of 21 wading birds species (2985 individuals) of 15 genera belonging to seven families in three orders were recorded in seven transect lines (Table 1). The highest numbers of species (16 species) were recorded in order Pelecaniformes and followed by order Charadriiformes (four species). The lowest number of species (one species) was recorded in order Gruiformes (Figure 2). Out of total, 11 wading birds were recorded as predators on the invasive species of golden apple snails. According to IUCN Red list, two near- threatened species of Black-headed Ibis *Threskiornis melanocephalus*) and Black-tailed Godwit *Limosa limosa* and one vulnerable species of Sarus Crane *Grus antigone* were observed in the study site during the research period (Plate 1).

Populations and flock size of wading birds

During the research period, 2985 individuals of wading birds were observed in the study site. The highest numbers of population were recorded in order Pelecaniformes (2689 individuals) and followed after by Gruiformes (210 individuals). The lowest numbers was recorded in order Charadriiformes (86 individuals) (Figure 3). The populations number of wading birds species of Asian Openbill (1825 individuals), Glossy Ibis (375 individuals) and Painted Stork (110 individuals) belonging to family Ciconidae and Threskiornithidae were recorded to be higher than other wading birds species. A total of 40 flocks were recorded in the study sites during the survey period. The highest numbers of flock were recorded in two wading birds species, such as Asian Openbill (11 flocks) and Glossy Ibis (5 flocks). The effects of the wading bird species on snail abundance were studied by flock size of wading birds. The flocks of wading birds were recorded in two kinds of sites (low wading birds pressure site and high wading birds pressure site). In low wading birds pressure site, 25 flocks of wading birds were recorded while 15 flocks were recorded in high wading birds pressure site. In low wading bird pressure site, the numbers of flock were higher but the population numbers of wading birds were lower. On the other hand, the population numbers of wading birds were higher but the flock numbers were lower in high wading birds pressure site (Table 2).

The role of the wading birds as predators on golden apple snail and their food types

In recorded 21 wading birds species, 11 wading birds were observed as predators on golden apple snail (GAS) (Plate 2). The food types of all wading birds were mollucs, crustaceans, crab, fish, insects, reptiles, amphibians, small mammals and aquatic plants by visual observation. Some wading birds were omnivores and they ate vegetable and animal matter. According to the results, five omnivores were recorded during the research period (Table 3). On the other hand, some wading birds ate fishes and which were main food for them. The main food types were different among wading bird species. In case of the diet types of wading birds, four folivores, three grainvores, 14 crustaceovores, 15 carnivores, 16 insectivores, 11 molluscivores, 13 picivores, and eight vermivores were recorded during the research period according to Lope *et.al*., 2003 (Figure 4). Out of total, five species of wading birds, such as Asian Openbill *Anastomus oscitans*, Glossy Ibis *Plegadis falcinellus*, Black- headed Ibis *Threskiornis melanocephalus* Painted Stork *Mycteria leucocephala* and Black- tailed godwit *Limosa limosa* were observed the higher eating rate on snails than other molluscivores because their main food type was snails. These wading birds searched for snails probed with their bills under and aquatic vegetation and rice stalks. The wading birds spent most of their foraging time in those snails' habitats. Mostly their feeding ground depending upon the presence or absence of the snails (GAS). All of molluscivores ate the adult snail's size than other size because it gave more profitability than small size snails.

The wading birds efficiency on golden apple snail (GAS) abundance

The golden apple snails were found throughout of the year in the study site and the abundance of GAS was more observed in pre and post raining season. The golden apple snail's egg masses were found mostly in the rainy season on the paddy plants and aquatic plants (Plate 2). In the cold season, the GAS burrows in the ground for hibernation. After rainy season, all paddy fields were burnt by landlord to grow the second crop and then there were shortly flooded afterwards by watering from irrigation canal where visited by wading birds. By the time, the wetlands habitats (paddy fields) allowed these snails to come out of their hibernating burrows grow and reproduce, and it was fascinating the wading birds as their feeding ground in those paddy fields. The wading birds, especially population abundance of molluscivores were totally depending upon snail abundance. In all observed of the wading birds, 11 species regarded as predators on golden apple snail. The wading birds of Asian Openbill *Anastomus oscitans*, Glossy

Ibis *Plegadis falcinellus*, Black- headed Ibis *Threskiornis melanocephalus* Painted Stork *Mycteria leucocephala* and Black- tailed Godwit *Limosa limosa* were observed to be higher the feeding rate than other wading birds species on golden apple snails (GAS). The highest numbers of population and flock size were recorded in Asian Openbill *Anastomus oscitans* (1825 individuals in 11 flocks) and follow by Glossy Ibis *Plegadis falcinellus* (375 individuals, in 5 flocks). These wading birds were recorded in two wading birds pressure sites (low and high wading birds pressure site). The population numbers of Asian Openbill were observed in higher in high wading birds pressure site than low wading birds pressure site. According to the data, the numbers of molluscivore bird species were recorded in lower but the individuals of bird were higher in each species. Based on the results, large flocks of wading birds could greatly reduce snail populations in their feeding ground in foraging time. Live snails and empty shells were observed in the wading birds' habitats after the foraging time.

Threats of golden apple snail (GAS) on wetland habitats

Wetland water, used for paddy field irrigation allows these snails to invade during irrigation and heavy rainfall events, and feed on sprouting rice seedlings resulting in extensive crop damage and other aquatic plants (Plate 2). The golden apple snail (GAS) destroyed the paddy fields only in a single night in some area. Moreover, it consumed large quantities of different plant species which depends by small wetland species for nesting, foraging and roosting in the study site. The loss of plants that used to provide income or habitat, this may led to high in water nutrient levels and algal blooms and, therefore, low water quality. At higher abundance, the snails have the capacity to completely eradicate most palatable plant species. Another important impact was the golden apple snail predation on other aquatic animals, mostly macroinvertebrates. Invasive aquatic invertebrates can have high negative environmental impacts. The results showed that golden apple snails were threat to this important wetland ecosystem.

Table 1 List of wading birds with their taxonomic status

Sr. no.	Scientific name	Common Name	Order	Family
1	*Anastomus oscitans*	Asian Openbill	Pelicaniformes	Ciconidae
2	*Mycteria leucocephala*	Painted Stork		Ciconidae
3	*Plegadis falcinellus*	Glossy Ibis		Threskiornithidae
4	*Threskiornis melanocephalus*	Black-headed Ibis		Threskiornithidae
5	*Nycticorax nycticorax*	Black-crowned Night-Heron		Ardeidae
6	*Ardeola grayii*	Indian Pond-Heron		Ardeidae
7	*Ardeola bacchus*	Chinese Pound-Heron		Ardeidae
8	*Ardea cinerea*	Grey Heron		Ardeidae
9	*Ardea purpurea*	Purple Heron		Ardeidae
10	*Bubulcus coromandus*	Eastern Cattle Egret		Ardeidae
11	*Ardea alba*	Great Egret		Ardeidae
12	*Mesophoyx intermedia*	Intermediate Egret		Ardeidae
13	*Egretta garzetta*	Little Egret		Ardeidae
14	*Ixobrychus sinesis*	Yellow Bittern		Ardeidae
15	*Ixobrychus cinnamomeus*	Cinnamon Bittern		Ardeidae
16	*Ixobrychus flavicollis*	Black Bittern		Ardeidae
17	*Grus antigone*	Sarus Crane	Gruiformes	Gruidae
18	*Vanellus cinereus*	Grey-headed Lapwing	Charadriiformes	Charadriidae
19	*Vanellus indicus*	Red -wattled Lapwing		Charadriidae
20	*Himantopus himantopus*	Black-winged Stilt		Recurvirostridae
21	*Limosa limosa*	Black -tailed Godwit		Scolopacidae

Table 2 Recorded number of wading birds and flock size in two wading birds pressure site

Sr. No.	Common Name	No. flock in low wading birds pressure site	No. flock in high wading birds pressure site	Total number of flock	Total number of individuals
1	Asian Openbill	3	8	11	1825
2	Painted Stork	1	1	2	110
3	Glossy Ibis	2	3	5	375
4	Black-headed Ibis	2		2	35
5	Black-crowned Night-Heron	1		1	47
6	Indian Pond-Heron	1		1	17
7	Chinese Pound-Heron	1		1	22
8	Grey Heron	1		1	14
9	Purple Heron	1		1	23
10	Eastern Cattle Egret	1	1	2	76
11	Great Egret	1		1	15
12	Intermediate Egret	1		1	33
13	Little Egret	1	1	2	68
14	Yellow Bittern	1		1	9
15	Cinnamon Bittern	1		1	13
16	Black Bittern	1		1	7
17	Sarus Crane	1	1	2	210
18	Grey-headed Lapwing	1		1	38
19	Red -wattled Lapwing	1		1	11
20	Black-winged Stilt	1		1	32
21	Black -tailed Godwit	1		1	5
	Total	**25**	**15**	**40**	**2985**

Table 3 List of recorded wading birds with their food types

Sr. no.	Common name	Vegetable matter		Animal matter						Omnivore	Food types
		Herbivore		Faunivore							
		FO	GR	CR	CA	IN	MO	PI	VE		
1	Asian Openbill			✓	✓		✓	✓	✓		mollusc, fishes, worms, reptiles amphibians, and
2	Glossy Ibis	✓		✓			✓		✓	✓	mollusc, crabs, worms and aquatic plants
3	Black-headed Ibis			✓			✓		✓		mollusc, insects, and worms
4	Painted Stork			✓	✓		✓	✓			mollusc, crustacean, fishes and crabs
5	Black-crowned Night-Heron	✓		✓	✓	✓		✓		✓	crustaceans, fishes, insects, reptiles, amphibian, and aquatic plants
6	Indian Pond-Heron			✓	✓	✓		✓			fishes, crustaceans, insects, and amphibians

Sr. no.	**Common name**	**Vegetable matter**		**Animal matter**						**Omnivore**	**Food types**
		Herbivore		**Faunivore**							
		FO	**GR**	**CR**	**CA**	**IN**	**MO**	**PI**	**VE**		
7	Chinese Pound-Heron			✓	✓			✓			fishes, crustaceans, insects, and amphibians
8	Grey Heron			✓	✓	✓		✓			fishes, crustaceans, insects, rodents and amphibians
9	Purple Heron			✓	✓	✓		✓			fishes, crustaceans, insects, rodents and amphibians
10	Eastern Cattle Egret				✓	✓			✓		insects, frogs, worms and reptiles
11	Great Egret			✓	✓	✓	✓	✓			fishes, frogs, crustaceans, mollucs and insects
12	Intermediate Egret			✓	✓	✓	✓	✓			fishes, frogs, crustaceans, mollucs and
13	Little Egret			✓	✓	✓	✓	✓	✓		frogs, fishes, crustaceans, insects and molluscs
14	Yellow Bittern				✓	✓		✓			fishes, insects and amphibians
15	Cinnamon Bittern				✓	✓		✓			fishes, insects and amphibians
16	Black Bittern				✓	✓		✓			fishes, insects and amphibians
17	Sarus Crane	✓	✓	✓	✓	✓	✓	✓		✓	crustaceans, molluscs, insects, fishes, reptiles, amphibians, seed, grain and aquatic plants
18	Grey-headed Lapwing		✓			✓	✓		✓	✓	insects, molluscs, worms, grains, and seed
19	Red -wattled Lapwing		✓			✓	✓		✓	✓	insects, molluscs, worms, grains, and seed
20	Black-winged Stilt			✓		✓					crustaceans and insects
21	Black -tailed Godwit	✓				✓	✓		✓		mollucs, insects, worms, amphibians and aquatic plants

Key

FO - Folivore IN - Insectivore MO - Molluscivore PI - Picivore

VE - Vermivore GR - Grainvore CR - Crustaceovore CA – Carnivore

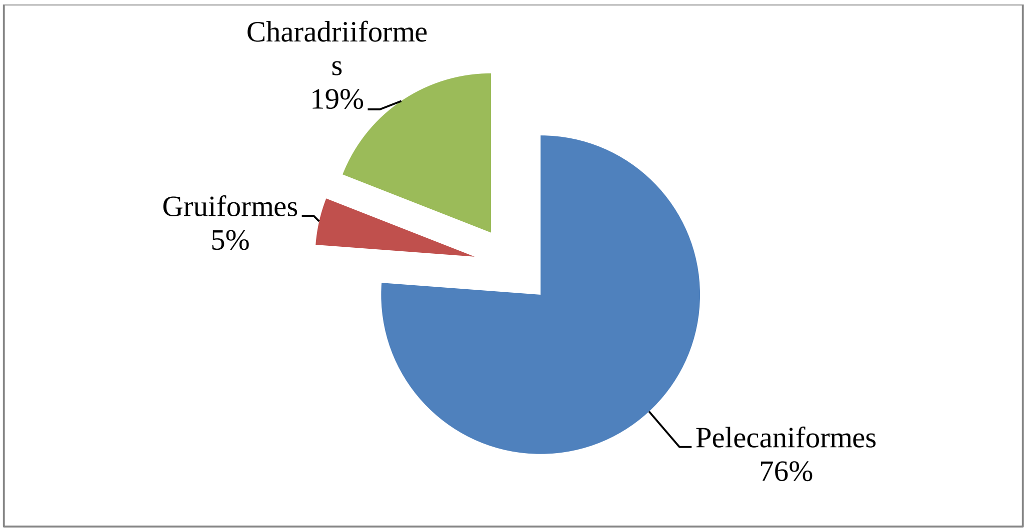


Figure 2 Recorded wading bird species percentage in order wise

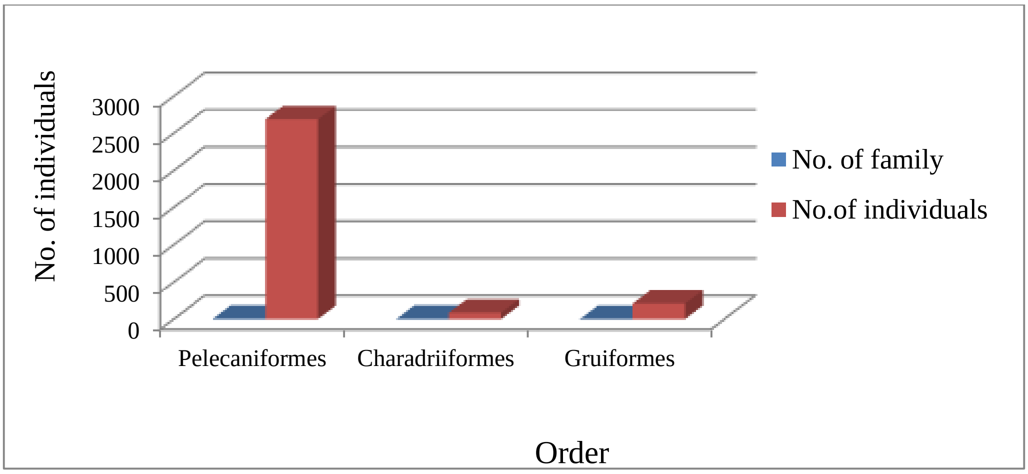


Figure 3 Recorded number of family and individuals of birds in different orders

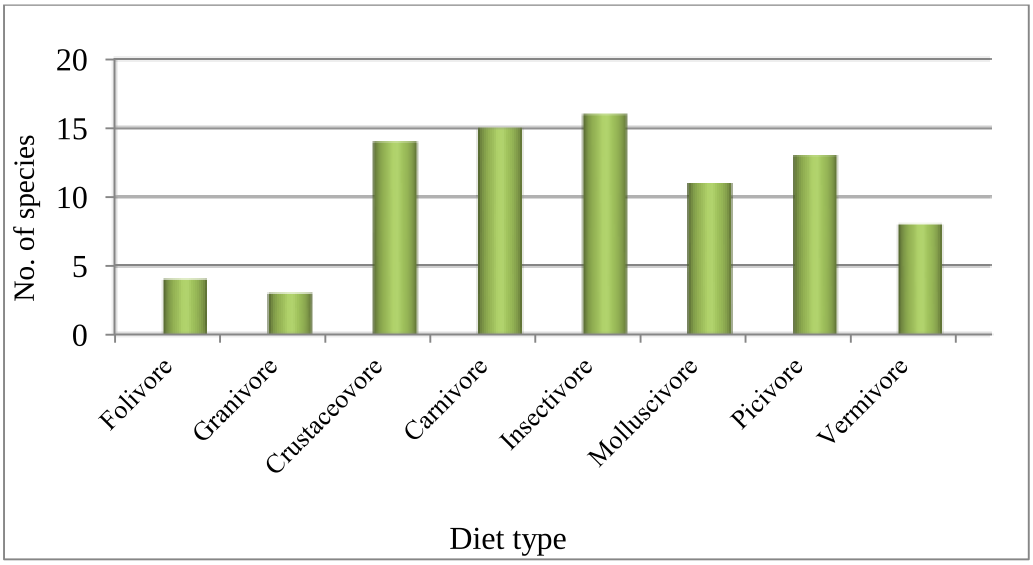


Figure 4 Recorded number of wading birds species with different diet types


A. *Anastomus oscitan*

B. *Plegadis falcinellus*


C. *Mycteria leucocephala*

D. *Threskiornis melanocephalus*


E. *Limosa limosa*

F. *Mesophoyx intermedia*


G. *Ardea purpurea*

H. *Grus antigone*

Plate 1 Recorded wading birds species in foraging ground

(A)

(B)

(C)

Plate 2 (A)Recorded photos of Golden apple snails and their empty shell

(B) Golden apple snails' egg masses on paddy plants and aquatic plants

(C) Golden apple snail damaged leaves of paddy plants and aquatic plants

Discussion

Wetlands are highly productive ecosystems that harbour great biodiversity and biological production that is important in the rural economy of many countries, especially in Southeast Asia (Carlsson *et al*., 2004). During the survey period, a total of 21 wading birds species of 15 genera belonging to seven families in three orders were recorded in seven transect lines of wetland habitats. The recorded wading birds' main food types were different among different species. Diet type categories were divided by their main food type. Based on the diet type category, 11 wading birds species were molluscivore and regarded as predators on the invasive golden apple snail (GAS). During the research period, 2985 individuals of wading birds were recorded in the wetland habitats. The highest population numbers were recorded in order Pelecaniformes (2689 individuals) and followed after by Gruiformes (210 individuals). The lowest numbers were recorded in order Charadriiformes (86 individuals). According to the data, the numbers of molluscivore bird species (11species) were recorded in lower but the individuals of bird were higher in each species. These wading bird species were recorded in all three orders. Their main food type was snail. The empty shell of snails left in the wetland habitats including paddy fields after the wading birds foraging. It may be assumed that the big flocks of wading birds could reduce the invasive snail population in their foraging ground.

Aquatic plants in freshwater systems provide substrate, refuge from predation, and a source of food for other aquatic organisms (Lodge *et al*., 1998). The invasion by the exotic golden apple snail *Pomacea canaliculata* in Southeast Asia is a threat to local agricultural economies as the snail consumes rice and naturally occurring aquatic plants (Halwart, 1994; Naylor, 1996). During the research period, the invasive gastropod of golden apple snails destroyed the paddy fields in a single night in some area. Likewise the aquatic plants were also eaten by golden apple snails. The results showed that golden apple snails were serious threat on wetland habitats. It was regarded as pests not only of rice but also of other aquatic plants of direct economic importance such as lotus, water hyacinth and water spinach. Aquatic plants in wetlands are thus an important part of rural everyday life in Southeast Asia, and many resident and transient organisms depend on wetland plants at some life stage (Mochida, 1991).

Most of the local farmers used molluscicide and hand picking for control the snails in the study site. Molluscicide used against invasive golden apple snail may also result in negative effects on organisms feeding on them. Current biological control with wading birds seems to be the promising option for sustainable GAS management. However, biocontrol must be added with other methods such as lowering water levels or draining the rice fields. Draining will not kill GAS because they are able to survive long periods of desiccation (Wada, 2004). By observing the field results, it may be assumed that, the wading birds especially molluscivores were predators on the invasive golden apple snail (GAS).

Conclusion

The invasion of the golden apple snail (GAS) *Pomacea canaliculator* and their destructive nature to the paddy land crop and wetland areas have become a major pest problem due to their growth and high reproductively, hence, causing drastic economic impact to the study site. The invasive GAS has become established and are numerous in various habitats, especially in paddy fields, this effects on people' livelihood. The results showed that the GAS has become a pest for pre-germinated rice crops and other aquatic plants in the study site. Most of the farmers prefer to choose chemical molluscicides which deliver fast and effective responses. However, the application has negative effect to the farmer's health and the ecosystem. Based on the data, the wading birds could potentially feed on the GAS. Therefore, a better and safer solution ought to be addressed. The research conducted for the wading birds as the most effective natural predators or biological control agents on the invasive golden apple snail (GAS) *Pomacea canaliculator*.

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SPECIES DIVERSITY OF ZOOPLANKTON AND SOME PHYSICO-CHEMICAL WATER PARAMETERS OF NAUNG TONG LAKE, KYAING TONG TOWNSHIP, EASTERN SHAN STATE

Khaing Khaing Aye[1], Moe Kyi Han[2]

Abstract

Investigation on the species diversity of zooplankton species and water parameters in Naung Tong Lake were conducted during March, 2019 to January, 2020. A total of 17 zooplankton species, of ten genera, six families and four orders were recorded. Based on the relation between water parameters and zooplankton, the moderate positive relationship was observed between pH and zooplankton population. Other parameters (such as water temperature, turbidity, DO and BOD) and zooplankton were poorly correlated. This study revealed that physico-chemical fluctuations were negative impact on the zooplankton species richness and abundance in this lake.

Keywords: zooplankton, Naung Tong Lake, Water parameters, species richness and abundance

Introduction

In freshwater ecosystem, the zooplankton forms an important faunal group, as most of them live on primary producers and makes themselves available to be eaten by higher organisms in food chains including fish and contribute significantly to the biological productivity of this ecosystem (Michael, 1973). Zooplankton constitute important food item of many omnivorous and carnivorous fishes. The larvae of carps feed mostly on zooplankton (Dewan *et al*., 1977) because zooplankton provides the necessary amount of proteins. Zooplankton plays an important role in indicating the water quality, eutrophication status and productivity of a freshwater body (Mikschi, 1989). Zooplankton communities are typically diverse and are highly sensitive to environmental variation. Due to short life cycle, zooplankton communities often respond quickly to environmental change (Sharma *et al.*, 2007). The changes in physico-chemical conditions of water can be reflected directly on the biotic community of ecosystem. Physico-chemical parameters influence both vertical and horizontal migration of aquatic organisms. It affects their distribution, diversity and feeding. Physico-chemical parameters of water are determined largely by the climate, geochemical and geomorphological conditions of catchment basin. Factors such as temperature, pH, DO, transparency, and electrical conductivity form part of abiotic components of an aquatic ecosystem. When water temperature in outside tolerable range, abundance of zooplankton is affected directly (Abdullahi, 1998; Imam *et al*., 2011). High acidic or high alkaline pH could result to the death of aquatic organisms including zooplanktons. Zooplankton requires oxygen for energy metabolism. Sensitivity to low oxygen concentration differs between species, various life stages (eggs, larvae and adults), and different life processes including feeding, growth and reproduction (Imam and Balarabe, 2012). Water transparency influences vertical migration of zooplankton, which affects their diurnal rhythms (Verma and Agarwal, 2007). Investigations of freshwater zooplankton community structure have significant potential for assessing aquatic ecosystem health. The objectives of the present study are;

- to record the occurrence of zooplankton species in Naung Tong Lake
- to investigate the monthly diversity indices value of zooplankton in study area
- to assess relation between water parameters and zooplankton population in study area

[1] Assistant Lecturer, Department of Zoology, Kyaukse University
[2] Dr, Professor, Department of Zoology, University of Mandalay

Materials and Methods

Study area

The present study was carried out in Naung Tong Lake. It is located in Kyaing Tong Town, the eastern part of Shan State. It lies between Latitude: 21º 17' 20.94" N - 21º17' 46.34" N and longitudes 99º 35' 51.08"E - 99 º35' 58.59" E. Water is stored in this lake within an area of 2526.92ft in length and about 711.67ft in width. In the flood season, its water level increases with a water depth about 1.824 m and in dry season, it reduces with a water depth about 1.117 m (Fig. 1).

Study period

The study period lasted from March, 2019 to January, 2020.

Collection of samples

Sample collection was carried out twice a month (second week and fourth week) on Saturdays, between 9:30 am-11:30 am. The samples were collected using plankton nylon net having 22 cm mouth diameter and 50 µm mesh size attached with one liter sampling bottle. Collections were made by horizontal hauling. The net was submerged and dragged hauled for about 10 seconds through a distance of 2 ft and hauled up for water sample. Into the collected water samples 10ml of 10% formalin solution was added before zoolplankton were studied. Before observation was made, it was ensured that all the preserved zooplankton samples were settled down. The amount of water samples were decanted to get approximately 10ml. Water sampling was carried out once in study area.

Identification of zooplankton

Samples were examined using a binocular compound microscope (Series-30002907 CETI China) under various magnifications: general screening of the specimen was made under the magnification of the 40X (4X x 10X), the whole specimen was viewed under 100X (10X x 10X). The specimens were photographed by using canon camera which is manually attached to the eyepiece of the microscope.

A pipette was used to suck up the subsamples from bottle and a drop was put onto a clean glass slides, and then covered with cover glass, and scanned under a compound microscope. Identification was according to Pennak (1953), Edmondson (1966) and other relevant literatures.

Data analysis

To calculate the species richness and diversity of zooplankton species in study area, the data was analyzed using four diversity indices: Shannon-Wiener (1948), Simpson (1949), Margalef's (1958) and modified Hill's ratio (1981).

The Shannon-Wiener diversity index, H' is calculated using the following equation:

For Shannon-Wiener index (1948)

$$H' = -\sum_{i=1}^{s}\left[\frac{n_i}{n}\right]\ln\left[\frac{n_i}{n}\right]$$

H' = index of species diversity

S = number of species

n_i = number of individuals in i^{th} species in the sample

n = total number of individuals in the sample

ln = Natural logarithm

Simpson's diversity index is a measure of diversity. In ecology, it is often used to quantify the biodiversity of a habitat. It takes into account the number of species present, as well as the abundance of each species.

For Simpson's index (1949)

$$D = \sum_{i=1}^{S} \frac{n_i(n_i-1)}{n(n-1)}$$

D = Simpson's index

n_i = number of individuals in the i^{th} species

n = total number of individuals

Species richness was determined using the formula of Margalef's index (1958)

$$d = \frac{S-1}{\ln(N)}$$

d = Margalef's species richness index

S = number of species

N = Total number of individuals

For Hill's diversity number (1973)

Number 0 : N0 = S Where, S = total number of species

N0 = number of all species in the sample

Number 1: N1 = $e^{H'}$ Where, H' = Shannon's index

N1 = number of abundant species in the sample

Number 2 : N2 = $1/D$ Where, D = Simpson's index

N2 = number of very abundant species in the sample

Species evenness or equitability (or relative species abundances) was determined by using the evenness index of modified Hill's ratio (1981).

$$E = \frac{\left(\frac{1}{D}\right)-1}{e^{H'}-1} = \frac{N2-1}{N1-1}$$

Where, E = Hill's evenness index

D = Simpson's index of diversity

H' = Shannon's index of species diversity

N1 = number of abundant species in the sample

N2 = number of very abundant species in the sample

Measurement of water parameters

Before the zooplankton hauling, the temperature (ºC) of water was measured by dipping the thermometer slightly under the surface of water for about 5 minutes and recorded during the sampling period. To analyse water parameter, the water samples were collected every month from study area with 1liter plastic bottles bandaged by the black tape from about 5.1 cm depth water surface. Water samples were examined in Water and Soil Examination Laboratory, Ministry of Agriculture, Livestock and Irrigation, Department of Fisheries, Aquaculture Division, Freshwater Aquaculture Research, Yangon.

Source: Google Earth (2018)

Figure 1 Location map of the study area (Naung Tong Lake)

Results

A total of 17 zooplankton species of ten genera, six families of four orders from Naung Tong Lake were recorded. Among them, 15 species of rotifers and two species of copepods were found in Naung Tong Lake (Table 1).

Monthly occurrence of zooplankton species

A total 4262 individuals that represent 17 species of zooplankton were collected during March 2019 to January 2020. The highest number of species (15 species) was observed in April. The lowest number of species (7 species) was observed both in March, 2019 and January, 2020. The lowest number of individuals (234) was observed in March, 2019. The highest number of individuals (525) was occurred in June, 2020 (Table 2, Fig. 2).

Some seasonal physico-chemical parameters of water sample in study area

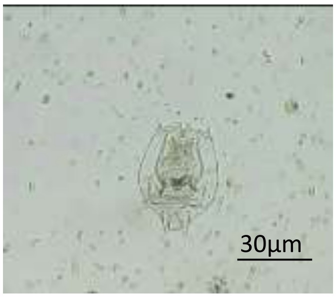
The maximum mean water temperature 30°C recorded in rainy season and minimum 21°C in cold season in Naung Tong Lake. In Naung Tong Lake, the minimum mean value of turbidity was 8.38 NTU in hot season and maximum 76 NTU in rainy season. The mean value of pH ranged between 7.1 to 8.5 during study period. The pH in this lake water was alkaline condition. The lowest mean value of DO was 2.0 ppm in hot season and the highest mean value 2.5 both in rainy and cold season of Naung Tong Lake. The lowest mean value of BOD was 3.5 mg/L in cold season and the highest 5 mg/L in hot season of Naung Tong Lake (Table 3).

Species richness and diversity indices value of recorded zooplankton species in naung tong Lake during March, 2019 to January, 2020

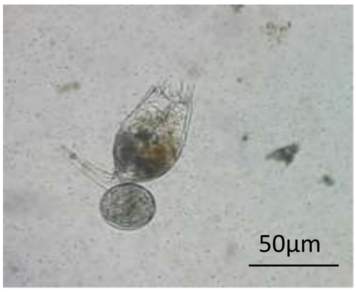
The Margalef index value was the highest d = 2.49 in April and the lowest d = 1.02 in January. Shannon-Wiener value was the highest in December (H' = 4.37) and the lowest in January (H' = 1.28), and the Simpson's index was the highest in March (D = 0.98). The lowest in January (D = 0.7). The highest evenness value was 0.24 in March and the lowest 0.01 in December were observed (Table 4).

A. *Rotaria neptunia*

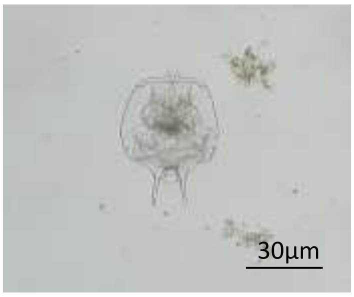
B. *Anuareopsis fissa*



C. *Brachionus angularis*

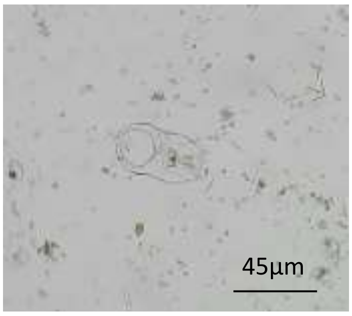


D. *Brachionus calyciflorus*

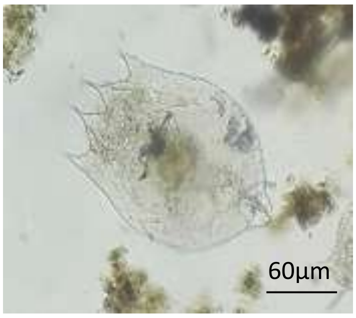


E. *Brachionus caudatus*

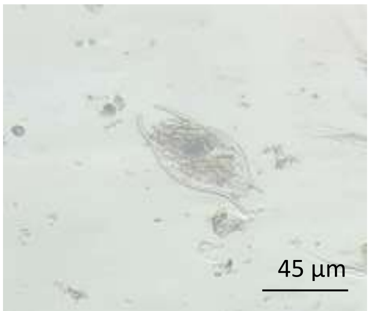
F. *Brachionus falcatus*



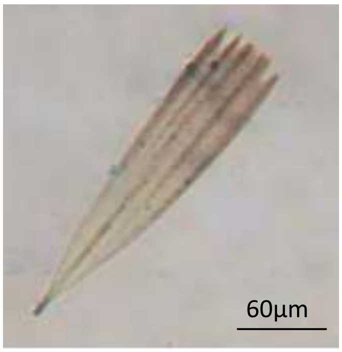
G. *Brachionus forficula*



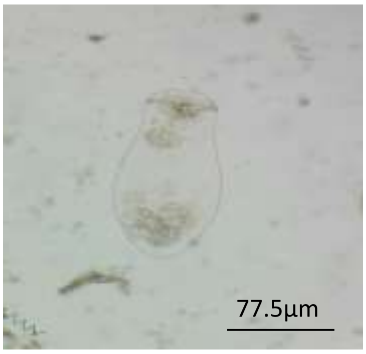
H. *Brachionus ruben*



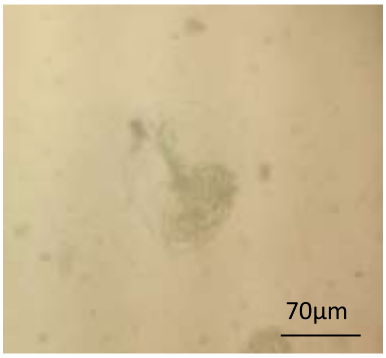
I. *Brachionus diversicornis*



J. *Notholca acuminate*



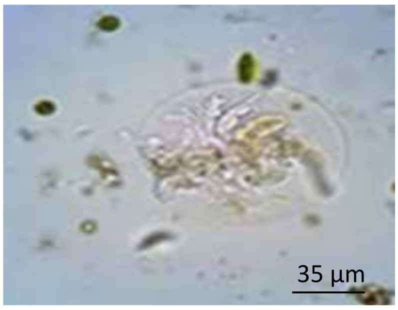
K. *Asplanchna brightwelli*



L. *Asplanchna herriki*

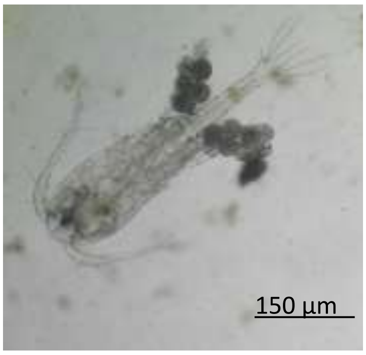
M. *Monostyla bulla*

N. *Pompholyx sulcata*

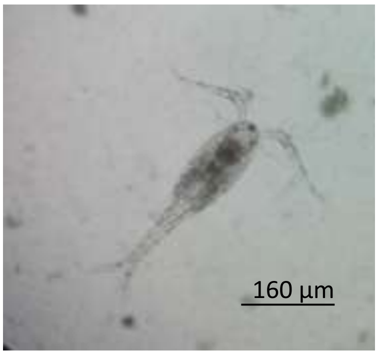


O. *Testudinella patina*

Plate 1 Recorded of some rotifer species



P. *Microcyclops* sp



Q. *Paracyclops* sp

Plate 2 Recorded of copepods species

Table 1 List of recorded zooplankton species in Naung Tong Lakes during study period

Order	Family	Species
Bdelloida	Philodinidae	1. *Rotaria neptunia*
Ploima	Brachionidae	2. *Anuareopsis fissa*
		3. *Brachionus angularis*
		4. *Brachionus calyciflorus*
		5. *Brachionus caudatus*
		6. *Brachionus falcatus*
		7. *Brachionus forficula*
		8. *Brachionus ruben*
		9. *Brachionus diversicornis*
		10. *Notholca acuminate*
	Asplanchnidae	11. *Asplanchna brightwelli*
		12. *Asplanchna herriki*
	Lecanidae	13. *Monostyla bulla*
Floscularíaceae	Testudinellidae	14. *Pompholyx sulcata*
		15. *Testudinella patina*
Copepoda	Cyclopoidae	16. *Microcyclops* sp.
		17. *Paracyclops* sp.

Table 2 Monthly occurrence of zooplankton individuals and species in Naung Tong lake during March, 2019 to January, 2020

No.	Species	Mar	April	May	Jun	July	Aug	Sep	Oct	Nov	Dec	Jan	Total number of individuals in each species
1	*Rotaria neptunia*	-	6	9	18	1	-	3	1	6	-	3	47
2	*Anuareopsis fissa*	2	10	10	3	-	2	-	2	3	8	3	43
3	*Brachionus angularis*	-	11	15	10	-	5	-	12	-	10	-	63
4	*Brachionus calyciflorus*	28	22	48	57	44	44	84	108	82	22	98	637
5	*Brachionus caudatus*	-	3	-	6	-	-	-	1	-	8	-	18
6	*Brachionus falcatus*	-	14	-	-	-	6	-	2	3	7	-	32
7	*Brachionus forficula*	-	-	3	-	10	-	1	-	-	3	-	17
8	*Brachionus ruben*	67	60	95	83	100	129	82	81	102	124	102	1025
9	*Brachionus diversicornis*	50	12	13	41	47	-	7	4	24	-	-	198
10	*Notholca acuminate*	-	5	3	19	1	11	3	5	15	2	4	68
11	*Asplanchna brightwelli*	-	2	-	34	2	1	-	6	3	-	-	48
12	*Asplanchna herriki*	-	-	-	5	-	-	-	-	-	-	-	5
13	*Monostyla bulla*	-	1	-	12	-	-	-	5	3	5	-	26
14	*Pompholyx sulcata*	1	1	-	-	-	3	-	-	-	-	-	5
15	*Testudinella patina*	-	2	1	-	3	1	-	-	2	-	-	9
16	*Microcyclops* sp.	38	23	28	85	56	33	45	42	51	19	8	428
17	*Paracyclops* sp.	48	106	144	152	163	172	131	167	213	161	136	1593
	Total number of individuals	234	278	369	525	427	407	356	436	507	369	354	4262
	Total number of species	7	15	11	13	10	11	8	13	12	11	7	17

Table 3 Some seasonal physico-chemical parameters of water sample in study area

Parameters	Hot season	Rainy season	Cold season
Temperature (ºC)	24	30	21
Turbidity (NTU)	8.38	76	51
pH	7.1	8.5	7.1
DO (ppm)	2.0	2.5	2.5
BOD (mg/L)	5	4.0	3.5

Table 4 Monthly species richness and diversity indices value of zooplankton in Naung Tong Lake during March, 2019 to January, 2020

Index	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan
No. of individuals	234	278	369	525	427	407	356	436	507	369	354
No. of species	7	15	11	13	10	11	8	13	12	11	7
d	1.10	2.49	1.69	1.91	1.48	1.66	1.19	1.97	1.76	1.69	1.02
D	0.98	0.79	0.76	0.84	0.24	0.71	0.75	0.25	0.75	0.69	0.7
H´	1.62	1.83	1.70	2.06	1.61	1.46	1.47	1.61	1.62	4.37	1.28
N1	5.05	6.23	5.47	7.84	5.02	4.30	4.30	5.00	5.05	79.04	3.59
N2	0.02	0.26	0.31	0.19	1.31	0.40	0.33	1.33	0.33	0.45	0.42
E	0.24	0.14	0.15	0.12	0.07	0.18	0.20	0.08	0.16	0.01	0.22

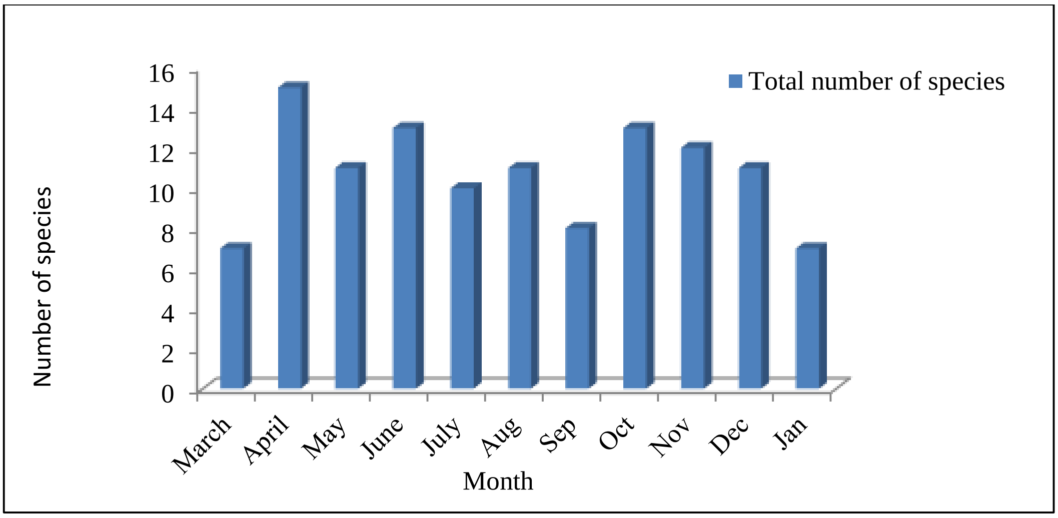


Figure 2 Monthly occurrence of zooplankton species during March, 2019 to January, 2020

Discussion

In the present study, a total of 17 zooplankton species in which 15 species of Rotifera and two species of Copepoda were recorded in Naung Tong Lake. During the study period, great variability in zooplankton abundance was recorded in the lake. Especially, rotifer was the most abundant group in lake.

Dumont (1999) recorded the higher species richness is characterized by longer food chain. At Naung Tong Lake, the highest species richness was recorded in cold season and the index value range 1.02 to 2.49.

Simpsons' index is used to quantify the biodiversity of habitats (Chatterjee, 2014). Whittaker (1965) reported that the Simpson diversity index is always higher where the community is dominated by less number of species and when the dominance is shared by large number of species (cited by Shah and Pandit, 2013). Vincent *et al.* (2012) reported the higher values of species diversity index suggest decreasing species richness with increasing trophic status. The similar trend was occurred in present study; Naung Tong showed higher species richness value.

The greater species diversity means longer food chain (Ludwik and Reynolds, 1998). Based on Shannon-Wiener legislation, the aquatic environment is classified as very good when H' is > 4, good quality 4 – 3, moderate quality 3 – 2, poor quality 2 – 1 and very poor quality < 1. Plafkin *et al.* (1989) reported that species diversity decreases when stress increases in the environment and a community dominated by a relatively few species indicate environment stress. According to the index, the H' index was 4.37 in Naung Tong lake indicating best water quality.

Amalesh *et al.* (2014) stated that species evenness is a measure of the relative abundance of each species in an area. Species evenness will be decreased if the population size of different species varies. Equitability takes a value between 0 and 1, with one being complete evenness. The index when applied in the present study, the value of evenness was ranged 0.01 to 0.24 at Naung Tong Lake. Overall results in the lake, Simpson index, Shannon-Wiener diversity index values and species evenness was highest in Naung Tong Lake. The highest Margalef's index was observed in Naung Tong Lake. Zooplankton diversity and abundance in Naung Tong Lake indicate that the lake water may be rich in nutrients.

Davies *et al.* (2009) reported that the fact that plankton distribution and abundance are affected by season, physical and chemical parameters and water movement. In present study, some physico-chemical water parameters were measured seasonally and relation with zooplankton were discussed in following chapter.

Mahar (2003) reported the water temperature is important in terms of its effect on aquatic life. During the study period, the average temperature is 30° C in Naung Tong Lake. Kamat (2000) and Gaikwad *et al*. (2008) recorded the water temperature in the range between 13.5°C and 32°C is found to be suitable for the development of the zooplanktonic organisms. According to the present result, temperature is not exceeded in 32°C. In the present study that the water temperature might also be the factor affecting the abundance of zooplankton individuals and species. Temperature is an important factor for zooplankton growth, and low temperatures reduce embryonic and post-embryonic development rates (Gillooly *et al*., 2002).

Lloyd *et al*. (1987) indicated a high correlation between increased turbidity levels and reduced zooplankton densities in Alaskan lakes (r = 0.96). Turbid lakes exhibited less than 5% of the zooplankton densities often associated with clear lakes. However, some literature suggests that increased turbidity is beneficial to large zooplankton (e.g., Fiksen and Giske 1995). In the present study, the highest turbidity level of Naung Tong Lake ranged 8.38 to 76 NTU. The relation between turbidity and zooplankton is found negative correlation in both lakes.

In the present study, the range of pH value was 7.1 to 8.5 in Naung Tong Lake. The low pH value was recorded in hot season. In this season zooplankton populations were found the lowest individuals when compare to another season. During study period, certain species of zooplankton were inclined at low pH while many of them prefer high pH medium. The value of pH was higher in the lake during rainy season. The results of the present study agree with the finding of the Pennak (1953) who reported that alkaline waters (above pH 7.0) contain relatively few species but large numbers of individuals.

Roy *et al*. (2010) recorded that the range of dissolved oxygen was 3 mg/L to 8 mg/L. The value of DO was range in 2.0 to 2.5 at Naung Tong Lake. The distribution of DO is affected by the solubility of many inorganic nutrients, which are governed by seasonal shifts from aerobic to anaerobic environments (Benson and Krause, 1980). The higher the concentration of dissolved oxygen, the better is the water quality.

Biological oxygen demand is a parameter to assess the organic load in a water body (ICMR, 1975, cited by Bhadja and Vaghela, 2013). According to Hynes (1960, cited by Banita *et al*, 2013), BOD values between 1-2 mg/L or less represent clean water, 2-7 mg/L represent slightly polluted water and more than 8 mg/L represent severe pollution. In the present study, BOD values range between 3.5 and 5 mg/L were recorded in Naung Tong Lake. According to the above author, Naung Tong Lake is slightly polluted. The high BOD may be due to the presence of high organic load released from industrial sewage (Jalilzadeh *et al*, 2008). Various water parameters of Naung Tong Lake with national and international standards guideline, pH, the levels of DO and BOD are within the permissible levels of drinking water standards except turbidity in Naung Tong Lake.

This study revealed the values of different physico- chemical conditions from the study area. The increase in number of zooplanktons was in accordance with temperature of its habitat. The study also showed that zooplankton species survive in the neutral condition. Thus, the status of the lake could said to be eutrophic as indicated by the diversity of zooplankton.

Physico-chemical conditions of the lake can be changed because of various anthropogenic effluents which release to the water. Therefore, conducting further studies in this area is essential to measuring the diversity of zooplanktons. The variation of zooplankton species was the indicator of lake water. The diversity indices are also creating a signal about the good health of aquatic environment. This study revealed that physico-chemical fluctuations was negative impact on the zooplankton species richness and abundance, thus, the need for the government to establish catchment management agency in order to curtail the menace that disrupt the aquatic ecosystem.

Conclusion

A total of 17 zooplankton species in ten genera, six families belonging to four orders were recorded. During the study period, phylum Rotifera was the most abundant group. Rotifers are generally abundant in eutrophic lakes and permanent dominant species have been reported. Therefore, it could be concluded that the present result revealed the characteristics of eutrophication and important bioindicator of water quality in the lake.

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HERPETOFAUNA OF TAWYAGYI PROTECTED AREA, WETLET TOWNSHIP, SAGAING REGION

Naing Win[1], Zaw Myo Win[2] and Thant Zin[3]

Abstract

Occurrence and distribution of herptofauna were conducted in Tawyagyi protected area embodied three study sites and commenced from July 2018 till April 2019. A total of 22 species of herptiles were recorded. The 22 species included five species of anurans (amphibia) confined to four genera and distributed among three families and a single order Anura. The remaining 17 species were reptiles distributed among two suborders, Serpentes (ophidia) snakes and Lacertalia (lizards), and confined to the order Squamata. Suborder Serpentes was represented by seven species of snakes distributed among seven genera and three families namely Colubridae, Elapidae and Viperidae. Suborder Lacertalia was represented by ten species of lizards distributed among seven genera and four families namely, Agamidae Gekkonidae, Scincidae and Varanidae. Among the recorded lizards *calotes htunwini* (Htunwin's forest lizard) happened to be the only endemic species.

Keywords: Herpetofauna, Protected Area, Endemic Species

Introduction

Amphibians and reptiles are considered important actors of biodiversity and good indicator of habitat quality as they are both present on terrestrial and aquatic habitats. Although very cosmopolitan, their highest diversity is in the tropics. Researches confirm the important pressure on reptile and amphibian population worldwide and particularly in Asia. In Southeast Asia, both reptile and amphibian populations suffer increasingly from habitat loss, intensive hunting for local consumption or trade and pollution (Calame, 2012).

Amphibians are valuable part of the biotic community. The dietary habitats of amphibians are important in ecosystem because as adults they consume vast quantities of insect and thus help to maintain a balance in the ecosystem (Duellman and Schlager, 2003). A single Blanchard cricket frog consumes approximately 4,800 insects per season (Frost, 2006). Areas where local anurans have been criminated have witnessed large population increase in some kinds of insects. Amphibians are important as prey item because they convert invertebrate "energy" they consume to useable energy in higher tropic level.

Due to their abundance and relative ease of capture, amphibians are included in the diets of a great variety of animals, especially many small mammals, birds, and many kinds of snakes (Frost, 2006).

Many amphibian and reptile populations are declining at unprecedented rates and some with extinction under the threats of global climate change, habitat loss, human exploitation, invasion species, pollution, and disease (Gibbon *et al.,* 2000; Stuart *et al.,* 2004). Yet, amphibians and reptiles are among the most understudied vertebrate taxa in Southeast Asia and the population status of most species is unknown (Rowley *et al.,* 2010). There is an urgent need to collect baseline data on population status and to initiate monitoring programs for amphibian and reptiles in the region to detect any responses to threats and environmental changes (Gibbons *et al.,* 2000) (Cited by Sung *et al.*,2011).

[1] Dr, Lecturer, Department of Zoology, Kyaukse University
[2] Dr, Assistant Lecturer, Department of Zoology Shwebo University
[3] Dr, Professor and Head, Department of Zoology, University of Mandalay

Myanmar, a country in the Southeast Asia is often considered to be the "last frontier of global biodiversity in mainland Asia" has a remarkably diverse fauna and flora and is home to many endangered reptile and amphibians. Myanmar also boasts to harbour one of the world's rarest chelonians, the Rakhine forest turtle. Moreover it is also a home for variety of lizard species and approximately 150 species of snakes and very few research works were conducted thoroughly in some protected areas covering only few species groups. Many protected areas and species have not yet been studied in detail.

The study area, Tawyagyi Protected Area is one of the protected areas of Myanmar and located in the Dry Zone belt of central Myanmar and has tropical steppe type of climate. The natural vegetations of the study area are generally tropical forest type. Although many animal species occupied and inhabited, no fauna of this area was investigated and reported previously.

This research was conducted with the following objectives;

- to identify and record the amphibians and reptiles in the study area
- to investigate the composition of amphibian and reptilian species
- to assess the relative abundance of amphibians and reptiles species

Materials and Methods

Study area and sites

Tawyagyi Protected Area is situated 19.31 km away from Wetlet Township, Sagaing Region, in central Dry Zone of Myanmar and has dry climate. The area has a length of 1.77 km from north to south and a width of 0.25 km from east to west. The area covers an area of 139 hectares. It is the first wildlife Sanctuary in Myanmar. The study area is located between 22º 15' and 22º 17' N and 95º 58' and 95º 59' E. Three study sites were allocated within protected area. Site I, Along the Ayeyawady river bank; Site II, Mixed deciduous forest and Site III, Hillock strewn open ground area.

Study period

The study was conducted during a period of from July 2018 to April 2019.

Study design

The study was conducted from July 2018 to April 2019. At each sampling site, an appropriate data collection line was designated to cover the area of each sampling site. One permanent transect line running from north to south with 50 m width was established in each study site. Herpetofauna was investigated during day and night. Two methods were used for the survey of herpetofauna; visual encountered survey method and pitfall method.

Visual encountered survey method

In visual encountered survey method a slow walking along each transect line was made. During walking, species and individuals of herptiles encountered on either side of transect were recorded. Data collection was made continuous three days for each study sites monthly. Collection of data was made two times per day, morning and evening. Some species were caught and kept as voucher specimens.

Pitfall method

Pitfall trapping is a sampling technique which is widely used in studies of seasonal occurrence, to examine spatial distribution patterns, to compare relative abundance in different micro-habitats, to study daily activity rhythms, and in community surveys. Three pitfalls were set up at each study sites. The fence of plastic sheet was measured 9.09 m in length and 0.60 m in height. A total of three pits were set up along the fence. Pitfalls were set up at least 100 m away from each other. Inspection of the herptiles was made every hour of morning 6:00 am to night 8:00 pm. The species encountered in the pitfall were recorded and some species were kept as voucher specimens. Pitfall method was used monthly to analyze the herpetofauna in the study area.

Identification and classification

Species identification was followed after Cox *et al.* (1998), O'shea and Halliday (2002) and Das (2010). For confirmation of species, relevant literatures and internet sources were also used.

Analysis of data

The collected data were analyzed. The relative abundance of each species was assessed based on the method stated by Bisht *et al.* (2004).

$$\text{Relative abundance (RA)} = \frac{\text{Total number of individuals in each species}}{\text{Total number of individuals in all observed species}}$$

The status of relative abundance were categorized as,

Uncommon (uC) = having relative abundance less than 0.0100

Common (C) = having relative abundance of 0.0100 and above but less than 0.0500

Very common (vC) = having relative abundance of 0.0500 and above

(**Source**: Google Earth, 2018)

Figure1 Location map of study area showing study sites

Results

A total of 22 species, 18 genera, ten families, two orders belonging to two classes of herptiles were recorded in three study sites of Tawyagyi Protected Area during July 2018 to April 2019. In 22 species, five species were amphibian and the rest were reptile (Table, 2).

Occurrence and abundance of herpetofauna

The highest number of species was that of order Squamata represented by 17 species confined to 14 genera and seven families. Among the amphibians, the order Anura was represented by five species confined to four genera and three families.

During the study period, among the anurans the highest (60.00%) was that of the family Dicroglossidae, followed by (20.00%) each of family Bufonidae and Microhylidae (Fig.2). Among the reptiles, family Colubridae revealed that the highest species composition (29.42%), family Agamidae and family Gekkonidae (23.53%) each and remaining four families Elapidae, Scincidae, Varanidae and Viperidae with (5.88%) each (Fig.3).

Visual encountered survey method

In study site I, both *Hemidactylus frenatus,* and *Hemidactylus mutilata* showed the highest 26 individuals and *Trimeresurus albolabris*, and *Blythia reticulate* observed with only two individuals were the lowest. The highest 15 species and 22 individuals were found in July and the lowest five species and seven individuals were found in April.

In study site II, *Hemidactylus brookii* showed the highest 18 individual and *Trimerisurus albolabris*, *Lycodon aulicus*, *Elaphe taeniura*, and *Calotes irawadi* were recorded with one individual. The highest 11 species and 16 individuals were observed in October and the lowest three species and four individuals were found in March and April.

In study site III, *Duttaphrynus melanostictus* showed the highest six individuals. *Boiga ochracea*, *Calotes irawadi and Calotes htunwini* revealed to be the lowest with one individual. The total number of seven species and eight individuals were noted in October and only one species and individual were found in February, March and April. Among them *Calotes htunwini* is endemic species observed only in site III.

In the present study of visual encountered survey method, the total numbers of species were 17 and total number of individuals are 144 in site I. In site II, total number of species were 20 and total number of individuals are 95. In site III, total number of species are 14 and total number of individuals are 39 (Table 1).

Pitfall method

Study site I, *Fejervarya limnochairs* and *limnonectes modestus* showed the three individuals and *Eutropis carinata* was recorded with only one individual. The total number of five species and five individuals were found in September and only one species and individual were recorded in July.

Study Site II, *Fejervarya greenii* was found with four individuals and *Duttaphrynus melanostictus*, *Fejervarya limnocharis* and *Varanus nebulosus* were observed with only one individual. The total numbers of species were highest in July, August and September. Four individuals were observed in October.

Study site III, all the reptiles recorded were found with only one individual.The highest total number of three species and three individuals were noted in August and the lowest only one species and individuals were recorded in July.

By pitfall method the number of species caught were eight and number of individuals were 17 in site I. In study site II, total number of species are seven and total number of individuals are 14. In site III, total number of species are six and total number of individual are six (Table 2).The number of species and individual recorded were more in visual encountered survey method than pitfall method. Based on the data of combined method, ten species of herptiles are considered as very common, six species are considered as common and six species are considered as uncommon (Table 3).

A.*Duttaphrynus melanostictus*

B.*Fejervarya greenii*

C.*Fejervarya limnocharis*

D.*limnonectes modestus*

E.*Kaloula puachra*

Plate 1 Recorded amphibian

A. *Blythia reticulata*

B. *Boiga ochracea*

C. *Elaphe taeniura*

D. *Lycodon aulicus*

E. *Xenochrophis piscator*

F. *Bungarus magnimaculatus*

G.*Trimeresurus albolabris*

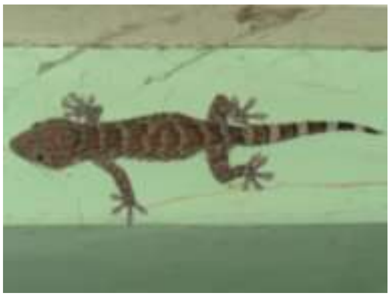
H. *Calotes htunwinii*

I. *Calotes irawadi*

J.*Calotes mystaceus*

K.*Leiolepis belliana*

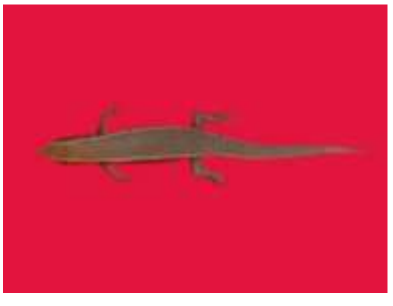
L. *Gehyra mutilata*



M.*Gekko gecko*

N. *Hemidactylus*

O. *Hemidactylus frenatus*



P.*Eutropis carinata*

Q. *Varanus nebulosus*

Plate 2 Recorded reptile species

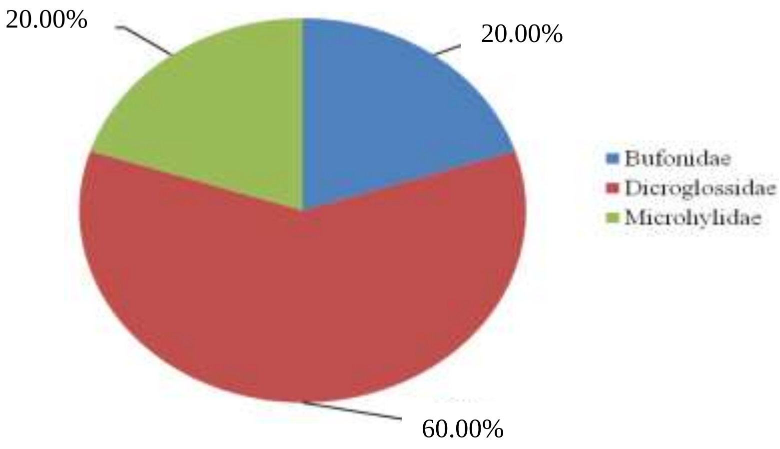


Figure 2 Percentage species composition of amphibians in different families

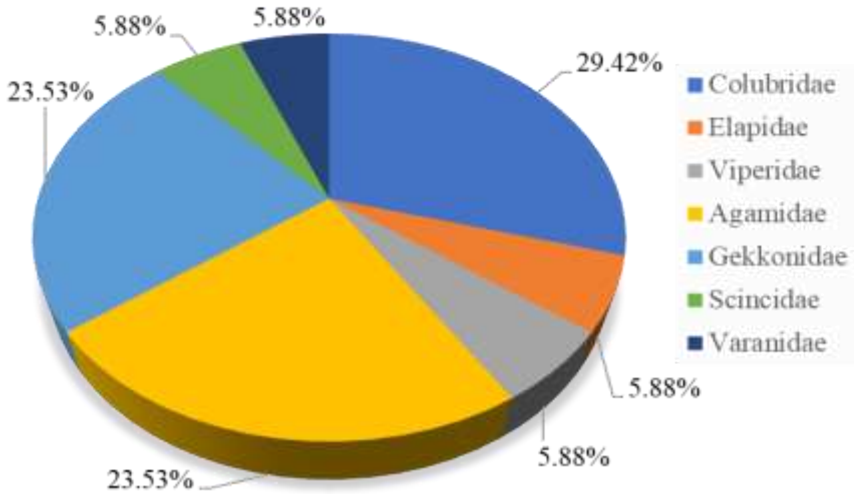


Figure 3 Percentage species composition of reptiles in different familie

Table 1 Species composition and abundance of herptiles in three study sites of study area (Visual encountered survey method)

Sr. No.	Class	Order	Family	Scientific name	Common name	Site I	Site II	Site III	Total
1	Amphibia	Anura	Bufonidae	1. *Duttaphrynus melanostiauts*	Commom toad	15	7	6	28
2			Discroglossidae	2. *Fejervary limnocharis*	Paddy frog	5	6	2	13
3				3. *Fejervary limnocharis*	Paddy frog	6	4	4	14
4				4. *Limnonectes modestus*	Grass frog	2	4	2	8
5			Microhylidae	5. *Kaloula pulchra*	Bull frog	6	6	3	15
6	Reptilia	Squamata	Elapidae	6. *Elaphe taeniura*	Beauty Ratsnake	5	1	-	6
7			Colubridae	7. *Boiga ochracea*	Tawny cat snake	-	2	1	3
8				8. *Blythia reticulata*	Iridescent snake	2	2	4	8

Sr. No.	Class	Order	Family	Scientific name	Common name	Site I	Site II	Site III	Total
9				9. *Lycodon aulicus*	Indian wolf snake	-	1	-	1
10				10. *Xenocrhophis piscator*	Chequered keel black	4	-	-	4
11				11. *Bungarus magnimaculatus*	Splendid krait	-	2	-	2
12			Viperidae	12. *Trimeresurus albolabris*	White-lipped Pit-viper	2	1	-	3
13			Agamidae	13. *Calotes htunwini*	Htunwin's forest lizard	-	-	1	1
14				14. *Calotes irawadi*	Ayeyarwady forest lizard	-	1	1	2
15				15. *Calotes mystaceus*	Blue forest lizard	4	9	5	18
16				16. *Leiolepis belliana*	Spotted ground lizard	4	3	2	9
17			Gekkonidae	17.*Gehyra mutilata*	Common four-clawed gecko	15	2	-	17
18				18. *Gekko gecko*	Tokay gecko	4	2	3	9
19				19. *Hemidactylus brookii*	Brooke’s House gecko	0	0	1	1
20				20. *Hemidactylus frenatus*	Asian House gecko	26	6	-	42
21			Scincidae	21. *Eutropis carinata*	Keeled Indian Mabuya	12	4	2	18
22			Varanidae	22.*Varanus nebulosus*	Southeast Asian Monitor lizard	6	4	3	13
Total number of individuals						144	95	39	278
Total number of species						17	20	14	22
Percent composition of species (%)						77.72	90.90	63.63	

Table 2 Abundance of herptiles in three study sites of study area (Pitfall method)

Sr. No.	Scientific name	Common name	Site I	Site II	Site III	Total
1.	*Duttaphrynus melanostiauts*	Common toad	2	1	1	4
2.	*Fejervarya greenii boulenger*	Paddy frog	2	4	1	7
3.	*Fejervary limnocharis*	Paddy frog	3	1	1	5
4.	*Limnonectes modestus*	Grass frog	3	2	1	6
5.	*Kaloula pulchra*	Bull frog	2	3	1	6
6.	*Leiolepis belliana*	Spotted Ground Lizard	2	2	1	5
7.	*Eutropis carinata*	Keeled Indian Mabuya	1	-	-	1
8.	*Varanus nebulosus*	Southeast Asian Monitor lizard	2	1	-	3
Total number of Individuals			17	14	6	37
Total number of species			8	7	6	8
Percent composition of species (%)			100	87.5	75	

Table 3 Relative abundance and status of herpetofauna by two methods in study area

Sr. No.	Scientific name	Common name	Individual by Visual encountered survey method	Individual by pitfall method	Total individual	Relative abundance	Status
1	*Duttaphrynus melanostiauts*	Common toad	28	4	32	0.1015	vC
2	*Fejervarya greenii*	Paddy frog	13	7	20	0.0634	vC
3	*Fejervary limnocharis*	Paddy frog	14	5	19	0.0603	vC
4	*Limnonectes modestus*	Grass frog	8	6	14	0.0444	C
5	*Kaloula pulchra*	Bull frog	15	6	21	0.0666	vC
6	*Blythia reticulata*	Iridescent snake	8	-	8	0.0253	C
7	*Boiga ochracea*	Tawny cat snake	3	-	3	0.0095	uC
8	*Elaphe taeniura*	Beauty Ratsnake	6	-	6	0.0190	C
9	*Lycodon aulicus*	Indian wolf snake	1	-	1	0.0031	uC
10	*Xenocrhophis piscator*	Chequered keel black	4	-	4	0.0126	C
11	*Bungarus magnimaculatus*	Splendid krait	2	-	2	0.0063	uC
12	*Trimeresurus albolabris*	White-lipped Pit-viper	3	-	3	0.0095	uC
13	*Calotes htunwini*	Htunwin's forest lizard	1	-	1	0.0031	uC
14	*Calotes irawadi*	Ayeyarwady forest lizard	2	-	2	0.0063	uC
15	*Calotes mystaceus*	Blue forest lizard	18	-	18	0.0571	vC
16	*Leiolepis belliana*	Spotted ground lizard	9	5	14	0.0444	C
17	*Gehyra mutilata*	Common four-clawed gecko	18	-	18	0.0571	vC
18	*Gekko gecko*	Tokay gecko	9	-	9	0.0285	C
19	*Hemidactylus brookii*	Brooke's House gecko	44	-	44	0.1396	vC
20	*Hemidactylus frenatus*	Asian House gecko	42	-	42	0.1333	vC
21	*Eutropis carinata*	Keeled Indian Mabuya	18	1	19	0.0603	vC
22	*Varanus nebulosus*	Southeast Asian Monitor lizard	13	3	16	0.0507	vC
	Total number of individual		278	37	315		

vC= very Commom, C=common, uC-uncommon

Discussion

A total of 22 species of herptiles belonging to 10 families and distributed under two orders were recorded in Tawyagyi Protected Area during study period which commenced from July 2018 till April 2019.

In the present study, the number of species was highest along the river bank in all the three study sites. This may be due to be presence of shrub, thorn, scrub, and tall and medium tree microhabitats allowing adequate shelter. The species that were predominant in site I were *Duttaphrynus melanostictus*, *Hemidactylus frenatus* and *Gehyra mutilata*, so that it is presumed that these species prefer the habitat type of this site.

During the study period some reptiles were observed in all kinds of habitats. However, *Xenochrophis piscator* was observed only in along the river bank.*Calotes htunwinii* was observed in mixed deciduous forest area only and it is endemic species of Myanmar.

Zug *et al.* (2003) stated that 87 species of frogs and toads were recorded in Myanmar. In the present study only five species of frogs and toads were recorded. *Fejervarya greenii* recorded was the newly recorded species in Myanmar.

Hemidactylus Gray, 1845 is one of the most species-rich genera of the family Gekkonidae and comprises about 80 recorded species. *Gekko gecko* is one of the most widely distributed reptile in the world. The results in this study indicated that *Hemidactylus brookii* and *Hemidactylus frenatus* were mostly found in all study sites.

The genus *Gehyra* Gray, 1834 is a speciose taxon of gekkonid lizard found in Australia, Madagascar, Asia, the Indo- Malayan Archipelago, New Guinea and Pacific Island. *Gehyra* comprises about 33 species (Uetz *et al.,* 2005). In the present study area, *Gehyra mutilata* was commonly found among the trees and old buildings existed along the Ayeyarwady river bank.

Six species of the genus *Hemidactylus* under family Gekkonidae are reported currently from Myanmar. It is the most geographically widespread and one of the most speciose (Kluge 2001).

Modern herpetologists tend to classify the world of lizard into 23 families. Of these, 11 families are represented in the Indian and Indochina regions. According to Smith (1935) only seven of the modern lizard families are found within the boundaries of Myanmar.

Under the family of Agamidae, Smith (1935) recorded five Myanmar genera with (16) local species. In the present work, four species of agamids under two genera were recorded, *Calotes mystaceus, C. irawadi, C. htunwinii* and *Leiolepis belliana.*

Smith (1935) also recorded six Myanmar genera with 17 local species under the Family Scincidae. In the present work only one of Smith's species *Eutropis carinata* could be recorded.

The Family Varanidae is represented by a single living genus. Six species have been known to occur in the Indo-Chinese subregion of which Smith (1935) has recorded four species from Myanmar. In the present work only one species *Varanus nebulosus* could only be collected from Tawyagyi Protected Area.

Smith (1943) recorded a total of 135 species of snakes within the boundaries of Myanmar, 89 species belonging to the family Colubridae and the remaining 46 species are non-colubrid snake. In the present study 5 species of Colubrid snake and only one species of Viperid and Elaphid are recorded and identified.

Conclusion

Tawyagyi Protected Area, composed a variety of microhabitats for herptiles. Structure and composition of various sized trees, shrubs, thorns, scrubs and scattered tree influence on distribution of herptiles species. Therefore, awareness, creation, conservation and rehabitation are essential for maintaining the habitats and herpetofauna of these study area.

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ANALYSIS ON THE CONTAMINATION OF TOXIC METALS IN THE MUSCLE OF SELECTED FISH SPECIES, WATER AND SEDIMENT FROM KAUNG-HMU-DAW IN (LAKE), SAGAING TOWNSHIP

Lay Lay Nwe[1], Myin Zu Minn [2], Thant Zin [3]

Abstract

A total of five fish species; two herbivores (*Labeo rohita* and *Oreochromis* sp.), two carnivores (*Glossogobius giuris* and *Channa striata*) and one omnivore (*Trichopodus pectoralis*) from Kaung-hnu-daw In (Lake), Sagaing Township, Sagaing Region was selected to assess the toxic metals such as lead (Pb), cadmium (Cd) and arsenic (As) during the study period from February 2019 to January 2020. Samples of fish muscles, water and sediment were collected on seasonal basis and analysed by Flame Atomic Absorption Spectrometer (FAAS) in Universities' Research Center (URC) at University of Yangon. The concentration levels of studied fish species except *T. pectoralis* was observed to be lower than that of FAO/WHO (2001) maximum permissible limits. Arsenic (As) concentration of *T. pectoralis* was found to be higher than the maximum permissible limit in cold season. Moreover, Cd, Pb and As concentration levels of water and sediment were detected far exceed than the FAO/WHO (2001) permissible limit.

Keywords: toxic metal, herbivore, carnivore, omnivore, permissible limit

Introduction

Nearly one billion people, most of them in developing countries, currently depend on fish for their primary source of protein (Toth *et al*., 2012). Fish is an important part of the human diet because of its high nutritional quality (Sioen *et al.*, 2007).

At present, the pollution has become a serious threat, and has brought hazards to the growing population as well as the environment. The speedy urbanization and industrialization has led to increase disposal of pollutants like heavy metals, radio nuclides, and various types of organic and inorganic substances into the environment (Praveena *et al.*, 2013).

Heavy metals refer to those metallic element which has density of about 5 gcm^3. Heavy metals such as copper, iron, chromium, zinc and nickel are essential metals since their play an important role in biological role in biological systems, whereas cadmium, lead, mercury and arsenic are non-essential metals, as they are toxic, even in trace amounts (Fernandes *et al.*, 2008). For the normal metabolism of the fish, the essential metals must be

taken up from water, food or sediment (Canh and Ath, 2003).

Toxic metals such as lead, mercury, cadmium and arsenic are biological contaminant of special concern due to a wide distribution in the environment and likely adverse effects for human health. The accumulation of toxic metals can have middle term and long term health risks and strict periodic surveillance of these contaminants is therefore advisable (Narvaes, 2002).

Water and sediment of the polluted sites contains various levels of heavy metals. Measurement of heavy metals in both water and sediment samples can show the condition of the ecosystem regard to heavy metal pollution. On the other hand, aquatic organisms are the target of heavy metal intoxication, which accumulate large volume of heavy metals in their tissues. Therefore, determination of heavy metals in the aquatic organisms' tissue may be valuable and informative (Saghali *et al.*, 2012).

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[1] Lecturer, Department of Zoology, Kyaukse University
[2] Dr, Pro-rector, University of Mandalay
[3] Dr, Professor (Head), Department of Zoology, University of Mandalay

Kaung-hmu-daw In (Lake) is a seasonal lake formed by the flooding of Ayeyawady River during the rainy season of each year and is bound on the east by Phu-kan Lake, on the south by Myay-thin Lake, on the west by Maung-ma-kan Lake and on the north by Kaung-hmu-daw pagoda. Since the Ayeyawady River and Kaung-hmu-daw Lake are indirectly connected, this lake receives not only water but also most of the fishes from the river; this inturn serve these fish for local people.

The present study was conducted with the following objectives:

- to measure the levels of heavy metals such as lead (Pb), cadmium (Cd) and arsenic (As) in the muscle tissues of five fish species, water and sediment of Kaung-hmu-daw In (Lake)
- to assess whether levels of metals recorded in water conforms to the acceptable limits of heavy metals by FAO/WHO (2001) standard

Materials and Methods

Study area

Kaung-hmu-daw In (Lake) situated at 21˚ 55' 50" and 21˚ 54' 33" North latitudes and 95˚ 55' 12" and 95˚ 56' 45" East longitudes in Ayeyawady River in Sagaing Township, Sagaing Region, Central Myanmar were chosen as the study area to determine toxic metal concentration in some water and sediment and muscle of selected fish species (Fig. 1).

Study period

The present study was carried out from February 2019 to January 2020.

Collection of specimens

Five commercially important fish species were selected for determination of seasonal toxic metal concentrations in their muscles (Plate 1). Samples of water and sediment were also collected seasonally from the study area for analysis of toxic metals.

Fish sample preparation

Selected fish specimens were washed by tap water until the contamination on the body surface was runoff. Sample for each treatment consisted of 5 to 20 number of individuals fish from the study site. Total length (cm) and body weight (g) of specimens were measured. After that, the specimens were scaled by clean stainless steel knife. Only the edible parts (the muscles) of the fish species were used and samples were treated or prepared as though they were for human consumption. The muscle tissue was removed and weighed. Muscle samples were put in petri dishes to dry at 90 ˚C until reaching a constant weight in an oven and dried samples were weight and stored in airtight containers.

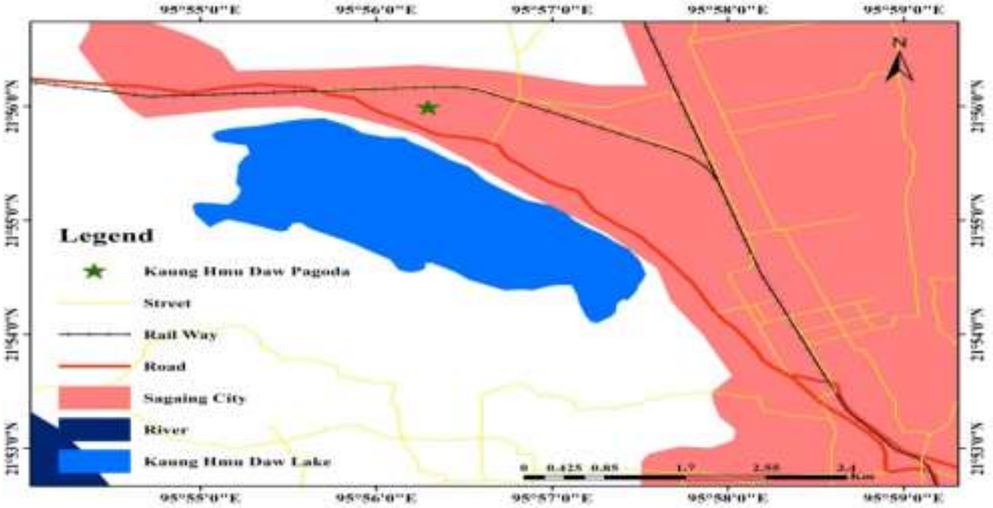
Water and sediment sampling

Water sample was seasonally collected with plastic bottle from the study site at depth of at least 35 cm depth. Metals analysis of water sample was filtered through a 0.45 micron Whatman filter. The samples were analyzed by FAAS.

The sediment sample was sun dried, grounded and sieved with 200 mm sieve to obtain a fine powder. The digested sediment sample was analyzed by FAAS.

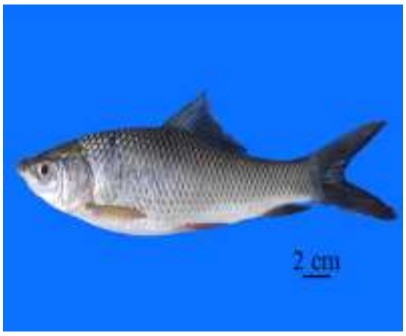
Analysis of metals

The concentration of metals in the fish samples, water and sediment were analyzed tri-replicates by Flame Atomic Absorption Spectrometer (FAAS) (Perkin Elmer AAanalyst 800 and Winlab-32 software) in Universities' Research Center (URC), Yangon. Seasonal variations of test results were compared with FAO/WHO (2001) maximum permissible limits.



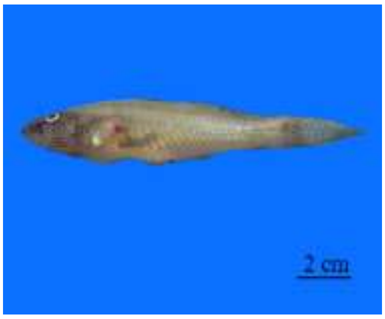
Source: ArcGIS Software

Figure 1 Location map of the study area, Kaung-hmu-daw In (Lake)

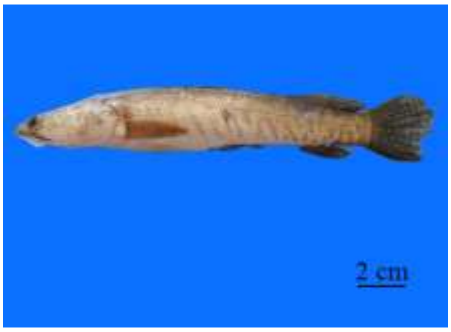


A. *Labeo rohita*

B. *Oreochromis* sp.



C. *Glossogobius giuris*



D. *Channa striata*

E. *Trichopodus pectoralis*

Plate 1 Selected study fish species

A. Muscle of fish sample

B. Dried fish muscle tissue

C. Pounder of the fish

D. Fish, water and sediment samples

E. Flame Atomic Absorption

Plate 2 Sample and apparatus used in sample analysis

Results

A total of five fish species from Kaung-hmu-daw In (Lake), Sagaing Township, Sagaing Region were selected to assess toxic metal contamination in the muscles. These selected fish species included two species of herbivores (*Labeo rohita*, *Oreochromis* sp.), two species of carnivores (*Glossogobius giuris*, *Channa striata*) and one species of omnivore (*Trichopodus pectoralis*) according to FishBase (2011). The selected fish species included three benthopelagic dwellers (*L. rohita, O.* sp. and *T. pectoralis*) and two bottom dwellers (*G. giuris* and *C. striata*) (Table 1).

In the present study, a total of 114 individuals comprising (30 individuals of two herbivorous fish species, 69 individuals of two carnivorous fish species and 15 individuals of one omnivorous fish species) were selected to test toxic metal contaminations in muscle tissue. The levels of metal content such as lead (Pb), cadmium (Cd) and arsenic (As): non-essential metals (toxic metals) were assessed in the muscle of the five species selected *(L. rohita, O. sp., G. giuris, T. pectoralis* and *C. striata*).

Lead (Pb) concentration levels of all studied species except *L. rohita* were higher in cold season than in other two seasons and Pb concentration 0.93 mg/kg of *L. rohita* in hot season. The levels of Pb in all studied fish species were below the maximum permissible limits (Pb in 1 mg/kg) established by FAO/WHO (2001) limits. Cadmium (Cd) concentration levels of all studied fish species were not detected in all seasons. In hot season, arsenic (As) concentration of 0.04 mg/kg recorded in *L. rohita*, 0.01 mg/kg in *G. giuris* and not detected in remaining three species. In rainy season, (As) was not detected in all studied fish species. In cold season, (As) concentrations of *T. pectoralis* was higher than FAO/WHO (2001) maximum permissible limits and not detected in other species (Table 2 and Fig. 2).

Lead, cadmium and arsenic concentrations of water environ was not detected in hot season. Lead and arsenic concentrations of water environ was found to be higher than those of FAO/WHO (2001) maximum permissible limit during rainy and cold seasons. Lead, and arsenic concentrations of sediment environ was observed to be higher than those of FAO/WHO (2001) maximum permissible limit in all seasons. Cadmium concentrations of sediment environ was observed to be higher than those of FAO/WHO (2001) maximum permissible limit in hot season but not detected in rainy and cold seasons (Table 4 and Fig. 4).

Table 1 Feeding types and habitats of studied fish species from Kaung-hmu-daw In (Lake)

Sr. No	Species studied	Feeding type	Habitats
1	*Labeo rohita*	Herbivore	Benthopelagic feeder
2	*Oreochromis* sp.	Herbivore	Benthopelagic feeder
3	*Glossogobius giuris*	Carnivore	Bottom feeder
4	*Channa striata*	Carnivore	Bottom feeder
5	*Trichopodus pectoralis*	Omnivore	Benthopelagic feeder

Table 2 Toxic metal contents (mg/kg) in the muscles of study fish species in hot, rainy and cold seasons compared with FAO/WHO (2001) limits

Species	Lead			Cadmium			Arsenic		
	Hot	Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold
Labeo rohita	0.93	0.643	0.659	ND	ND	ND	0.036	ND	ND
Oreochromis sp.	ND	0.618	0.65	ND	ND	ND	ND	ND	ND
Glossogobius giuris	ND	0.607	0.657	ND	ND	ND	0.005	ND	ND
Channa striata	ND	0.601	0.639	ND	ND	ND	ND	ND	ND
Trichopodus pectoralis	0.1	0.615	0.637	ND	ND	ND	ND	ND	1.077
Mean	0.52	0.617	0.648	-	-	-	0.021	-	1.077
SD	0.6	0.016	0.01	-	-	-	0.022	-	-
FAO/WHO (2001) limits	1			0.2			0.26		

Table 3 Toxic metal contents in muscle tissues of studied fish species of different feedingtypes in hot, rainy and cold seasons

Feeding types	Species	Lead			Cadmium			Arsenic		
		Hot	Rainy	Cold	Hot	Rainy	Cold	Hot	Rainy	Cold
herbivore	*Labeo rohita*	0.93	0.643	0.659	ND	ND	ND	0.04	ND	ND
	Oreochromis sp.	ND	0.618	0.65	ND	ND	ND	ND	ND	ND
	Mean	0.93	0.631	0.655	ND	ND	ND	0.04	ND	ND
	SD	-	0.018	0.006	-	-	-	-	-	ND
carnivore	*Glossogobius giuris*	ND	0.607	0.657	ND	ND	ND	0.01	ND	ND
	Channa striata	ND	0.601	0.639	ND	ND	ND	ND	ND	ND
	Mean	ND	0.604	0.648	-	-	-	0.01	-	-
	SD	ND	0.004	0.013	-	-	-	-	-	-
omnivore	*Trichopodus pectoralis*	0.1	0.615	0.637	ND	ND	ND	ND	ND	1.077
	Mean	0.1	0.615	0.637	-	-	-	-	-	1.077

Table 4 Analysis of toxic metals in water and sediment of study area with the maximum permissible limits proposed by FAO/WHO (2001)

Sample	Toxic metalas	Hot season	Rainy season	Cold season	FAO/WHO (2001) guideline
Water	Lead	ND	0.633	0.615	0.5 mg/L
	Cadmium	ND	ND	ND	0.01 mg/L
	Arsenic	ND	6.043	3.694	0.01 mg/L
Sediment	Lead	30.75	0.768	0.682	0.5 mg/kg
	Cadmium	1.22	ND	ND	0.01 mg/kg
	Arsenic	0.089	10.12	16.8	0.01mg/kg

*ND = not detected

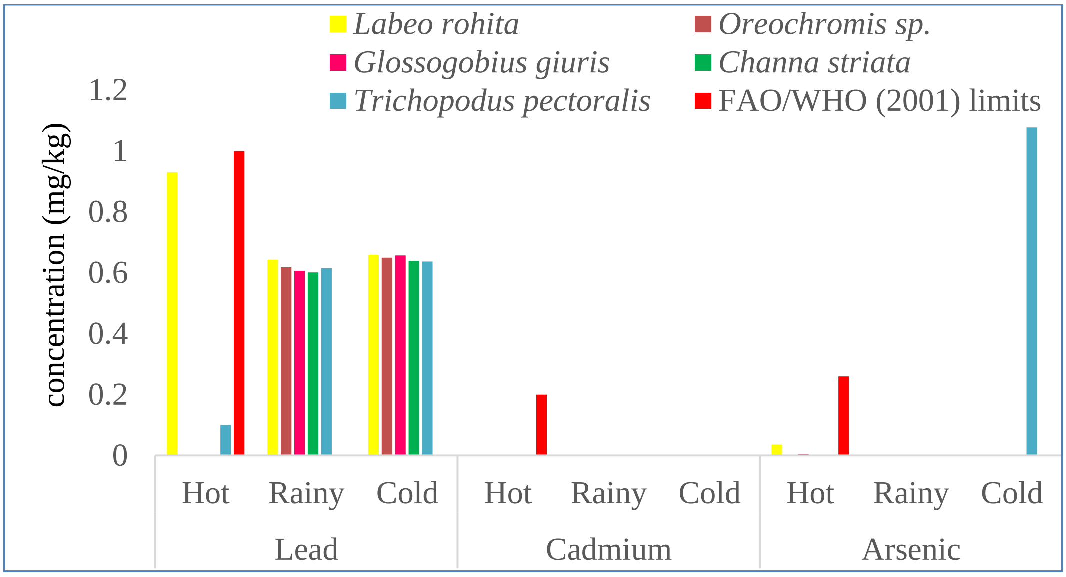


Figure 2 Comparison with FAO/WHO (2001) limits for toxic metal concentrations in studied fish muscles during hot, rainy and cold seasons

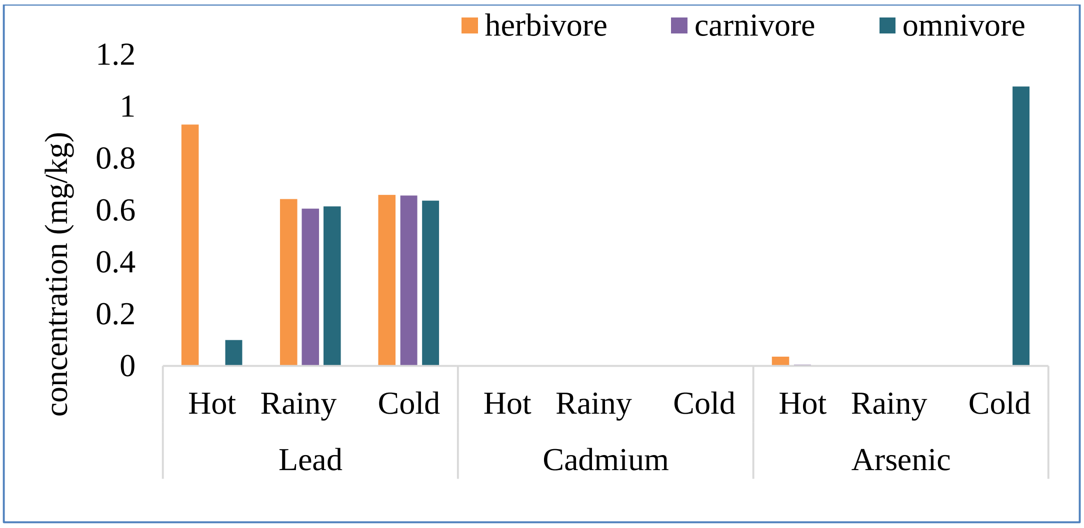


Figure 3 Toxic metal concentrations in studied fish species of different feeding types

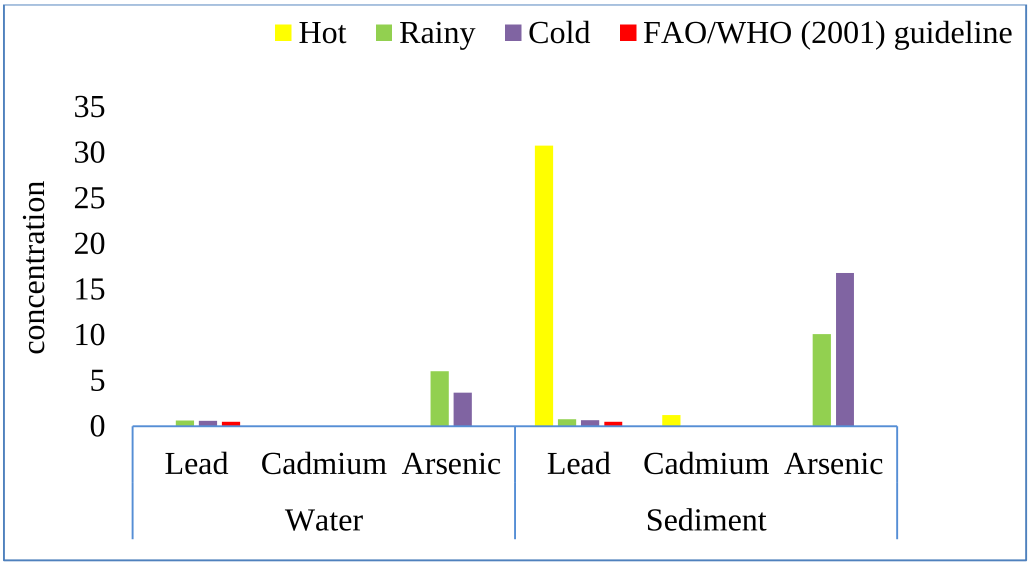


Figure 4 Toxic metal concentrations in water and sediment of study area compared with FAO/WHO (2001) limits during hot, rainy and cold seasons

Discussion

A total of selected five fish species; two herbivores, two carnivores and one omnivore fishes from Kaung-hmu-daw In (Lake), Sagaing Township, Sagaing Region were analysed for the presence of lead, cadmium and arsenic in the muscles during hot, rainy and cold seasons between February 2019 to January 2020.

Lead is toxic heavy metals. Lead can cause neurological and behavioral disorders, especially in children, anemia, impaired kidney and testicular function (Brazilay *et al*., 1999). In the present study, concentration levels of Pb were found below the FAO/WHO (2001) maximum permissible limits (1.0 mg/kg) in all seasons.

Nyein Chan San (2015) studied *Channa striata* from Chindwin River, Monywa Segment. He stated that Pb level far above the FAO/WHO permissible limits. The possible explanation for this could be difference in sizes and ages of fish species, sampling period and area conditions.

Cadmium is a non-essential element known to have a high toxic potential. Cadmium toxicity in human may affect some organs such as kidney, lung, bones, brain as well as central nervous system (Castro-Gonzalez and Mendez-Armenta, 2008). In present study, Cd was not detected in studied fish species Thus, consumption of fish from Kaung-hmu-daw In (Lake) could not pose any Cd induced health hazard.

Khin Myint Mar (2011) studied 25 fish species including *Oreochromis* sp. and *Channa striata* from Gaw Wein landing site of Ayeyawady River, Mandalay segment to determine for lead ,cadmium and mercury levels. She described that Pb and Cd concentration levels do not constitute a risk to human health. The results of the present study agreed with Khin Myint Mar.

Pandey *et al*. (2014) described that arsenic (As) can cause the cancer of skin, lung, liver, lymph, nasal passage, kidney, bladder, prostate and haematopoietic systems of humans. In the present study, arsenic (As) concentration of all species studied except one species, *T. pectoralis* were found below the maximum permissible limit. From the finding of this study, concentration levels of arsenic (As) in *T. pectoralis* was found to be over FAO/WHO (2001) permissible limit in cold season. The presence of arsenic (As) in fish species depends on the feeding types, habitat, age and development of fishes, and other physiological factors.

Sawyer *et al*. (2003) stated that arsenic is quite widely distributed in natural water and is often associated with geological sources, but in some locations anthropogenic inputs, such as the use of arsenical insecticides and the combustion of fossil fuels, can be extremely important additional sources. Most of the arsenic (As) compound is used in manufacture of agricultural products such as insecticides, herbicides, fungicides and algaecides.

Hakason (1984) described that the concentrations of toxic heavy metals (Pb, Hg, Cd) in fish is affected by many biological factors such as species, sex, age, feeding type and environmental factors, such as the season of the year, pH value of water, temperature, dissolved oxygen and salinity.

From the finding of this study, Pb and As concentration levels of water and sediment were detected far exceed than the FAO/WHO (2001) maximum permissible limits (0.5 mg/kg and 0.01 mg/kg) in all seasons. Cadmium concentration level of sediment was observed to be higher than FAO/WHO (2001) maximum permissible limit (0.01 mg/kg) in hot season. It may be due to the use of various agricultural fertilizers, insecticides and pesticides from the surrounding crop field near the area, farm animal wastes and industrial activities.

Therefore, the present results indicated that all the metal levels except arsenic (As) in all fish species studied were detected to be lower than the FAO/WHO (2001) maximum permissible limits. Especially, *T. pectoralis* of arsenic concentration was observed to be higher than those of FAO/WHO (2001) permissible limits. Therefore, Kaung-hmu-daw In (Lake) fishes except *T. pectoralis* are generally safe for the human consumption with respects to the levels of Pb, Cd and As do not constitute a risk for human health. However, water and sediment of study area were contaminated with toxic metal levels above FAO/WHO (2001) permissible limits. Therefore, these results further corroborate the idea that there is a potential for lead (Pb) and arsenic (As) pollution to occur because of the presence of paddy cultivation in Kaung-hmu-daw In (Lake) environ. Therefore, it was concluded that the fish are burdened with arsenic (As) yet, so a danger should be considered due to the agricultural and industrial development in this region threatening the aquatic environs with the effluent and pesticide usages were considerably polluted. Hence, Kaung-hmu-daw In (Lake) should be monitored periodically to assess the level of pollution and maintain the friendly nature of the In.

Conclusion

The contamination of toxic metals in muscle tissues of five studied fish species, water and sediment of Kaung-hmu-daw In (Lake), Sagaing Township were analysed. Except T. pectoralis, toxic metal levels of all studied fish muscle were detected within FAO/WHO (2001) maximum permissible limits. Although water and sediment of study area were detected with some toxic metals levels above FAO/WHO (2001) limits. Thus it was concluded that the water and sediment from study area were generally nor secure from toxic metal contamination. Therefore, the present study will be able to give valuable information.

Acknowledgements

We are very thankful to Dr. Aung Khin Myint, Rector and Dr. Su Su Win, Pro-rector, Kyaukse University, Dr. Pa Pa Moe, Professor (Head) and Dr. Tin Moe Win, Professor, Department of Zoology, Kyaukse University for their interest and encouragement of our research work.

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OCCURRENCE AND SPECIES COMPOSITION OF MACROINVERTEBRATES FAUNA FROM UPSTREAM AND DOWNSTREAM OF MIN YE DAM, ZAWGI RIVER SEGMENT, KYAUKSE TOWNSHIP, MANDALAY REGION

Mie Mie Myint [1], Myo Thandar Thone[2] and Thant Zin [3]

Abstract

A total of 24 macroinvertebrate species, 24 genera belonging to 20 families, ten orders and three phyla were also collected and identified in four study sites of upstream and downstream of Min Ye Dam, Zawgi River Segment during the study period from August to December 2019. During the study period, the highest number of species composition was observed in Hemiptera (25.00%), followed by Mesogastropoda (16.66%), Coleoptera (16.66%), Odonata (12.50%), Araneae (8.33%), Haplotaxida, Veneroida, Decapoda, Ephemeroptera and Diptera (4.17%) respectively. In the present study, macroinvertebrates fauna was the highest at upstream of dam (site I and II) and the lowest at downstream of dam (site III and IV). In upstream, tall grasses predominated and the bed composed of silt. The downstream was found washing and bathing, activities of human and sand and gravels occupied the bed of stream. The macroinvertebrates fauna were higher in upstream than downstream of Min Ye Dam from Zawgi River, and is allocated to the habitat compatibility.

Keywords: Hemiptera, Mesogastropoda, Coleoptera, Odonata, Araneae

Introduction

Aquatic macroinvertebrates are organisms that live in the water (aquatic), are visible with the naked eye without the use of a microscope (macro), and lack an internal skeleton (invertebrate). Examples of aquatic macroinvertebrates include insects, worms, snails, mollusks and crustacean. Aquatic macroinvertebrates are typically found living under rocks or logs or living in congregated leaf packs. Aquatic macroinvertebrates are an integral part of the food chain. Many macroinvertebrates feed on organic material such as leaves and algae. Other higher order organisms such as birds, fish and larger insects then feed on aquatic macroinvertebrates. Macroinvertebrates are widely recognized as the best biological indicators for stream health. Because of their short life cycles (generally one year of which most is spent in the water) and relative immobility. Aquatic macroinvertebrates are good indicators of stream health. Their survival is directly linked to their habitat. Aquatic macroinvertebrates cannot quickly move to another stream if the one they are currently living in becomes polluted (Carmen *et al*., 2015).

Macroinvertebrates that live in rivers include worms, snails, beetles, dragonflies, mayflies, stoneflies and yabbies. Pollution and other changes caused by human activities in and around a river will determine what types of macroinvertebrates live there. For this reason, river macroinvertebrates are widely used to indicate the biological health of a river. Healthy rivers are typically unpolluted and have many different macroinvertebrates habitats, so in a healthy river one would expect to find many different types of macroinvertebrates including species that are sensitive to water pollution. Unhealthy rivers may be highly polluted or may have lost most types of macroinvertebrate habitats. Such river may have only few types of hardy, pollution tolerant macroinvertebrate species (New South Wales (NSW) government, 2000).

Although, Zawgi River is permanent with the water body throughout the year and the river is inhabited with form and different kinds of macroinvertebrates, no information was available

[1] Lecturer, Department of Zoology, Kyaukse University
[2] Dr, Lecturer, Department of Zoology, University of Mandalay
[3] Dr, Professor (Head), Department of Zoology, University of Mandalay

about the occurrence of macroinvertebrates in this area. Therefore, the present study was carried out with the following objectives:

- to identify and record the benthic macroinvertebrates fauna in the allocated segment of Zawgi River
- to determine the species composition and occurrence based on the monthly collected species

Materials and Methods

Study area and sites

The study area is the upstream and downstream of Min Ye Dam, Zawgi River Segment, Kyaukse Township, Mandalay Region. It lies at 21˚ 35′ 49.04″ N and 96˚ 08′ 38.08″ E. It is 1.8 km long.

Four study sites were selected in Zawgi River Segment, two in upstream and two in downstream of Min Ye Dam. Site I is located at upstream of Min Ye Dam of Zawgi River (21˚ 35′ 47.0″ N and 96˚ 08′ 24.0″ E). Site II is located at upstream of Min Ye Dam of Zawgi River (21˚ 35′ 56.0″ N and 96˚ 08′ 24.0″ E). Site III is located at downstream of Min Ye Dam of Zawgi River (21˚ 35′ 56.0″ N and 96˚ 08′ 24.0″ E). Site IV is located at downstream of Min Ye Dam of Zawgi River (21˚35′ 56 ″ N and 96˚ 08′ 15.0″ E).

Study period

The present investigation for macroinvertebrate was carried out from August to December 2019.

Specimen collection and preservation

Macroinvertebrates were collected once per month and between 7:00 am and 8:00 am in four study sites. They were collected using nylon dip net (or) pond net for 15 minutes at each site. They were preserved in 70% alcohol solution for further study. They were sorted, counted, identified under a stereo microscope and photographic records were made in the laboratory of Zoology Department at University of Mandalay.

Identification of the specimens

Identification of the collected macroinvertebrate species were made according to Edmondson (1959), Koh (1989), Gerber and Gabriel (2002), Perez *et al*. (2004), Wade *et al.* (2004), Mekong River Commission (2006), Wegner (2011) and Umar *et al.* (2013). The classification of macroinvertebrate was followed after Mekong River Commission (2006).

Sources: Google Earth (2019)

Plate 1 Map of study area showing the study sites

Results

A total of 24 species of macroinvertebrates fauna belonging to three phyla, six classes, ten orders and 20 families were recorded in study areas (Table 1).

Species occurrence and composition of recorded macroinvertebrates

A total of 24 macroinvertebrates species, 24 genera belonging to 20 families and ten orders were collected during the study period. The ten orders are Haplotaxida, Mesogastropoda, Veneroida, Araneae, Decapoda, Ephemeroptera, Odonata, Hemiptera, Coleoptera and Diptera (Plate 2). Among these orders, Haplotaxida, Veneroida, Decapoda, Ephemeroptera and Diptera were represented by a single species, genus and family each. Under Araneae two species, two genera and two families were recorded and three species, three genera and three families of Odonata were found. Mesogastropoda was found four species, four genera and two families. Coleoptera was found four species, four genera and four families. Hemiptera was found six species, six genera and four families.

In site I, the highest number of species was found in September (seven species) and the lowest in October (two species). In site II, the highest number of species was found in October (nine species), followed by August and September (eight species) and the lowest in December (four species). In site III, the highest number of species was found in September, October and December (five species) and the lowest in November (four species). In site IV, the highest number of species was found in December (eight species) and the lowest in November (one specie). The total numbers of macroinvertebrate species were different at four study sites. The highest number of species in upstream of dam (site I and II) and the lowest number of species in downstream of dam (site III and IV) were recorded. (Table 2, Fig. 1)

Among the ten orders, the percentage species composition of recorded macroinvertebrate fauna was found to be highest (25.00%) under the order Hemiptera, followed by (16.66%) in Mesogastropoda and Coleoptera, (12.50%) in Odonata, (8.33%) in Araneae, and Haplotaxida, Veneroida, Decapoda, Ephemeroptera and Diptera in each of (4.17%) respectively. (Table 3, Fig. 2)

Table 1 List of macroinvertebrate species recorded in the study area (August to December, 2019)

Phylum	Class	Order	Family	Genus	Species	Common name
Annelida	Clitellata	Opisthopora	Megascolecidae	*Pheretima*	1. *Pheretima posthuma*	Earthworm
Mollusca	Gastropoda	Mesogastropoda	Ampullariidae	*Pila*	2. *Pila globosa*	Apple snail
			Thiaridae	*Thiara*	3. *Thiara scabra*	Pagoda snail
				Tarebia	4. *Tarebia granifera*	Quilted melania snail
				Melanoides	5. *Melanoides tuberculata*	Red-rimmed melania snail
	Bivalvia	Veneroida	Corbiculidae	*Corbicula*	6. *Corbicula fluminea*	Asian clam
Arthropoda	Arachnida	Araneae	Salticidae	*Phidippus*	7. *Phidippus audax*	Jumping spider
			Tetragnathidae	*Tetragnatha*	8. *Tetragnatha montana*	Shadow stretch-spider
	Crustacea	Decapoda	Palaemonidae	*Macrobrachium*	9. *Macrobrachium lamarrei*	Grass shrimp
	Insecta	Ephemeroptera	Heptageniidae	*Thalerosphyrus*	10. *Thalerosphyrus* sp.	Flat-headed mayfly
		Odonata	Coenagrionidae	*Coenagrion*	11. *Coenagrion puella*	Damselfly larvae
			Gomphidae	*Gomphus*	12. *Gomphus adelphus*	Dragonfly larvae
			Libellulidae	*Pachydiplax*	13. *Pachydiplax* sp.	Dragonfly or blue dasher
		Hemiptera	Nepidae	*Ranatra*	14. *Ranatra linearis*	Water scorpions
			Notonectidae	*Notonecta*	15. *Notonecta* sp.	Back swimmer
			Pleidae	*Paraplea*	16. *Paraplea* sp.	Pygmy backswimmer
			Gerridae	*Gerris*	17. *Gerris lacustris*	Water strider
				Ventidius	18. *Ventidius distanti*	Water strider
				Ptilomera	19. *Ptilomera* sp.	Water strider
		Coleoptera	Dytiscidae	*Eretes*	20. *Eretes sticticus*	Predaceous diving beetle
			Noteridae	*Hydrocanthus*	21. *Hydrocanthus* sp.	Burrowing water beetle
			Curculionidae	*Listronotus*	22. *Listronotus* sp.	Under water weevil
			Psephenidae	*Pseuphenus*	23. *Pseuphenus* sp.	Water-penny beetle
		Diptera	Culicidae	Unidentified pupae	24. Unidentified pupae	Mosquito

Table 2 Monthly recorded macroinvertebrate species in four study sites

Sr. No	Species	Site I					Site II					Site III					Site IV				
		Aug	Sep	Oct	Nov	Dec	Aug	Sep	Oct	Nov	Dec	Aug	Sep	Oct	Nov	Dec	Aug	Sep	Oct	Nov	Dec
1	*Pheretima posthuma*	+	+	–	+	–	–	+	+	+	+	–	–	–	–	–	–	–	–	–	–
2	*Pila globosa*	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–
3	*Thiara scabra*	–	+	–	–	+	+	+	–	–	+	–	–	–	–	+	–	–	–	–	+
4	*Tarebia granifera*	–	–	–	–	–	+	–	–	–	–	–	–	–	–	+	–	–	–	–	+
5	*Melanoides tuberculata*	+	+	–	+	+	+	+	–	+	+	–	–	–	+	+	–	+	–	–	+
6	*Corbicula flumineia*	–	–	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	+
7	*Phidippus audax*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
8	*Tetragnatha montana*	–	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	–
9	*Macrobrachium lamarrei*	+	+	–	–	+	+	+	–	–	–	–	+	+	+	+	–	+	–	–	+
10	*Thalerosphyrus* sp.	–	+	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	–
11	*Coenagrion puella*	–	–	–	–	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	–
12	*Gomphus adelphus*	+	–	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–	–
13	*Pachydiplax* sp.	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
14	*Ranatra linearis*	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–
15	*Notonecta* sp.	–	–	–	–	–	–	–	–	–	–	–	+	+	+	–	+	+	–	–	+
16	*Paraplea* sp.	–	–	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
17	*Gerris lacustris*	–	–	–	–	+	–	–	–	–	–	–	+	–	+	–	+	+	–	–	–
18	*Ventidius distanti*	–	+	+	–	–	+	+	–	–	–	–	–	–	–	–	+	–	+	–	+
19	*Ptilomera* sp.	–	+	+	+	–	+	–	–	–	–	–	–	–	–	–	+	–	–	+	+
20	*Eretes sticticus*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
21	*Hydrocanthus* sp.	–	–	–	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–	–
22	*Listronotus* sp.	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
23	*Pseuphenus sp.*	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–
24	*Diptera pupae*	–	–	–	–	–	–	–	–	–	–	+	–	+	–	–	+	+	+	–	–
	Total species	4	7	2	3	5	8	8	9	5	4	2	5	5	4	5	5	5	2	1	8

+ = observed, - = not observed

Table 3 Percent species composition of macroinvertebrate in different orders during the study period

Sr. No	Order	Number of Family	Number of genus	Number of species	Species composition (%)
1.	Haplotaxida	1	1	1	4.17
2.	Mesogastropoda	2	4	4	16.66
3.	Veneroida	1	1	1	4.17
4.	Araneae	2	2	2	8.33
5.	Decapoda	1	1	1	4.17
6.	Ephemeroptera	1	1	1	4.17
7.	Odonata	3	3	3	12.50
8.	Hemiptera	4	6	6	25.00
9.	Coleoptera	4	4	4	16.66
10.	Diptera	1	1	1	4.17
		20	24	24	100

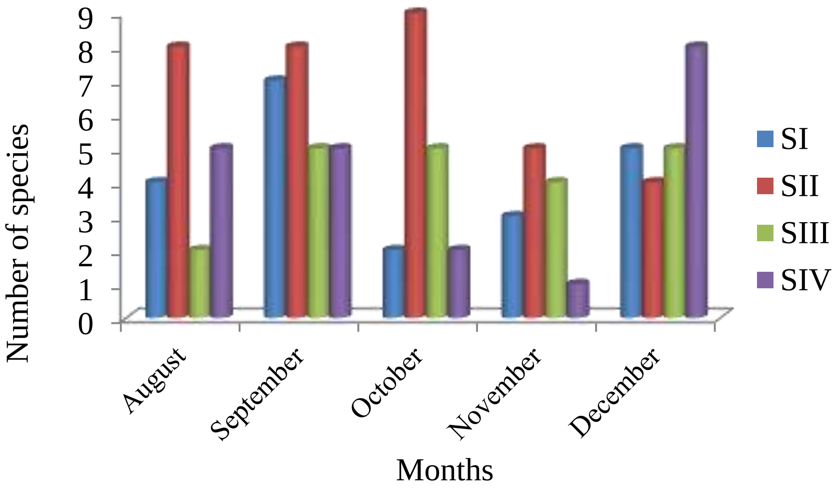


Figure 1 Occurrence of macroinvertebrate species in four study sites from August to December 2019

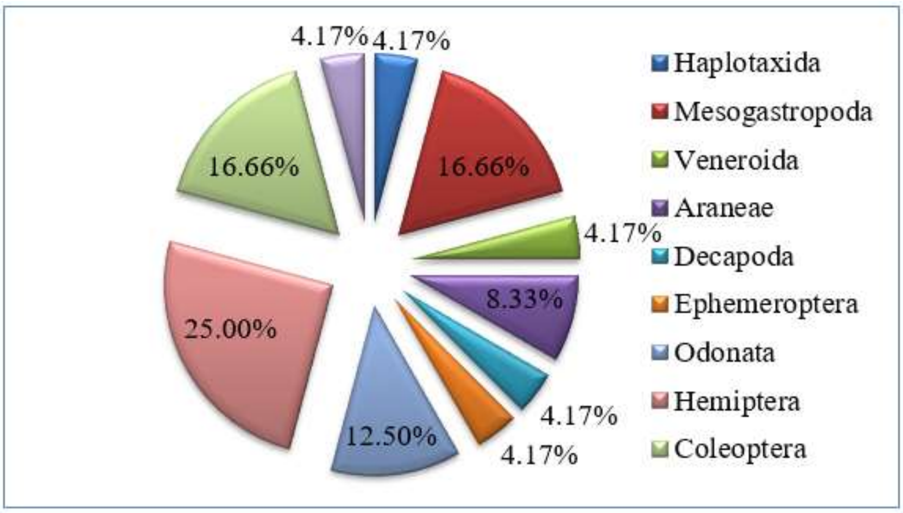


Figure 2 Percent species composition of macroinvertebrate in different orders during study period

A. *Pheretima posthuma*

Apertural view of the shell

Abapertural view of the shell

B. *Pila globosa*

Apertural view of the shell

Abapertural view of the shell

C. *Thiara scabra*

Plate 2 Recorded macroinvertebrate species

Apertural view of the shell

Abapertural view of the shell

D. *Tarebia granifera*

Apertural view of the shell

Abapertural view of the shell

E. *Melanoides tuberculata*

Apertural view of the shell

Abapertural view of the shell

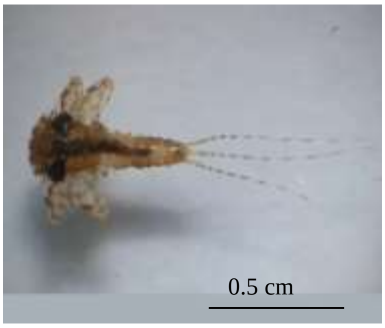
F. *Corbicula fluminea*

Plate 2 Continued

G. *Phidippus audax*

H. *Tetragnatha montana*

I. *Macrobrachium lamarrei*

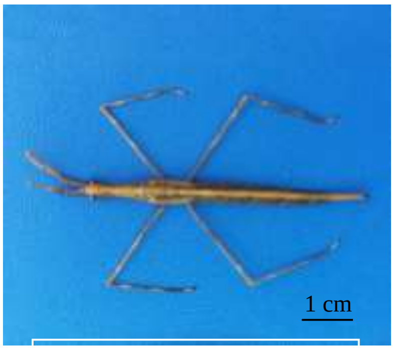


J. *Thalerosphyrus* sp.

K. *Coenagrion puella*

L. *Gomphus adelphus*

M. *Pachydiplax* sp.



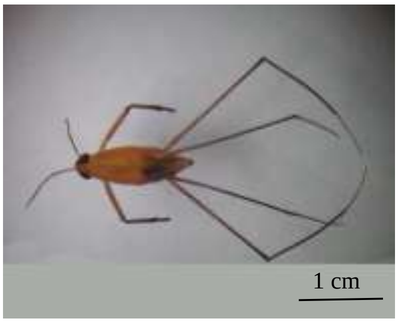
N. *Ranatra linearis*

O. *Notonecta* sp.

P. *Paraplea* sp.

Q. *Gerris lacustris*

R. *Ventidius distanti*



S. *Ptilomera* sp.

T. *Eretes sticticus*

U. *Hydrocanthus* sp.

V. *Listronotus* sp.

W. *Psephenus* sp.

X. Unidentified pupae (mosquito)

Discussion

A total of 24 species belonging to three phyla, six classes, ten orders, 20 families and 24 genera were recorded in four study sites of upstream and downstream of Min Ye Dam, Zawgi River Segment, Kyaukse Township, Mandalay Region from August to December 2019.

The highest species composition were recorded in Order Hemiptera with six species and lowest in Order Haplotaxida, Veneroida, Decapoda, Ephemeroptera and Diptera with only one species respectively.

Ei Ei Khaing Nyein (2015) reported that order Hemiptera revealed the highest species composition. The order Coleoptera and Diptera were the least species composition in Meiktila Lake, Meiktila Township. In present study, Order Hemiptera found to be highest and Diptera was least species composition.

Khin Lay Yee (2016) stated that the highest number of species and individual was occurred in order Hemiptera and lowest in order Ophisthoptera from Paleik In, Sintkaing Township. In present study showed that the highest number of species was recorded in Order Hemiptera.

Dosi, *et al*. (2018) revealed that in total 3,257 individual macroinvertebrates were collected. These specimens represented 37 species from 20 families and eight orders. More than 50% of the macroinvertebrates captured were aquatic beetles (Order Coleoptera), Aquatic bugs (Hemiptera) represented 26% of captures. Ten percent of the macroinvertebrates specimens were dragon/damselflies (Odonata). Flies (Diptera), mayflies (Ephemeroptera), shrimps (Decapoda) and aquatic moths (Lepidoptera) were less commonly found in Maludam National Park, Sarawak.

Akindele and Liadi (2014) reported that nineteen taxa of macroinvertebrates were recorded comprising three phyla, four classes and 17 families in Aiba Stream, Iwo and Southwestern Nigeria. Shah, *et al*. (2011) stated that in total, 50 taxa, belonging to 15 orders were recorded for littoral zone of the reservoir. The recorded higher number of taxa (family level) belonged to order Heteroptera (water bugs), Diptera (flies), and class Mollusca in Jagadishpur Reservoir, Taulihawa country. Barman and Gupta (2015) reported that the study revealed presence of 21 species of aquatic insects belonging to 14 families and 7 orders. The order Hemiptera was found most diverse and relatively abundant in Bakuamari stream, Chakrashila Wildlife Sanctuary, Assam, North East India. In present study, a total of 24 species of three phyla, six classes, ten orders and 20 families were recorded. The highest species composition were recorded Order Hemiptera.

In the present study, macroinvertebrates fauna highest in upstream of dam (site I and II) and lowest in downstream of dam (site III and IV) were recorded. The substrate of upstream was mainly composed on stones pebbles, rocks, clay, sand and organic matter. It was the desirable habitat for macroinvertebrates. In addition, there are many trees and tall grasses on the river bank. Bank vegetation is also evaluated in terms of provision of stream shading and refuge for fish and macroinvertebrates. Downstream habitat and water condition were degraded due to human settlement, disposal of domestic and agricultural wastes, surrounding land use and local geology which contributed to poor species diversity. Therefore, reducing number of macroinvertebrates were recorded in site III and IV.

Conclusion

A total number of 24 macroinvertebrate species of 24 genera, 20 families, ten orders, six classes belonging to three phyla were recorded in four study sites of upstream and downstream of Min Ye Dam, Zawgi river segment in Kyaukse Township. Study sites of upstream showed high species richness of aquatic macroinvertebrates than downstream sites due to local geology, stream habitat condition, human settlement and surrounding land use. Therefore, this study show that the needs to protect the water resources included the habitats of the Zawgi river segment.

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OCCURRENCE OF INDIGENOUS HONEYBEE SPECIES AND THEIR DISTRIBUTION IN TWO TOWNSHIPS, MAGWAY REGION

Cherry Soe[1], Thida Wai[2], Lai Lai Phyu[3], Nyo Nyo Lwin[4], Aye Mya Phyu[5]

Abstract

A total of four indigenous species from Magway University Campus in Magway Township and Yae Poke village in (Minbu/ Saku) Township, Magway Region were recorded during the period from June 2019 to January 2020. Three species of honeybees *Apis florea*, *A. dorsata*, *A. cerana* and one species of stingless bee, *Trigona iridipennis* were collected during the study period. Of those species *Apis cerana* was not found in Magway University Campus. All four species were recorded from Yae Poke village. A total of 123 honeybee nests were recorded from two study sites. Among them *A. florea* nest was recorded as the highest number and *A. cerana* nest was found the lowest number in the study period. In the present study, *A. florea* was found the most widespread indigenous honeybee species distribute in Magway region.

Keywords: Indigenous honeybee species, Honeybee nest, Stingless bee

Introduction

Honeybees are known to be the fascinating creatures and greatest friend of man. The bees are eusocial creatures and taught us how we should live and cooperate with each other in the society. People admire honeybees for their diligence, unity, sacrifice, tolerance, cooperation, coordination, division of labour, social service and usefulness (Agarwal, M.L. and Singh, R. 2018). There are about 25000 species of bees found in the world. They include honeybees, bumble bees, stingless bees, and solitary bees. Bees are the most efficient and most important pollinators of many cultivated crops and wild flora. A large number of bees on a crop ensure of good pollination that results in higher yields and better quality produce (Hepburn and Radloff, 2011).

Honeybees live in colonies where the young are nursed and fed by adults with a mixture of honey and pollen throughout the year. Honeybee colonies are large compared to other bees consisting of 5,000-80,000 individuals depending upon the species. Honeybees start their foraging early in the morning and cease late in the evening, working many hours a day. Honeybees have evolved a special communication system by which thousands of foragers can be deployed when a good food source is present. Most importantly, honeybees provide honey, beeswax and other bee products. They are found in different geographical areas and are adapted to different altitudes and climates (Free, 1993).

Nine species of honeybees, including the giant honeybees or rock bees (*Apis drosata* and *A. laboriosa*), the little honeybee (*A. florea*), the small dark honeybees (*A. andreniformis*), the Asian honeybee (*A. cerana*) and European honeybee (*A. mellifera*), are found in Asia (Hepburn, 2011). In Myanmar, the five indigenous honeybee species are *A. dorsata* (Giant Asain honeybee), *A. laboriosa* (Rock honeybee), *A. cerana (*Asian hive honeybee/Cavity nesting honeybee*), A. florea* (Little honeybee) *A. andreniformis* (Dwarf honeybee), and one exotic species is *A. mellifera* (European honeybee) (Petersen,2005).

A. *dorsata* is found throughout the Asian region up to 2,000m. It builds single comb nest in the open on branch of tall trees and tall buildings and chimneys, in shady places during summer

[1] Assistant Lecturer, Department of Zoology, Magway University
[2] Lecturer, Department of Zoology, Magway University
[3] Lecturer, Department of Zoology, Magway University
[4] Professor Head, Department of Biology, Yangon University of Education
[5] Lecturer, Department of Zoology, University of Yangon

and sunny places during winner. *A. dorsata* produce harvestable amounts of honey and is an important pollinator of many crops and other plants (Hepburn,2011). *A.florea* is one smallest honeybee species is called the dwarf bee. It also builds single comb nests on branches of bushes, hedges, small trees and chimneys, etc. This species is found in the plains and in hilly areas up to 500m, *A. florea* is another pollinator of agricultural crops (Hepburn,2011). *A. cerana*, the Asian hive bee or Himalayan hive bee, is widespread up to 3,000m throughout Asia. It has the gentle temperament, an industrious nature and good hygiene qualities (Verma, 1990).

Trigona iridipennis is one of the most primitive honeybees, widely distributed in Asian countries. It is highly social and live in perennial colonies (Leonhardt *et al*, 2007), most abundant and effective pollinator of various flowering plants occurs in both natural habitats, agro ecosystems (Roubik, 1989). Forests provide excellent resources for bees and beekeeping, and bees are a vital part of forest ecosystems. The distribution and abundance of indigenous honeybee species in Magway region has not been so far. Thus present study was conducted to record the occurrence and distribution of indigenous honeybee in two study area in Magway Region.

Materials and Methods

Study areas and period

Two study sites were selected in Magway Region. Magway University Campus is located on Taungdwin road and South of the Magway at 20º 08.24′N and 094º 56.14′E. Yae Poke Village is located on Salin-Pwint Phyu highway at 20º 13.55′N,094 º 48.82′E. The study was carried out during June 2019 to January 2020.

Specimen collection

Specimen collection was done through direct observation and interviews with the local residents. Field visits were made to two townships and interviewed honey hunters and dealers to investigate the occurrence of honeybees in places such as markets, gardens, vegetable farm yard, the forest area and building where bees usually come to collect pollen and nectar. The insect net with a long- handle was used for collection of bees from tall trees. When collect from bee hive, honey bees were driven from the comb with smoke, some honeybees were caught using net and a piece of comb was also taken for further study of the structure of comb.

Measurement of specimens

Collected bees were preserved in 70 percent ethanol for further identification. From the preserved specimens, 10 honeybees with well distended abdomen from each caste were used for measurements. The collected specimens were measured by scale showing micro-centimeter. The wings, legs, antenna and head with mouth parts appendages were cut off from the body and kept on the slide and observed under the dissecting microscope.

Identification

The honeybees were identified according to Bingham (1897) and Marks (2013). Identification of species was made based mainly on the morphometric characters of each species.

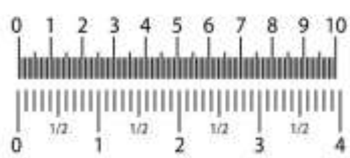
A. Magway University Campus B. Yae Poke Village**Plate 1** Map of study sites (Source: Google Earth, 2019)

A. Magway University Campus B. Yae Poke Village

Plate 2 Study sites

A. Insect Net

B. Dissecting Microscope



C. Ruler
(**Source:** VectorStock.com/23492502)

D. Digital Camera
(Cannon SX 430 IS 45 X)

E. Ethanol

F. Specimen Box

Plate 2 Materials used in result work

Results

A total of four honeybee species, *Apis florea*, *A. cerana*, *A. dorsata* and *Trigona iridipennis* belong to two genera under one family in one order were recorded in two study sites during the study period.

Morphometric characters of studied indigenous honeybees

Apis florea - Body length 10.8±1.18 mm (n=10); head, thorax and abdomen dull and opaque; slightly pubescent, the basal abdominal segments more or less red; younger workers are pale in color; the pubescence on the head and thorax white, on the posterior tarsi ferruginous golden; wings hyaline and iridescent.

Apis dorsata - Body length 15.15± 0.71 mm(n=10), Head, thorax and abdomen with short pubescent; head, thorax, legs and apical three segments of the abdomen black; the basal three segments of the abdomen honey-yellow; legs beneath, especially the posterior tibiae and tarsi, with short ferruginous pubescence; wings pale fuscous.

Apis cerana - Body length 12.55±0.5 mm(n=10), Head, thorax and abdomen smooth and shining, sparsely pubescent. Head thorax and apical abdomen segment black; the scutellum and basal five segments of the abdomen yellowish brown; legs rufo-fuscous, pubescence cinereous; wings hyaline and iridescent.

Trigona iridipennis - Body length 4.02±0.47 mm(n=10), predominantly blackish brown. Head is dark brown and is sparsely cover with yellowish-brown hairs. Compound eye is brown, and ocelli are brown and large. The abdomen is dark brown.

Habitat types and nests location of indigenous honeybee species

The nest of *A.florea* (dwarf honeybee) is a single and open nest, attached in the bushes and the branches of small trees. *Apis dorsata* (giant honeybee) also builds usually single and open nest, it is found overhanging from the branches of high trees and building. *Apis cerana* is cavity nesting bee, builds multiple comb and close type nest in tree hollows, house cavities as well as in the ground of cultivated fields and farmland. *Trigona iridipennis* builds nests in cavities of old logs and hollow trunks of old trees (Table 1).

A. *Apis Florea* (worker)

B. *Apis cerana* (worker)

C. *Apis dorsata* (worker)

D. *Trigona iridipennis* (worker)

Plate 3. Indigenous honeybee species collected from study areas

Monthly occurrence of honeybee species in two study sites

Apis florea (dwarf honeybee), *A. cerana* (cavity nesting honeybee), *A. dorsata* (giant honeybee) and *Trigona iridipennis* (stingless bee) were recorded in Yae Poke village. *Apis cerana* was not found in Magway University Campus during the study period.

In Magway University Campus, nests of *A. florea* were recorded throughout the study period. The nests of *A. dorsata* also found throughout the study period except in November. The species *Trigona iridipennis* was found only in November and January. *Apis cerana* was not recorded in this study site (Table.2).

In Yae Poke village, *A. florea* species was found during the study period except in July. *Apis dorsata* was found in the months of July, August and December. *Apis cerana* was recorded only in July and December. The species *Trigona iridepennis* was recorded in July, September, November, December and January (Table.3).

Distribution of studied honeybee nests found in two study sites

In the present study, nests of *Apis florea* were abundance of number 96 nests as 78% of total recorded honeybee nests, followed by number 13 nests as 11% in *A. dorsata*, 12 nests of *Trigona iridipennis* 8% and the lowest number 2 nests of *A. cerana* 3% were recorded in two study sites (Table. 4).

For percentage of individual species in each study site, *A. florea* was recorded that the highest percentage by 85.33% in Magway University Campus and 66.67% in Yae Poke village. *A. dorsata* was recorded that the highest percentage by 12% in Magway University Campus, followed by 8.33% in Yae poke village. *A. cerana* was recorded that highest percentage by 4.17% in Yae Poke village and 0% in Magway University Campus. *Trigona iridipennis* was recorded that the highest percentage by 20.83% in Yae Poke village, followed by 2.67% in Magway University Campus (Figure.1, Table. 4).

A. Nest of *Apis florea*

B. Nest of *Apis cerana*

C. Nest of *Apis dorsata*

D. Nest of *Trigona iridipennis*

Plate 4 Nests of indigenous honeybee species

Table 1 Habitat types and nests location of studied indigenous honeybee species

Scientific Name	Common Name	Local Name	Habitat Types	Nest Location
A. florea	Dwarf honeybee	Yin Pya	Single comb/open type	Bushes, branches of small tree
A. dorsata	Giant honeybee	Pya Gyi	Single comb/open type	Branches of high trees, building
A. cerana	Cavity nesting bee	Thit Khaung Pya	Multi comb/close type	Tree hollows, house cavities, ground of cultivated fields and farmland
T. iridipennis	Stingless bee	Myat Hmwe Lain	Cavity nest/close type	Cavities of old logs, hollow tree trunks of old trees

Table 2 Monthly occurrence of honeybee species in Magway University campus (2019-2020)

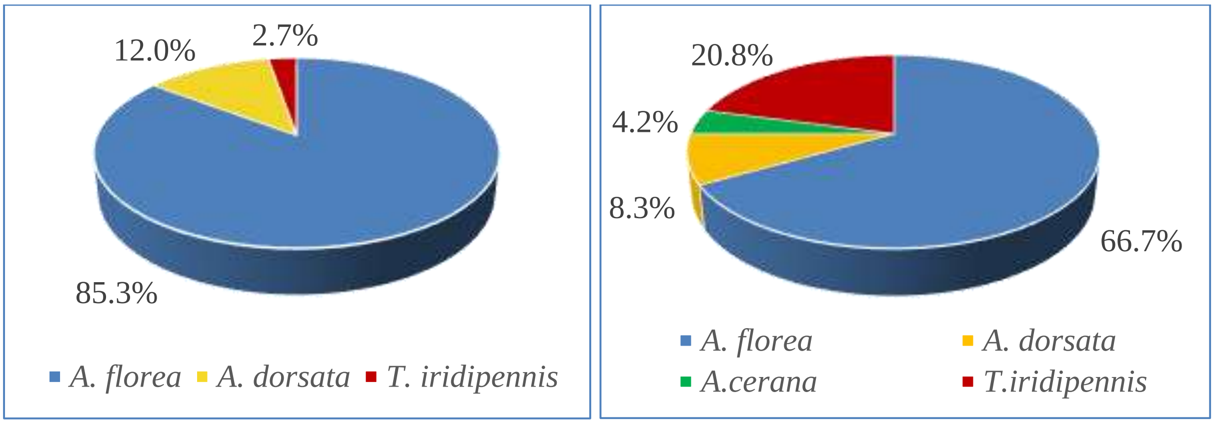
Monthly	*A. florea*	*A. cerana*	*A. dorsata*	*Trigona iridipennis*	Total
June	√	-	√	-	2
July	√	-	√	-	2
August	√	-	√	-	2
September	√	-	√	-	2
October	√	-	√	-	2
November	√	-	-	√	2
December	√	-	√	-	2
January	√	-	√	√	3

Table 3 Monthly occurrence of honeybee species in Yae Poke village (2019-2020)

Month	***A. florea***	***A. cerana***	***A. dorsata***	***Trigona iridipennis***	**Total**
June	√	-	-	-	1
July	-	√	√	√	3
August	√	-	√	-	2
September	√	-	-	√	2
October	√	-	-	-	1
November	√	-	-	√	2
December	√	√	√	√	4
January	√	-	-	√	2

Table 4 Distribution percentage of individual species in two study sites

Species	Magway University Campus		Yae poke Village		Total
	Number of nests	Distribution Percentage(%)	Number of nests	Distribution Percentage (%)	
Apis florea	64	85.33	32	66.67	96
Apis dorsata	9	12	4	8.33	13
Apis cerana	-	0	2	4.17	2
Trigona iridipennis	2	2.67	10	20.83	12
Total	75	100	48	100	123



A. Magway University Campus B. Yae Poke Village

Figure 1 Percentage of studied honeybee species found in the two study sites

Discussion

A total of four species of honeybee (*Apis florea*, *Apis cerana*, *Apis dorsata* and *Trigona iridipennis*) belong to two genera, one family and one order were recorded in during study period.

The presence of honeybee species and their distribution in two study sites of Magway Region were recorded. In the present work, three species of genus *Apis* (honeybees) and only one species of genus *Trigona* (stingless bees) were recorded from Yae Poke village. Indigenous honeybee species *A. florea, A. dorsata and Trigona iridipennis* were found in Magway University campus. But *A. cerana* was not found in this site in the present study.

Hepburn (2011) studied that a nest of *A. florea* consist of a single comb, typically built in small tree. *A. florea* nests in the open, but nests are camouflaged. Bradbear (2009) noted that they are very small-sized species of bees, and their single comb nests are small too(Bradbear,2009). In this study, *A. florea* is small bees, single comb nests, attached in the bushes, branches of small tree and median-sized plants.

Bradbear (2009) also stated that *A. dorsata* bees are large, and their nests consist of single large combs suspended from a branch, cliff face or building. In this work, *Apis dorsata* is large bee, the nest is large comb, open in air, and build overhanging from the branches of high tree and buildings.

Gupta (2014) described that *A. cerana* is the Asiatic honeybee or the oriental honeybee because they are only found in Asia. *A. cerana* builds multiple parallel comb in a wide variety of available cavities, including, for example, tree or palm hollows, rock crevices, and house wall cavities. In present study, *A. cerana* is cavity nesting bee, builds multiple parallel comb, which is found in tree hollow, disuse wells and house cavity.

Bradbear (2009) stated that stingless bees are also present throughout tropical and southern sub-tropical Asia. Gupta (2014) noted that they live usually in insects in hollow trunks, tree branches, underground cavities, or rock crevices. In this study, *T. iridipennis* is smallest bee, build nest in cavity of old logs, hollow trunks of old trees.

In Myanmar, Nyo Nyo Lwin (2007) described that seven species of honeybee such as *A. dorsata, A. cerana, A. cerana cerana, A. cerana indica, A. florea, A. andrineformis* and *A. mellifera*, two species of stingless bees such as *T. iridipennis and T. laeviceps* were recorded.

Myint Myint Htwe (2013) stated that the four species of honeybees *A.dorsata, A. cerana, A. florea, A. andrineformis* and one species of stingless bees were recorded in Central Myanmar. In this work, the three species of honeybees *A. florea*, *A. dorsata, A. cerana*, and one species of stingless bees, *T. iridipennis* were recorded in the study period and area.

In the present study, a total of 123 nests of honeybee were recorded during study period. In Magway University Campus, a total of 75 nests in two honeybee species *(A. florea, A. dorsata*), one species of stingless bee *(T. iridipennis)* were collected, and the lower number 48 nests in four species (*A. florea, A. dorsata, A. cerana* and *T. iridipennis)* were recorded from Yae Poke village.

During the study period, the indigenous honeybee species are abundantly found in December and January due to the rich food sources and fine weather while the lowest in June as the availability of floral resources is insufficient of this study sites.

Among two study sites, the indigenous honeybee species was found more abundant in Magway University Campus due to plentiful honeybee food sources such as seasonal cultivated plants, variety wild plants and abundance nesting plants, preferable weather conditions in this area. In Yae Poke village, the inadequate of food sources and nesting plants.

Conclusion

The indigenous honeybee species are the benefit pollinator as well as rich diversity of forests in this region. They are also help to increases crop yields and improves crop quality for the local people. Moreover, the local people obtain the incomes from the bee products. In this way, it benefits the local people.

In the other, the wild honeybee species are threatening the several conditions such as loss of their habitats, competition of introduced species, hazards of pesticides, overhunting, etc. According to these factors, the loss of indigenous honeybee species should be protected and vital role of indigenous honeybees should be maintained. Seasonal cultivated crops and variety of wild plants in this tropical region serve as provide bees forage. The conservation of the indigenous honeybees is the main component in the preservation of the native ecosystem of this region.

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MORPHOMETRIC VARIATIONS OF TWO SNAKEHEADS (FAMILY-CHANNIDAE) FROM BAGO RIVER, YANGON REGION BASED ON LANDMARKS AND TRUSS NETWORK ANALYSIS

Kyi Kyi Win[1], Aye Aye Aung[1], Khin Thazin[2], Phyu Phyu Su Mon[2] & Moe Moe Dwe[3]

Abstract

The present study was conducted by observing landmark-based morphometric variation of the two snakeheads, *Channa panaw* and *Channa striata* of family Channidae from the same stock of Bago river, Yangon Region. A total of 20 specimens of each of two *Channa* species were collected during December 2019 to March 2020. Set up the ten morphological landmarks and twenty one truss distances were measured. The significance of morphological differences of study species were carried out analysis of variance (ANOVA). Significantly differences were found in twenty one truss measurements ($p < 0.001$) were observed. Wilk's lambda of discriminant functional analysis, values of study species were nearly 0 and differences strongly significant (range is $0 < \lambda < 1$). The eigenvalues of factor analysis (FA) was indicated four factors of *Channa panaw* and three of *Channa striata*. The principal analysis (PCA) indicated size and shape variation and explained percentage of total variance, 86.602 and 87.723. Discriminant function analysis (DFA) accounted for four morphological indices (94.6 %, 3.9 %, 1.1 % and 0.4%) in *Channa panaw* and three morphological indices in *Channa striata* (73.3%, 25.0 % and 1.7 %) of with group variability. About 95% of individuals of two *Channa* species into their original group were correctly classified in discriminant space, as determined by discriminant function analysis. All statistically analyses were done using SPSS Package Version 26.

Keywords: Landmarks, Truss network, Morphometric variations, FA, PCA, DFA

Introduction

The snakeheads of the family Channidae comprises of two genea. The genus *Channa* comprises of 33 species in Asia (Courtenay and Williams, 2004). In India, 12 species including *Channa amphibeus* (McClelland 1845), *C. auranti-maculata* (Musikasinthorn 2000), *C. barca* (Hamilton 1822), *C. bleheri* (Vierke 1991), *C. diplogramma* (Day 1865), *C. gachua* (Hamilton 1822), *C. marulius* (Hamilton 1822), *C. melano-stigma* (Geethakumari & Vishwanath 2011), *C. orientalis* (Bloch & Schneider 1801), *C. punctate* (Bloch 1793), *C. stewartii* (Playfair 1867), *C. striata* (Bloch 1793) are reported. Fishes within this genus are characterized by an elongated cylindrical body, long and entirely soft-rayed dorsal and anal fins, a large mouth with well-developed teeth on both upper and lower jaws, and an accessory air-breathing apparatus known as the supra-brachial organ (Musikasinthorn, 1998, 2003). Snakeheads are of considerable interest as food fish and in aquarium trade and also as predators (Courtenay and Williams, 2004). Due to lack of information on its distribution, biology and population trends, this species was assessed as Data Deficient (Britz, 2010). Morphometric is defined as the study of quantitative analysis such as size and shape of living organisms, which for understanding the taxonomy as well (Park *et al.*, 2013). Morphometric parameters of a fish species has a major role to ensure whether there is any disparity between same species of different geographic region (Naeem *et al.*, 2012). The morphometric relationships between various body parts of fish can be used to assess the well-being of individuals and to determine possible difference between separate stocks of the same species (King, 2007). Landmark is a point of correspondence on an object that matches between and within population (Swain & Foote, 1999). The distance between the landmarks provide more comprehensive coverage of form for greater discriminating power (Strauss and Bookstein, 1982). The truss

[1] Dr, Lecturer, Department of Zoology, Dagon University
[2] 2 MSc Students, Department of Zoology, Dagon University
[3] Dr, Professor (Head), Department of Zoology, Dagon University

network system is considered superior to traditional morphometric that use morphometric traits to represent the complete shape of fish, which has been commonly used in the field of fish taxonomy and fisheries management. Truss network systems are powerful tools for stock identification. A sufficient degree of isolation may result in notable morphological, meristic and shape differentiation among stocks of a species which may be recognizable as a basic for identifying the stocks (Turan, 2004). So far the research dealt with the morphometric variations between different stocks in different localities and between different species was scare. The present research is therefore designed;

- to examine the morphometric differences of within and between the species of *Channa*

Materials and Methods

Study area

The study area was chosen at Thatyapinchaung village of Bago river, Yangon Region. It was located between latitude 16˚ 54′ 57.8″ N and longitude 96˚ 20′ 15.2″ E.

Study period

The study was lasted from December 2019 to March 2020.

Collection of specimens

A total of 40 snakeheads comprising *C. panaw* and *C. striata* (20 specimens for each species) were collected with the help of fishermen immediately after catch. Specimens were transferred into the ice-box for further identification. Identified and classified of the collected species were follow after Talwar and Jhingran, 1991, Courtenay and Williams, 2004 and Froese and Pauly, 2018.

Truss network analysis

Pattern of size and shape variation of snakeheads, *C. panaw* and *C. striata* were evaluated by means of truss network analysis. The specimen was laid on the drawing graph sheet into a right body position. This sheet was sandwiched between plastic film sheet and pop block. A total of ten morphological landmarks were identified on the specimens (Table 1). The choosing landmarks were pierced with long round head pin. After removing the specimen from the drawing graph sheet, these landmarks were joined with the help of permanent marker as a series of a truss network. Measurements were taken 21 inter-landmarks between ten showing a degree of correspondence landmarks (i.e. 1 to 2, 2 to 3 etc.) using a standard truss network protocol (Table 2 and Figure 1) (Strauss and Bookstein, 1982).

Statistical analysis

Prior to the analysis, size effects from the data set were eliminated. Variations were attributed to body shapes and size differences of the fish. In this research, there were significant network morphometric variation among all measured characters and total length of the fish. Therefore, it was necessary to remove size-dependent variations for all the characters. An allometric formula givened by Elliott *et al*. (1995). $M_{adj} = M\ (L_s / L_o)^b$, where M is the original measurement, M_{adj} = The size-adjusted measurement, L_s is the overall mean of the total length samples in each analysis, L_o is the total length and b is the slope of the regression coefficient, was calculated for each character from the observed data as the slope of log M on log L_o. The efficiency of the size adjustment transformations was assessed by testing the significantly correlation between a transformed variable and the TL. Analysis of variance (ANOVA) was carried out to test the significance of morphological differences. In addition to size-adjusted data were standardized and submitted to multivariate techniques such as Factor Analysis (FA), Principal Component

Analysis (PCA) and Discriminant Functional Analysis (DFA) in SPSS software (version 26) (Veasey *et al.*, 2001).

Table 1 Morphological landmarks selected for the study fish

Landmark	Particular of landmark
1	Tip of the snout
2	Upper end of the operculum
3	Origin of the dorsal fin base
4	End of the dorsal fin base
5	Upper origin of the dorsal fin
6	Lower origin of the dorsal fin
7	End of the anal fin based
8	Origin of the anal fin based
9	Origin of the pelvic fin base
10	Lower end of the operculum

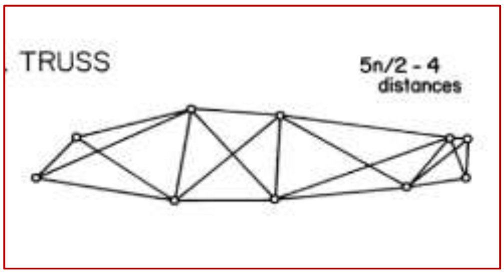


Figure1 The truss box of the 10 external landmarks design patterned

Landmark (n=10)

Truss distance $= \frac{5n}{2} - 4 = \frac{5 X 10}{2} - 4$

$= \frac{50}{2} - 4 = 25 - 4 = 21$

(Strauss and Bookstein, 1982)

Table 2 Morphometric measurements made for two *Channa* species

Measurement number	Network measurement	Distance code	Distance
1	1-2	HL	Head Length
2	2-3	BL1	Body Length 1
3	3-4	DFL	Dorsal fin length
4	4-5	BL2	Body Length 2
5	5-6	CL	Caudal Length
6	6-7	BL3	Body Length 3
7	7-8	AFL	Anal fin Length
8	8-9	BL4	Body Length 4
9	9-10	BL5	Body Length 5
10	1-10	ML	Mouth Length
11	2-8	BD1	Body diagonal 1
12	2-9	BD2	Body diagonal 2
13	2-10	HD	Head diagonal
14	3-8	BD3	Body diagonal 3
15	3-9	BH1	Body height 1
16	3-10	BD4	Body diagonal 4
17	4-7	BH2	Body height 2
18	4-8	BD5	Body diagonal 5
19	4-9	BD6	Body diagonal 6
20	5-7	BD7	Body diagonal 7
21	5-8	BD8	Body diagonal 8

Results

The length range and mean with standard deviation for each specimen were shown in table 3. Among the morphometric measurement, one-way ANOVA test showed that all truss measurements of two species were highly significant ($P < 0.001$) with Wilk´s lambda value were highly significant (nearly zero) of discriminant function (Table 4).

Twenty-one truss morphometric measurements of *C. panaw* and *C. striata* (Plate 1) yielded four factors and three factors explaining 86.602 % of the total variance (60.542 % for factor 1 and 10.966 % for factor 2, 9.130 for factor 3 and 5.964 for factor 4) and 87.72% of the total variance (66.58% for factor1, 12.99% for factor2 and 8.15% for factor3) in the entire dataset with eigenvalues of 13.319, 2.413, 2.009, 1.312 and 14.648, 2.858, 1.749 respectively (Table 5 and Figure 2A and 2B).

As the extraction in PC analysis of *C. panaw*, PC1 was the four truss network measurements of weak factors loading (BL2, BL3, BL5 and HD), among them the low standardized score was BL3 (-0.095) showed and component plot in rotated space also revealed the low neuroticism. Variable BL2 in PC1 and PC2 was weak factor loading, thus variable was also showed that the low neuroticism. These results showed size variation of morphological structure was BL2 and BL3 (Table 6 and Figure 3A).

PC2 in *C. panaw* was the four truss network measurements of weak factors loading as BL1, BL2, BL5 and ML but low variable was BL1 (-.294), showed the low standardized and component plot in rotated space also did not revealed the low outgoingness in table 6 and figure 3A. These results indicated that no shape variation of morphological structure.

As the extraction in PC1 of *C. striata* was the three truss network measurements of weak factor loading (BL1, AFL and BD5). Among them the low standardized score was AFL and BD5 showed and component plot in rotated space also revealed the low neuroticism in table 6 and figure 3B. These finding examined that size variation of morphological structure was AFL and BD5.

PC2 in *C. striata* was the six truss network measurements of weak factors loading as HL, BL2, CL, AFL, BD5 and BD6 but these low standardized scores and component plot in rotated space also did not revealed the low outgoingness (Table 6 and Figure 3B). The outcome of present results indicated that no shape variation of morphological structure.

The two studied species showed four and three morphological indices defining 94.6% (DF1), 3.9% (DF2), 1.1% (DF3) and 0.4% (DF4) the morphological differences in *C. panaw* and 73.3% (DF1), 25.0% (DF2) and 1.7% (DF3) the morphological differences in *C. striata*. The truss distances with important loading on DF1 were shown with total variance of 94.6% and 73.3% in both studied species and the largest absolute correlation between each variable and any discriminant function of DF1 the total variance were not shown in *C. panaw* and *C. striata* (Table 7).

All of these distances were described as morphometric measurements cover the whole body of the fish. The largest absolute correlation between each variable and any discriminant function of DF2 elucidated 3.9% in *C. panaw* and 25.0% in *C. striata* of the total variance were four network measurement as significance loading BD2, BD3, BH1 and BH2 in *C. panaw* and seven network measurements as BL3, BL4, BD1, HD, BD4, BD6 and BD7 in *C. striata* (Table 7).

The DF3 elucidated 1.1% in *C. panaw* and 1.7% in *C. striata* of the total variance were seven network measurements as significance loading DFL, BL2, CL, BL3, BL4, HD and BD6 in *C. panaw* and fourteen network measurements in *C. striata* (Table 7).

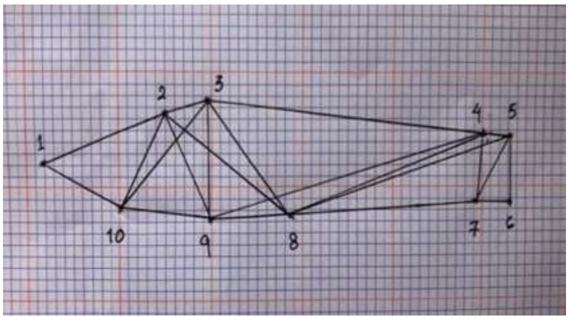
The rest ten measurements contributed to DF4 elucidated 0.4% in *C. panaw* of total variance (Table 7).

Although the largest absolute correlation between each variable, a correct classification of individuals into their original population of *C. panaw* and *C. striata* varied 98.5% by discriminant analysis (DF1 and DF2) and 95% of individual group cases (Table 8) and 98.3% by discriminant analysis (DF1 and DF2) and 95% of individuals group cases (Table 9) could be classified in their correct a priori grouping according to plotting of canonical discriminant functions of *C .panaw* and *C. striata*, which showed a more of them (20 individuals) overlapping for both morphometric (total length) and truss 21 measurements in discriminant space except total length 150 & 151 in *C .panaw* and total length 176 and 190 in *C. striata* (Fig. 4A and 4B).

Table 3 Descriptive data of snakeheads *C. panaw* and *C. striata*

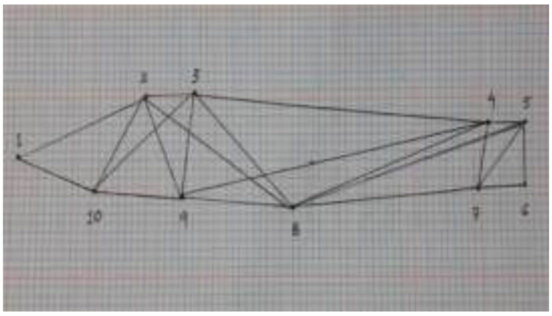
Source of fish sample	Species	Sample size	Total length (mm)	
			Range	Mean ± SD
Bago river	*Channa panaw*	20	135-197	162.20 ± 17.82
	Channa striata	20	150-270	209.95 ± 30.08

A. Location of landmarks of *C. panaw* (Musikasinthorn, 1998)



B. Scheme of truss network used *C. panaw*

C. Location of landmarks of *C. striata* (Bloch, 1793)



D. Scheme of truss network used of *C. striata*

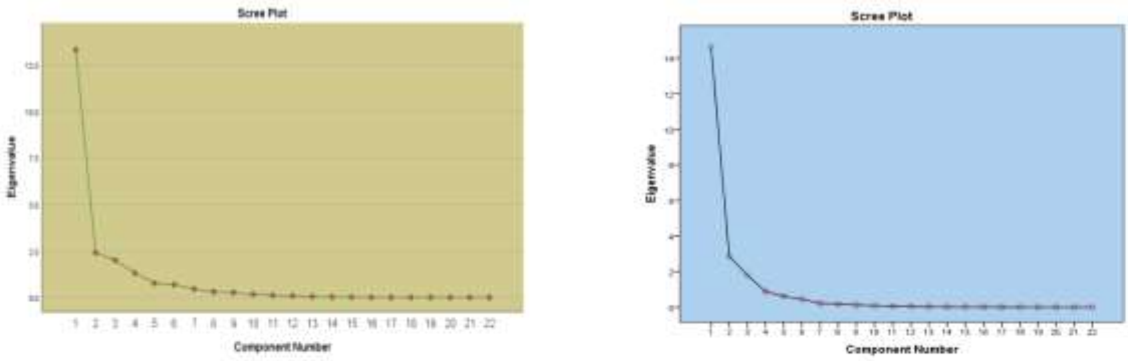
Plate 1 Morphometric analysis of *C. panaw* and *C. striata*

Table 4 Analysis of variance (ANOVA) testing differences within each species *C. panaw* and *C. striata*

Measurement numbers	Distance code	df		F-value		Wilk´s lambda		P-value	
		1	2	*C. panaw*	*C. striata*	*C.panaw*	*C.striata*	*C.panaw*	*C.striata*
1	HL	1	38	943.72	563.89	0.04	0.06	0.000*	0.000*
2	BL1	1	38	1363.11	786.94	0.03	0.05	0.000*	0.000*
3	DFL	1	38	410.37	215.88	0.09	0.15	0.000*	0.000*
4	BL2	1	38	1445.39	850.70	0.03	0.04	0.000*	0.000*
5	CL	1	38	1335.75	814.34	0.03	0.05	0.000*	0.000*
6	BL 3	1	38	1442.05	820.10	0.03	0.04	0.000*	0.000*
7	AFL	1	38	657.72	443.84	0.06	0.08	0.000*	0.000*
8	BL 4	1	38	1144.72	546.92	0.03	0.07	0.000*	0.000*
9	BL5	1	38	1174.34	703.33	0.03	0.05	0.000*	0.000*
10	ML	1	38	1128.72	679.98	0.03	0.05	0.000*	0.000*
11	BD1	1	38	847.30	387.03	0.04	0.09	0.000*	0.000*
12	BD2	1	38	1130.13	659.02	0.03	0.06	0.000*	0.000*
13	HD	1	38	1150.54	718.52	0.03	0.05	0.000*	0.000*
14	BD3	1	38	982.58	502.62	0.08	0.07	0.000*	0.000*
15	BH1	1	38	1130.46	688.88	0.03	0.05	0.000*	0.000*
16	BD 4	1	38	984.52	608.13	0.04	0.06	0.000*	0.000*
17	BH2	1	38	1303.46	805.17	0.03	0.05	0.000*	0.000*
18	BD5	1	38	596.55	401.65	0.06	0.09	0.000*	0.000*
19	BD6	1	38	336.92	188.38	0.10	0.17	0.000*	0.000*
20	BD7	1	38	1234.64	747.23	0.03	0.05	0.000*	0.000*
21	BD8	1	38	489.39	322.98	0.07	0.11	0.000*	0.000*

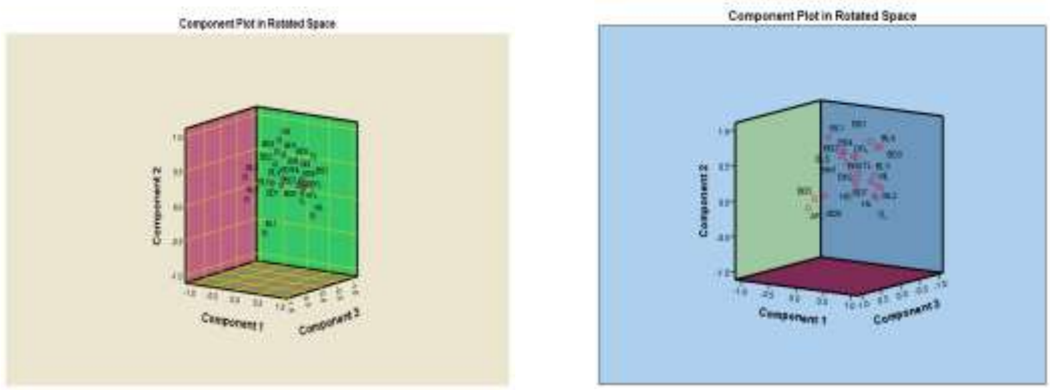
Table 5 Eigen values, percentage of variance and percentage of cumulative variance in *C. panaw and C. striata*

Factors	*C. panaw*			*C. panaw*		
	Eigenvalues	(%) of Variance	(%) of Cumulative variance	Eigenvalues	(%) of Variance	(%) of Cumulative variance
1	13.319	60.542	60.542	14.648	66.580	66.580
2	2.413	10.966	71.508	2.858	12.990	79.570
3	2.009	9.130	80.638	1.749	8.153	87.723
4	1.312	5.964	86.602	-	-	-



A. *Channa panaw* B. *Channa striata*

Figure 2 Showing of Eigen values by scree plot of two species of *Channa*



A . Channa panaw B. *Channa striata*

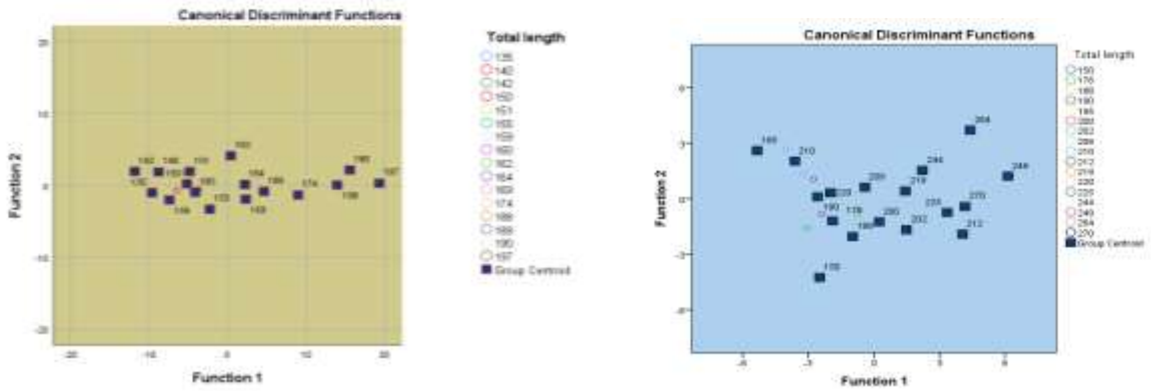
Figure 3 Score plots on size-corrected truss variables of two species of *Channa*

Table 6 Result of factors extraction in PC analysis after varimax normalized rotation in *C. panaw* and *C. striata*

Network measurement	Distance code	*C. panaw*				*C. striata*		
		PC 1	PC 2	PC 3	PC 4	PC 1	PC 2	PC 3
1-2	HL	.635	.606	-.085	.326	0.871	0.164	0.323
2-3	BL1	.368	-.294	.741	.030	-0.125	0.861	0.094
3-4	DFL	.891	.373	.121	.087	0.541	0.705	0.404
4-5	BL2	.056	.177	.855	.054	0.869	0.249	0.154
5-6	CL	.715	.608	-.110	-.042	0.845	0.124	0.250
6-7	BL3	-.095	.462	.692	-.036	0.696	0.407	0.092
7-8	AFL	.855	.337	.201	.237	0.124	0.006	0.982
8-9	BL4	.510	.453	.367	-.411	0.587	0.747	-0.231
9-10	BL5	.067	.290	.064	.915	0.332	0.754	0.403
1-10	ML	.862	-.114	-.065	-.259	0.851	0.304	0.250
2-8	BD1	.624	.396	.582	-.024	0.513	0.850	-0.070
2-9	BD2	.388	.657	.445	.296	0.350	0.697	0.439
2-10	HD	.217	.931	.065	.206	0.645	0.388	0.479
3-8	BD3	.533	.674	.173	-.165	0.599	0.769	-0.113
3-9	BH1	.438	.771	.245	.072	0.437	0.611	0.442
3-10	BD4	.300	.802	.265	.349	0.403	0.753	0.442
4-7	BH2	.610	.680	.106	-.028	0.668	0.334	0.524
4-8	BD5	.852	.397	.177	.205	0.221	0.146	0.951
4-9	BD6	.832	.446	.251	-.004	0.581	0.689	0.387
5-8	BD7	.650	.350	.042	-.352	0.812	0.360	0.344
5-9	BD8	.820	.370	.331	.200	0.375	0.198	0.896

Table 7 Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions of landmark distance of *C. panaw* and *C. striata*

Network measurement	Distance code	*C. panaw*				*C. striata*		
		DF 1 (94.6 %)	DF 2 (3.9 %)	DF 3 (1.1 %)	DF 4 (0.4%)	DF 1 (73.3%)	DF 2 (25.0%)	DF 3 (1.7%)
1-2	HL	.240	.586	-.340	-.695*	.444	.246	.861*
2-3	BL1	.026	-.034	.275	.960*	-.101	.691	-.716*
3-4	DFL	.505	-.319	-.710*	.374	.399	.600	-.694*
4-5	BL2	.050	-.082	.995*	-.034	-.593	-.004	.805*
5-6	CL	-.202	-.559	-.803*	-.027	-.438	-.065	.897*
6-7	BL3	-.404	.617	.668*	-.098	-.150	.775*	-.613
7-8	AFL	.112	-.026	.171	.979*	.490	.552	-.675*
8-9	BL4	-.155	-.188	.956*	.162	.575	.818*	.021
9-10	BL5	-.228	-.225	-.266	-.909*	.548	.418	-.724*
1-10	ML	.289	.238	.422	.826*	.212	.491	.845*
2-8	BD1	.211	.158	.603	.753*	.231	.735*	-.637
2-9	BD2	-.067	.971*	-.022	-.229	.147	.372	-.917*
2-10	HD	-.231	.542	-.604*	-.536	.151	.837*	-.525
3-8	BD3	.524	.648*	-.206	.513	.479	.378	-.793*
3-9	BH1	.055	.988*	-.103	-.103	.146	.349	-.926*
3-10	BD4	-.337	.549	-.485	-.591*	.202	.742*	-.639
4-7	BH2	.057	.661*	.447	-.600	-.081	.654	-.752*
4-8	BD5	.272	.048	.023	.961*	.296	.606	-.738*
4-9	BD6	.281	-.186	.929*	.150	.369	.691*	-.622
5-8	BD7	-.081	.189	-.119	.971*	-.440	.783*	-.439
5-9	BD8	.237	-.018	.677	.697*	.341	.626	-.701*



A. *Channa panaw* B. *Channa striata*

Figure 4 Sample centroids of discriminant function scores based on total length of two species of *Channa*

Table 8 Showing classification results of canonical discriminant function based on all truss measurements of *Channa panaw*

		Classification results Predicted group membership																
Total length		135	140	142	150	151	155	159	160	162	164	169	174	188	189	190	197	Total
Original %	135	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	140	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	142	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	150	.0	.0	.0	75.0	25.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	151	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	155	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	159	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	160	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
	162	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	.0	100.0
	164	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	.0	100.0
	169	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	.0	100.0
	174	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	.0	100.0
	188	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	.0	100.0
	189	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	.0	100.0
	190	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	.0	100.0
	197	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0	100.0

95 % of original grouped cases correctly classified

Table 9 Classification results of canonical discriminant function based on all truss measurements of *Channa striata*

		Classification results Predicted group membership																
Total length		150	176	188	190	195	200	202	209	210	212	219	220	225	244	249	264	270	Total
Original %	150	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
	176	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
	188	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
	190	0	50	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	100
	195	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	100
	200	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	0	100
	202	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	0	100
	209	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	100
	210	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	100
	212	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	100
	219	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	100
	220	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	100
	225	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	100
	244	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	100
	249	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	100
	264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	0	100
	270	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100	100

95 % of original grouped cases correctly classified

Discussion

In the present study, among the 21 truss measurements, all were highly significant different (p<0.001) within species of same stock. Rahman *et al.* (2014) found all truss morphometric measurements significantly different (p<0.001) in the Old Brahmaputra river. Kashyap *et al.* (2014) reported that all truss measurements were found to be highly significant (p <0 .001) of Freshwater Murrel, *Channa punctatus* from Northern and Eastern Regions of India.

Morphometric studies by statistical methods were based on a set of traditional measurements which were providing uneven and biased aerial coverage of the entire body form of the specimen explained Sathianandan (1999). Truss network provides a more systematic and geometric characterization of fish shape. Analysis of truss network measurements in the present study was done by principal component analysis (PCA). The study of PCA analysis indicated that the scores on the scatterplot of *both species* which were size variation in morphological structure but no shape effect variation in their populations.

Joseph (2000) explained that PCA does not require any prior information about the groups in the analysis of truss data. In this research, eigen values of the four and three principal components were over one by *C. panaw* and *C. striata* respectively. These eigenvalues were conducted a principal components analysis to determine how many important components are present data. Yakubu and Okunsebor (2011) explained that the morphological divergence is exclusively associated to body shape but not to size. Traits related to size can make the result error if not removed from the data during morphometric analysis. Two truss measurements (BL2 and BL3) of *C. panaw* and (AFL and BD5) of *C. striata* were skewed on the analysis but the size effect data was removed using allometric transformation.

These two studied species. were accepted on this analysis because missing measurements was adjusted on the analysis. PC analysis was used to observe the intraspecific variation by score plots, that PCs showed significant differences were in this research. PC1 of morphometric data as multivariate index of size and PC2 as shape indices was interpret by Cadrin, 1999. Therefore, the results of PC analyses clearly highlighted fish species and variability pattern relation.

In discriminant functional analysis (DFA), the first DF accounted for much more of the within-group variability than did the remaining other DFs. It was obvious that the other DFs explained much less of the variance than the first DF. According to no significance loading of morphological indices in DF1 (Discriminant Function Analysis) of both studied species in the present study, suggested that no shape variation of *Channa panaw and Channa striata.* About 95% of individuals of both studied species into their original group were correctly classified in discriminant space, as determined by discriminant function analysis. This finding is supported by Kashyap *et al.*, (2016) stated about in group classification using DFA, 100 percent of all the samples were correctly classified into their original subpopulation.

Conclusion

Truss network system has emerged as a new tool with more effective strategies for descriptions of size and shape, better data collection and diversified analytical tools. They offer powerful tool for testing and displaying morphological differences when combined with multivariate statistical method (FA, PCA and DFA). These analyses can be effectively applied to assess the variation of stock within each species in the present study. The present results were preliminary baseline information of studied species of same population in Bago River for further studies. More research based on genetic studies and investigations of the causes of environmental factors will be needed in future.

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MORPHOLOGICAL IDENTIFICATION AND REPRODUCTIVE CONDITIONS OF PRAWNS (DECAPODA: PALAEMONIDAE) FROM TRIBUTARIES OF THANLWIN RIVER, KAYIN STATE

Nan Wai Wai Phyo[1], Kay Lwin Tun[2]

Abstract

Freshwater prawn *Macrobrachium* species (Bate, 1868) is a large and cosmopolitan crustacean genus of high economic importance worldwide. The present study was investigated the morphological identification of freshwater prawns of the genus *Macrobrachium* in the Thanlwin River, Kayin State and their abundant were studied from June 2016 to May 2018. A total of 16 species belonging to two genera under the family Palaemonidae were recorded. Among them, five species; *Macrobrachium rosenbergii, M. villosimanus, M. malcolmsonii, M. birmanicum* and *M. idea* were selected to study the seasonal changes of their reproductive condition. *Macrobrachium lanchesteri* and *M. lamarrei* were carrying eggs throughout the year while *M. malcolmsonii*, *Macrobrachium rosenbergii* and *M. villosimanus* carried egg from May to November, 2018. The highest numbers of eggs in all prawn species were found in August and September, rainy season. The reproductive condition of the studied prawn species give valuable informing for breeding design and resource conservation programs for *Macrobrachium* in Myanmar.

Keywords: Thanlwin river, freshwater prawn, *Macrobrachium*, fecundity, morphological identification

Introduction

Prawns and shrimps are widely distributed in marine, fresh and brackish water environments ranging from tropical to polar regions. Most of prawns and shrimps belonging to the family Penaeidae are able to live in brackish waters with a very low salinity, while most members of the family Palaemonidae inhabit freshwater, but tolerate brackish and even marine environments (Jones, 1965). Both families belong to the order Decapoda, showing five pairs of legs.

A total of 3,047 species of prawns and shrimps are so far recoded worldwide (Jones, 1965). Of these, the most commercially important species belong to the superfamily Penaeoidea (Dore and Frimodt, 1987), while less than 300 species are of economic interest at all. Only about 100 species constitute significantly to the annual world catch (Chan, 1998). The taxonomy of the palaemonid freshwater prawns of Myanmar were reviewed by Cai and Ng (2002) and 19 species were recorded. These belong to three genera, with 17 species of *Macrobrachium* and one species of each, *Palaemonetes* and *Leptocarpus*.

In Myanmar, freshwater prawns are of economic importance for inland fisheries and an economically important food for local consumption. A total of 26 *Macrobrachium* species was found in Myanmar and of which one species was recorded as a new species. Hla Phone and Suzuki (2004) reconfirmed ten species of the genus *Macrobrachium* from Myanmar and recorded the new species of *Macrobrachium patheinense*. Twenty-seven species of *Macrobrachium* were reported up to date from Myanmar.

Macrobrachium rosenbergii De Man, 1879, is one of the most valuable freshwater species in Myanmar. While many freshwater taxa are important as sources for artisanal fisheries (Holthuis, 1980), *M. rosenbergii* is especially noteworthy, being an important aquaculture species in many countries in Asia-Pacific, such as Bangladesh, China, India, Malaysia, the Philippines, Vietnam and Thailand (New, 2005).

[1] PhD Student, Department of Zoology, University of Yangon
[2] Professor, Fisheries and Aquaculture, University of Yangon

It is necessary to further investigate the occurrence, life cycle, reproduction and growth rates as well as the habitat utilization of *Macrobrachium* species in different area of Myanmar, since research and development efforts of freshwater prawns have been principally conducted in delta regions of Myanmar.

Thanlwin River located Karin States and it is important river for fisheries sectors in the area. The river is about 2,815 kilometres long that flows from the Tibetan Plateau into the Andaman Sea in Southeast Asia. In Myanmar, it crosses northeastern Myanmar, Shan, Kayah, Mon and Kayi States before entering the Andaman Sea. Thanlwin River and it tributaries has deposition of top soil due to flooding and it provided the feeding and breeding ground supporting species richness of Palaemonidae. Although prawn resources have been exploited for many years, there is not much scientific information available on the areas. Freshwater prawns are very common in this region and are often dominant in all kinds of local water bodies. They are ecologically significantly important and several species are commercially important for human consumption (Than Than Soe, 2012).

The present study was undertaken to collect, identify and reproductive condition of prawn species occurring in Thanlwin River, Kayin State

Materials and methods

Study area and study site

Thanlwin River, Ma Gyi Kyun village was selected as the study area. The Thanlwin River which flows through Hpa-an Township is the 2815 km long. It is commercially useful for navigation, fishery and sand extraction. Ma Gyi Kyun is important river bank in Thanlwin River as it provides the productive agricultural land for the farmers residing villages along the bank of the river and as well as for the fishermen (Fig. 1).

Study period

The field survey and specimens collection were carried out from June 2016 to May 2018.

Specimen collection to estimate the population of prawn species

To estimate the population of prawn at study sites, the biggest prawn wholesale in the study site was selected. In the early morning of sample day, when fishermen came back from the fishing ground and sold the prawn to the wholesale, the total weight were noted for the day. Out of their weight, 3.2 kg of shrimp was randomly collected from the pile of prawns from wholesale. Shrimps were separated according to their species and number of individuals was also counted.

Morphological Identification of the species

Collected prawn species were identified according to Holthuis (1950), Holthuis (1980) and Jayachandran (2001) and Cai and Ng (2001). Extra features of shrimp are carefully checked. The key characters of prawns were done by examining the shape and size of the second pleopod, number of rostrum teeth in dorsal and ventral of rostrum. The length of second periopods and telson were measured. For telson, present and absent of spines were recorded (Fig. 2).

The total length (from tip of rostrum to posterior end of telson) of the prawns was measured in centimeters (cm) with a plastic ruler and individual weight was taken in grams (g) using a digital balance.

Determination of Sex ratio

Five shrimp species, *M. rosenbergii*, *M. malcolmsonii*, *M. villosimanus*, *M. lamarrei* and *M. lanchesteri* were selected to study the sex ratio. Initially a total of 20 individuals from each species were selected and measured their length.

The sex of each specimen was determined by visual observation at the base of the fifth pair of periopods (Hart *et al*., 2003). In males, the base of the fifth pair of periopods approximates to the middle while that of females is wide (Plate 2). All prawns were sexed by examination the shape of the endopod of the second pleopods (swimming legs) and the present or absent of the appendix masculine (Plate 2). The numbers of collected male and female specimens were recorded.

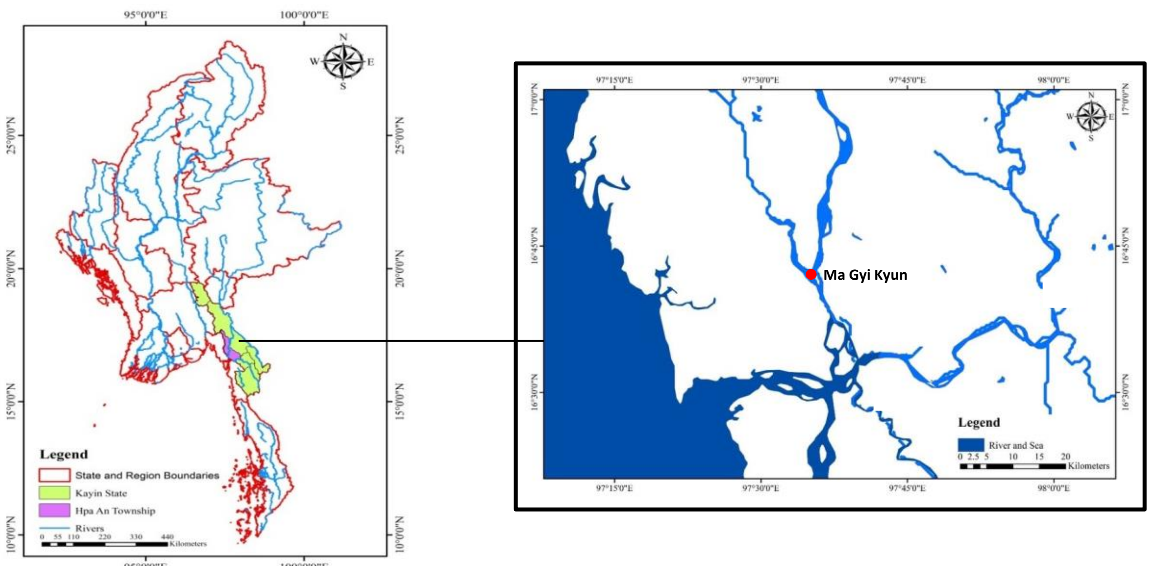
Egg size

During their breeding period, twenty eggs from each ovigerous female were randomly selected and the diameter of eggs was measured. Mean length and width were also calculated.

Fecundity estimation

Ovigerous females were collected and were individually packed in plastic bags and transferred to laboratory. The weights of the prawns were noted and eggs were removed by forceps and blotted with filter paper to remove the excess of water. To estimate fecundity, all eggs were removed from the pleopods and weighted. Then the eggs were weighed accurately on possible nearest to 0.1g. And then, the number of eggs was counted by using the stereo microscope to facilitate visualization and counting (Plate 3). The fecundity was also estimated. From the weight of the egg mass, the total number of egg present in the brood was calculated using this formula (Zar, 1999).

$$F = \frac{\text{Number of eggs}}{\text{Weight of the sample}} \times \text{Total egg mass weight}$$



(**Source:** Geography Department, University of Yangon)

Figure 1 Map of study site. Thanlwin River (Ma Gyi Kyun)

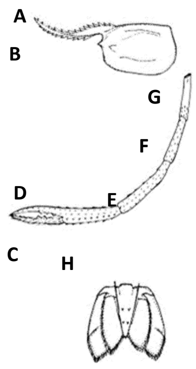
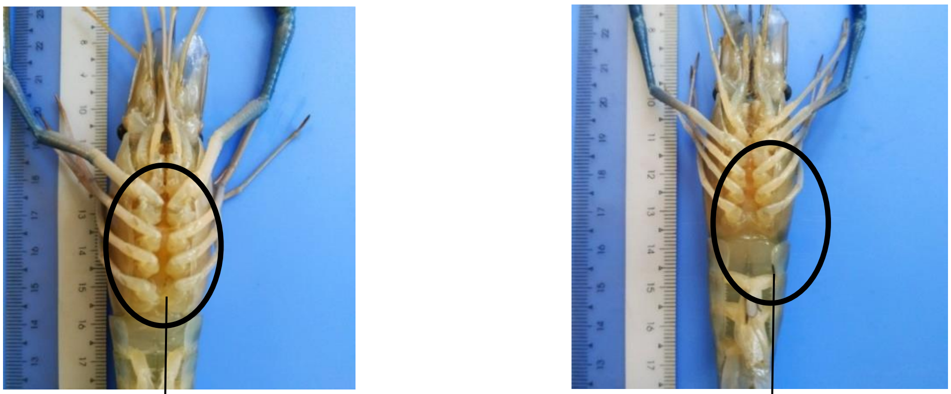


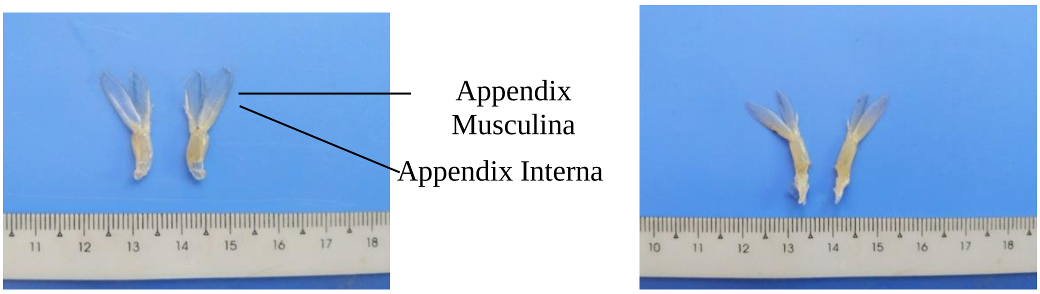
Figure 2 Descriptive characters of *Macrobrachium rosenbergii*. A. number of teeth of upper rostrum, B. number of teeth of lower rostrum, C. length of finger, D. length of palm, E. length of merus, F. length of carpus, G. length of ichium, H. present and absent of spine in telson.



Narrow space fifth pair of periopods (Male)

Wider space Fifth pair of periopods (Female)

Plate 1 Differentiation characters of male and female prawn



Male

Female

Plate 2 Comparison of second pleopods (Male and Female)

Environmental conditions at study area

Environmental conditions such as rainfall and temperature were recorded during the study period, 2016-2018.

Results

Occurrence of prawn species during the study period

A total of 16 prawn species and 2 genera belonging to one family were recorded from tributaries of Thanlwin River. The recorded 16 species were described in Table 1 and Plate 3.

Table 1 Occurrence of freshwater prawns (Palaemonidae) in tributaries of Thanlwin River during the study period

Sr No.	Scientific name	Common Name
1	*Macrobrachium birmanicum*	Birma river prawn
2	*Macrobrachium dayanum*	Kaira river prawn
3	*Macrobrachium equidens*	Rough river prawn
4	*Macrobrachium idae*	Slender river prawn
5	*Macrobrachium inflatum*	Not available
6	*Macrobrachium javanicum*	Java river prawn
7	*Macrobrachium lamarrei*	Kuncho river prawn
8	*Macrobrachium lanatum*	Not available
9	*Macrobrachium lanchesteri*	Riceland Prawn
10	*Macrobrachium malcolmsonii*	Monsoon river prawn
11	*Macrobrachium mirabile*	Short leg river prawn
12	*Macrobrachium neglectum*	Shrimps Shrimps
13	*Macrobrachium palaemonoides*	Not available
14	*Macrobrachium rosenbergii*	Gaint river prawn
15	*Macrobrachium villosimanus*	Dimue river prawn
16	*Leptocarpus fluminicola*	Gange delta prawn

Macrobrachium birmanicum (♂)

Macrobrachium dayanum (♀)

Macrobrachium equidens (♀)

Macrobrachium idae (♂)

Macrobrachium inflatum (♀)

Macrobrachium javanicum (♂)

Macrobrachium lamarrei (♀)

Macrobrachium lanatum (♂)

Macrobrachium lanchesteri (♀)

Macrobrachium malcolmsonii (♂)

Plate 3 Recorded prawn species in the tributaries of the Thanlwin River, Kayin State

Macrobrachium mirabile (♀)

Macrobrachium neglectum (♀)

Macrobrachium palaemonoides (♀)

Macrobrachium rosenbergii (♂)

Macrobrachium villosimanus (♀)

Leptocarpus fluminicola (♀)

Plate 3 Recorded prawn species in the tributaries of the Thanlwin River, Kayin State (Continue)

Seasonal variation of prawn species in different study sites

In Ma gyi kyun, a total of 16 species with 962,862 individuals of prawns were observed from June 2016 to May 2018. The highest number of individuals of *M. lamarrei* (21,340 individuals) was found in May 2017 and the lowest number of individuals of *M. malcolmsonii* (28 individuals) was recorded in November 2017 during the study period.

Among study species, *M. rosenbergii, M. malcolmsonii, M. birmanicum, M. idae, M. villosimanus, M. lanchesteri, M. lamarrei, M. dayanum, M. mirabile, M. palaemonoides and Leptocarpus fluminicola* were recorded throughout the year. The remaining species; *M. javanicum, M. lanatum, M. inflactum, M. neglectum* were not found from December 2016 to January 2017 and November 2017 to February 2018. *M. equidens* was not recorded from December 2016 to April 2017 and November 2017 to April 2018. The number of individual in all situations decreased in 2018 when it was compared to 2016 and 2017(Fig. 3nd 4).

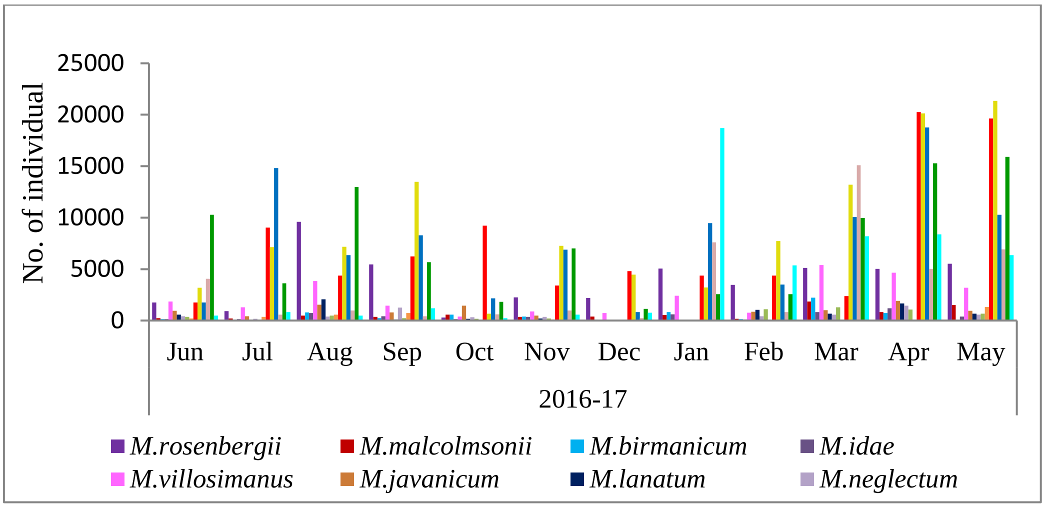


Figure 3 Seasonal variation of freshwater prawns (Palaemonidae) in Ma gyi kyun (2016-2017)

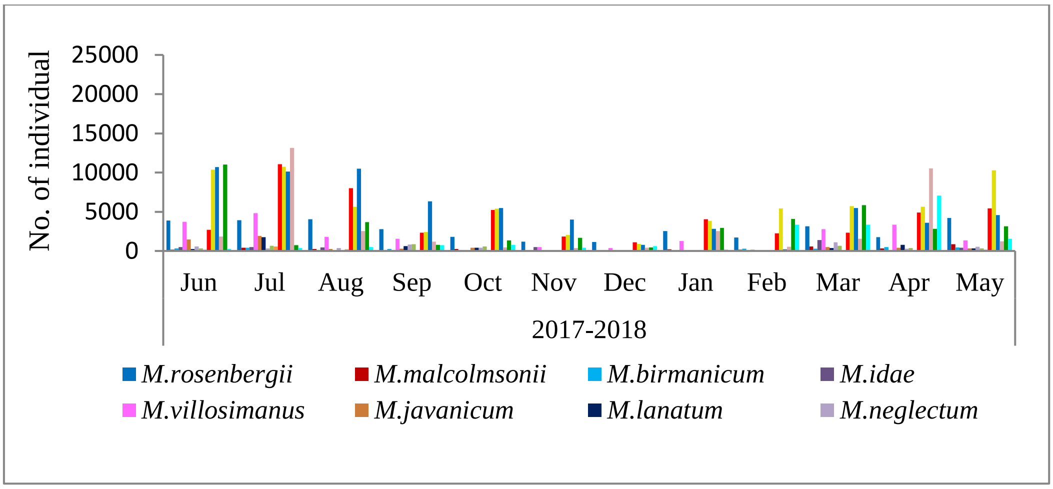


Figure 4 Seasonal variation of freshwater prawns (Palaemonidae) in Ma gyi kyun (2017-2018)

Reproductive condition of *Macrobrachium* species

Among the recorded species, five economically important species *M. rosenbergii, M. malcolmsonii, M. villosimanus, M. lamarrei and M. lanchesteri* were selected and their reproductive condition such as sex ratio, fecundity and egg size were recorded.

Sex ratio of collected species in Ma Gyi Kyun

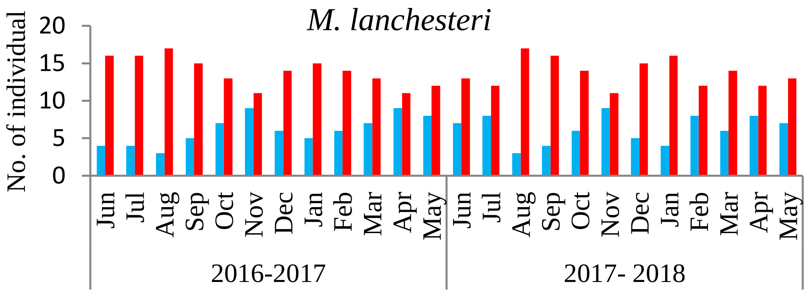
In Ma gyi kyun, a total of 148 males and 332 females of *Macrobrachium lanchesteri* were collected during June 2016 to May 2018. Female individuals were higher than that of male in all observed months. The highest significant different sex ratio of male and female (1: 6) was observed in August 2016 and 2017 (Fig. 5).

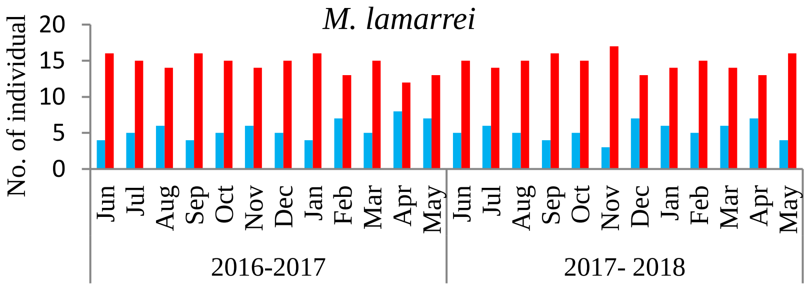
A total of 129 males and 351 females of *Macrobrachium lamarrei* were recorded during June 2016 to May 2018. Female individuals also dominated during study period. The highest significant different sex ratio of male and female (1: 6) was observed in November 2017.

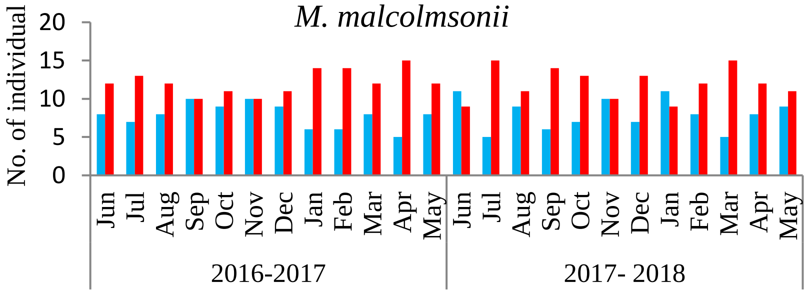
A total of 190 males and 290 females *Macrobrachium malcolmsonii* was found during June 2016 to May 2018. Female individuals were observed higher than male except in June 2017 and January 2018. The same individuals of male and female were recorded in September and November 2016 and November 2017. The highest significant different sex ratio of male and female (1: 3) was observed in April and July 2017 and March 2018.

A total of 192 males and 288 females *Macrobrachium rosenbergii* was recorded during June 2016 to May 2018. Female individuals were observed than male except in June 2016 and January to April 2018. The highest significant different sex ratio of male and female (1: 4) was observed in March 2017.

A total of 131 males and 349 females *Macrobrachium villosimanus* was collected during June 2016 to May 2018. Female individuals were observed higher than male in all months during study period. The highest significant different sex ratio of male and female (1: 9) was observed in August 2016 (Fig. 5).







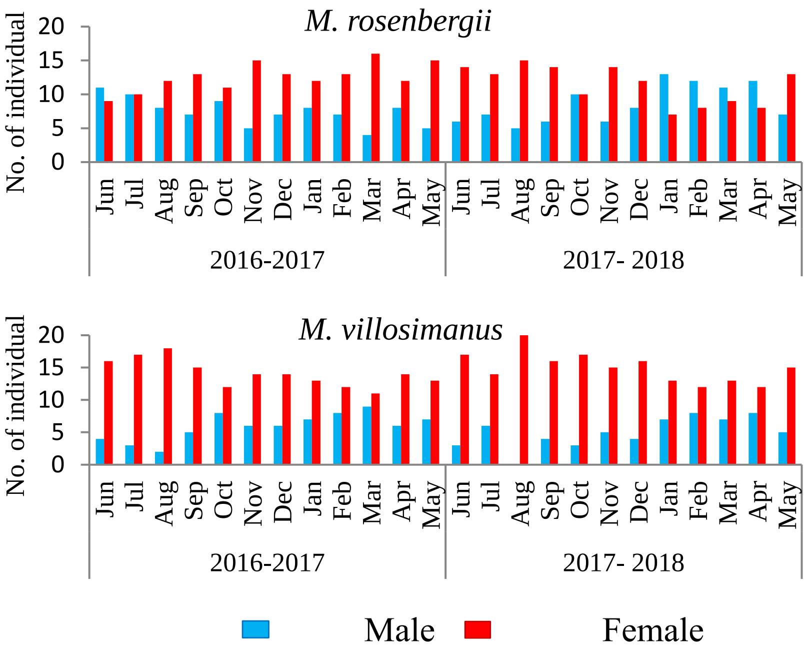


Figure 5 Sex ratio of collected species in Ma Gyi Kyun

Fecundity and egg size of selected species

Fecundity (total number of eggs carried) of five *Macrobrachium* species (*M. rosenbergii*, *M. malcolmsonii*, *M. villosimanus*, *M. lanchesteri* and *M. lamarrei*) was estimated through random sampling using egg counting method. Besides, to measure the diameter of egg of selected species, 20 egg were randomly collected and their diameter were measured.

In Ma Gyi Kyun, the high number of eggs in *M. lanchesteri* (280.8 ± 80.61) was found in July 2017 and the small amount of egg (82.4 ± 32.66) in April 2017 and 2018. The mean diameter of egg size (Short axis × Long axis) was 0.812 ± 0.035mm × 0.847± 0.005mm. The mean fecundity of *M. lanchesteri* was 204 ± 96 eggs (Fig. 6).

The high number of eggs in *M. lamarrei* (299.2 ± 28.44) was found in November 2017 and the small amount of egg (107.6 ± 24.43) in February 2018. The mean diameter of egg size (Short axis × Long axis) was 0.852 ± 0.007mm × 0.922± 0.170mm. The mean fecundity of *M. lamarrei* was 219 ± 91 eggs.

The high number of eggs in *M. malcolmsonii* (28078 ± 277919.6) was found in June 2016 and the small amount of egg (6669.8 ± 3472.73) in May 2018. The ovigerous females were not found in November 2016 to April 2017 and November 2017 to April 2018. The mean diameter of egg size (Short axis × Long axis) was 0.507 ± 0.051mm × 0.597± 0.054mm. The mean fecundity of 60 ovigerous females was 13935 ± 12476 eggs.

The high number of eggs in *M. rosenbergii* (67419.2 ± 13652.03) was found in August 2016 and the small amount of egg (17614.8 ± 7254.473) in November 2017. The mean diameter of egg size (Short axis × Long axis) was 0.515 ± 0.038mm × 0.610± 0.042mm. The mean fecundity of 70 ovigerous females was found 30250 ± 22463 eggs.

The high number of eggs in *M. villosimanus* (34814 ± 20745.8) was found in June 2017 and the small amount of egg (5878.8 ± 4638.457) in August 2016. The mean diameter of egg size (Short axis × Long axis) was 0.491 ± 0.023mm × 0.573± 0.043mm. The mean fecundity of 70 ovigerous females was 14981 ± 13893 eggs (Fig. 6 and plate 4).

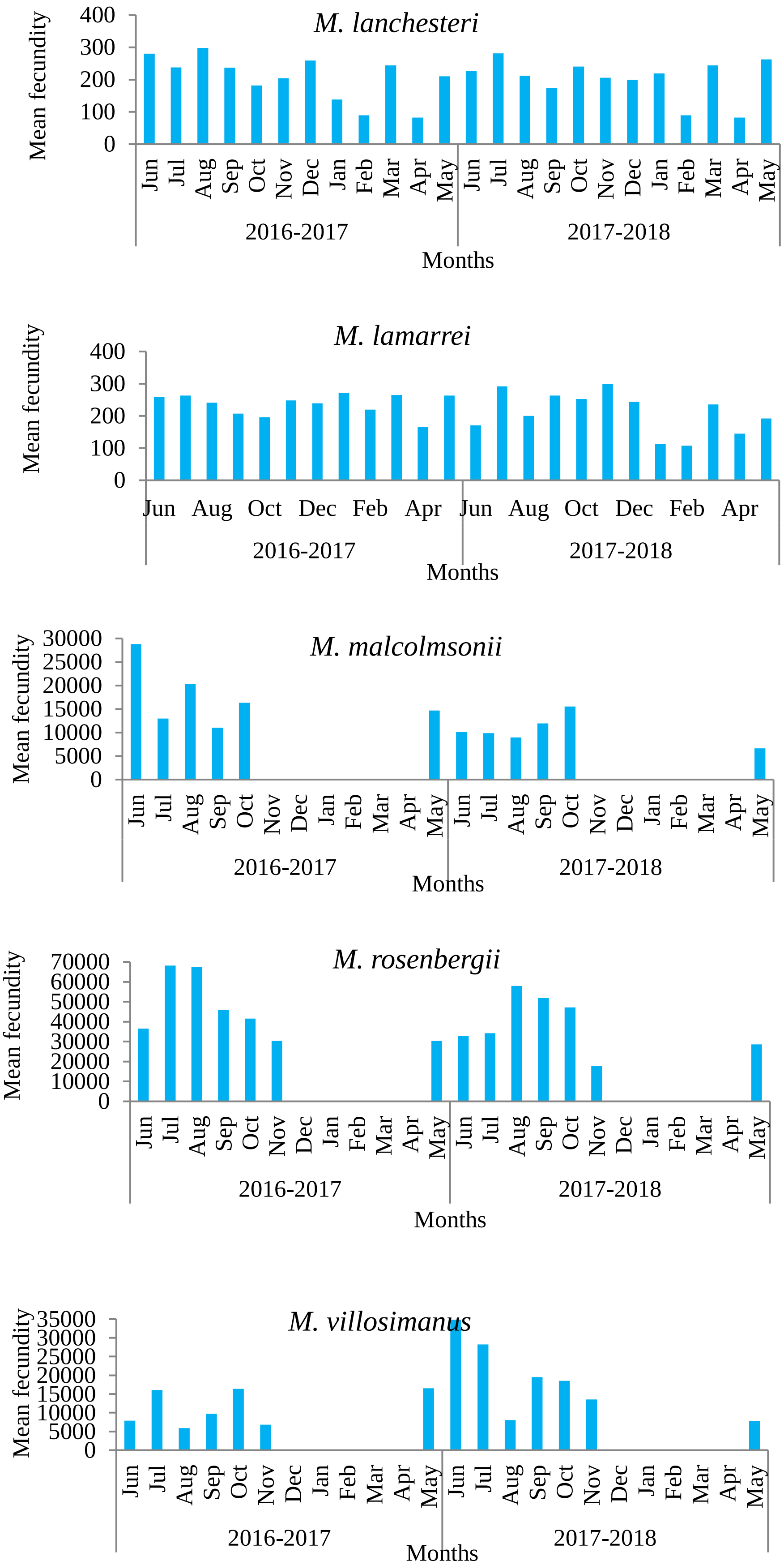
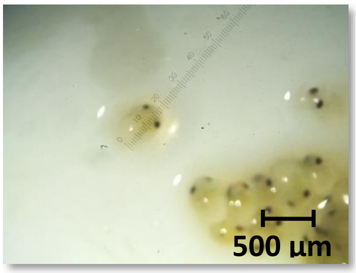


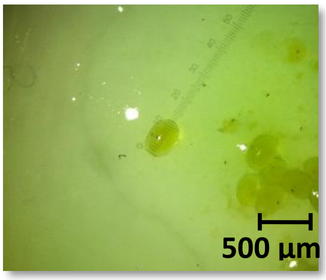
Figure 6 Mean fecundity of *Macrobrachium* species in Ma Gyi Kyun

Table 2 Egg diameter (mm) of *Macrobrachium* spp. in Ma Gyi Kyun

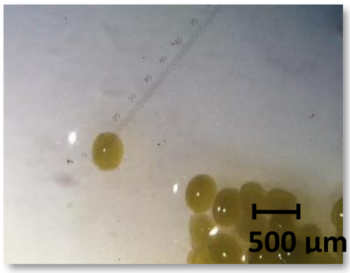
	M. lanchesteri		***M. lamarrei***		***M. malcolmsonii***		***M. rosenbergii***		***M. villosimanus***	
	Short Axis	Long Axis	Short Axis	Long Axis	Short Axis	Long Axis	Short Axis	Long Axis	Short Axis	Long Axis
Mean ± SD	0.812 ± 0.035	0.847 ± 0.005	0.852 ± 0.007	0.922 ± 0.170	0.507 ± 0.051	0.597 ± 0.054	0.515 ± 0.038	0.610 ± 0.042	0.491 ± 0.023	0.573 ± 0..043
Min ± Max	0.830 ± 0.747	0.830 ± 0.855	0.830 ± 0.863	0.872 ± 1.660	0.415 ± 0.581	0.830 ± 0.747	0.498 ± 0.581	0.581 ± 0.664	0.415 ± 0.498	0.498 ± 0.664



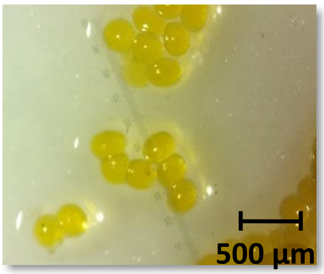
M. lanchesteri



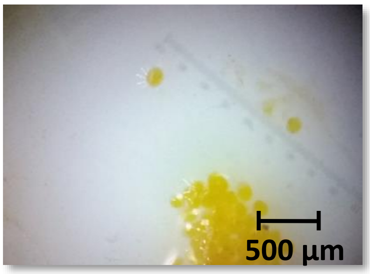
M. lamarrei



M.malcolmsonii



M. rosenbergii



M. villosimanus

Plate 4 Egg size of *Macrobrachium* spp. in Ma Gyi Kyun

Environmental conditions at studied area

Environmental conditions such as rainfall and temperature were recorded during the study period. Temperature ranged from 20.5 to 24.5˚ C. The rainfall was up to 36.2 to 43.02 mm during the rainy season (Table 3).

Table 3 Environmental conditions of rainfall and temperature during 2016-2018

Months	2016-2017		2017-2018	
	Mean temperature (˚C)	Rainfall (mm)	Mean temperature (˚C)	Rainfall (mm)
Jun	24.4	29.34	24.57	24.93
Jul	24	29.65	24.76	43.02
Aug	24	36.23	24.31	28.51
Sep	24.3	27.51	24.97	29.49
Oct	24.2	11.97	26.64	12.72
Nov	23.1	0.24	27.30	TR
Dec	21.3	0	27.96	0.63
Jan	20.59	2.12	20.5	0
Feb	18.97	0	20	0
Mar	21.55	0	22.5	0
Apr	24.89	3	24.7	1.5
May	25.43	12.63	24.7	9.7

Discussion

The morphological identification of freshwater prawns in the tributaries of Thanlwin River and Gyaing River, Hpa-an Township, Kayin State were carried out from June 2016 to May 2018. A total of 16 species belonging to two genera under the family Palaemonidae were recorded in the present study.

Than Than Soe (2012) observed the biology of prawn and shrimp populations in Thanlwin River Mouth and Adjacent Coastal Waters. She recorded 46 species of freshwater and marine water shrimp fauna belonging to 14 genera and 6 families. Of which, 26 species of Caridea: 22 Palaemonidae, 3 Atyidae, 1 Hippolytidae and 19 species of Penaeidea: 18 species of Penaeidae and 1 Solenoceridae and 1 species of Sergestidae were recorded. Among 22 Palaemonidae, seventeen species of *Macrobrachim* and *Leptocarpus fluminicola* were collected.

The present finding was agreement with Than Than Soe (2012) who stated that the highest number of species was recorded in family Palaemonidae along Thanlwin River mouth and adjacent Coastal Waters.

Win Win Myint (1988) described some freshwater and estuarine prawns of Mon State. She recorded six species of estuarine prawns and ten species of freshwater prawns. Khin Nwe Mu (1980) also studied the taxonomic characters of some prawns from Ngawun River Mouth, Pathein. She recorded ten species of freshwater prawns and nine species of brackish water prawns in her study area.

Thanlwin River has deposition of top soil due to flooding and it provided the feeding and breeding ground supporting species richness of Palaemonidae. In the present study, 16 species of Palaemonidae were recorded. It may be due to the fact that the present study focused on wide range of study site in Thanlwin River. The number of individuals in all situations decreased in 2017-2018 when it was compared to 2016-2017. It may be due to higher water level in Thanlwin River and its tributaries because of the heavy rainfall in Hpa-an environs during 2017-2018.

A total of 9,600 specimens from four study site, in which five species (1655 male and 3145 female) were recorded in 2016-2017 and (1591 male, 3209 female) in 2017-2018. From natural population, proportion between male and female was changed monthly in the studied species at all study sites. Occurrence of seasonal changes in population proportion was observed in all studied prawn species. The different sex ratio was occurred in all seasons. Number of all female prawn species was greater than that of the male in all study sites during study periods.

Tawari-Fufeyin *et al*., (2005) reported that sex ratio may not always be static, as they vary from season to season or from year to year within the same population. Shwe Lei Win (2013) stated that since the occurrence of the highest female sex-ratio coincided with highest percentage of ovigerous female, the reproductive period of *M. naso* could be determined from January to March (end of cool and commencement of hot seasons) and wet season in Inle' Lake. Oh *et al*., (2002) reported that the sex ratio in *Exopalaemon modestus* population was favour to females. Sex ratio may be related to the growth and longevity of shrimp population.

Fecundity of five economically important of prawns was recorded two years study periods (June 2016 to May 2018). Fecundity was estimated by volumetric methods and by direct counting of the number of eggs found on the pleopods of a berried female.

The fecundity of *M. malcolmsonii* has been reported to range from 3500 - 94000 of 54 - 165mm female (Ibrahium, 1962, Rajyalakshmi, 1980: cited by Sharma and Subba, 2005). New and Singholka (1982) reported that *M. rosenbergii* hatch between 100,000 - 700,000 eggs during the spawning season when they are mature. Rao (1998) estimated the fecundity of *M. rosenbergii* by counting the number of eggs on the pleopods and it ranged between 20,000 and 70,000 eggs.

In the present work, the mean fecundity of *M. rosenbergii* was lower number in 3,433 to107,254. Similarly, the mean fecundity of *M. malcolmsonii* was ranged from 4,200 to 63, 550 while it was 800 to 40, 060 in *M. villosimanus*. The fecundity of above three freshwater prawns was found to be relatively low when compared to Sharma and Subba, (2005) and New and Singholka (1982). Freshwater shrimps exhibit variation in fecundity from species to species. Fecundity also varies according to hydrographic region (Mashiko, 1990). In addition, the efficiency of egg production, i.e. the number of egg produced per female unit body weight, may be age and/or size dependent and is generally assumed to increase with female size (Malecha, 1983). The different geographical region and food availability may be influence on the fecundity of prawn.

In this study, ovigerous females of *M. lanchesteri* and *M. lamarrei* might be the dominant species because it was abundantly found in study site and this species is capable of reproducing throughout the year. However, in other three species, *M. rosenbergii*, *M. malcolmsonii* and *M. villosimanus*, ovigerous females were found only from May to November. Among the five *Macrobrachium* species, *M. rosenbergii* is the most economically important species and they are culture widely in Yangon and Ayeyarwaddy Divisions. Fecundity of *M. rosenbergii* was highest among the studied species. Because of high fecundity, producing of post larvae within the limited period is higher than other species. Mass production post larvae of *M. rosenbergii* can be produced in hatchery because of its high fecundity rate.

In the present study, morphology, fecundity and egg size of *M. malcolmsonii* is very similar to *M. rosenbergii*. *M. malcolmsonii* will be potential candidate for aquaculture farmer in the future.

Conclusion

This study has demonstrated the morphological parameters for the classification of *Macrobrachium* species. A total of 16 prawn species and 2 genera belonging to one family were identified and recorded from tributaries of Thanlwin River. Among them, five species *Macrobrachium rosenbergii, M. villosimanus, M. malcolmsonii, M. birmanicum* and *M. idea* were economic important species with high market demand. Reproductive biology of five species was also studied to estimate their fecundity during the spawning period. The number of females was higher than the number of males in all species. The most economically important species, *M. rosenbergii* has highest fecundity among the studied species.

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PREVALENCE OF COMMON INTESTINAL PARASITES IN ZEBU CATTLE OF MYIN MHWE VILLAGE, SAGAING REGION

Nyein Thi Aung[1] Myo Pa Pa Thet Hnin Htwe Aung[2], Nyein Nyein Hlaing[3]

Abstract

The gastro-intestinal tract (GIT) of animals harbour a wide variety of parasites mainly helminthes, which causes clinical and sub-clinical parasitism. These parasites adversely affect the health status of animals and cause enormous economic losses to the livestock industry. This study was conducted to detect seasonal variations in prevalence of common intestinal helminths in Zebu cattles of Sagaing Region. A total of 50 faecal samples were collected by simple random sampling method. Prevalence of infection was highest (52%-74%) at all seasons in Zebu. During the study period, four species of common intestinal parasites (four species of worms, *Strongyloides* sp., *Trichuris* sp., *Fasciola hepatica, Ascaris*) and one protozoan *Eimeria* sp. were occurred in Zebu cattles. *Strongyloides* sp were at high infection level. *Strongyloides* sp. and *Fasciola hepatica* were found in all ages. In this study, Zebu (or) ploughing cattle in Myin Mhwe village were found to be infected with intestinal parasites.

Keywords: Zebu cattle, intestinal parasites, seasonal prevalence

Introduction

Livestock production constitutes one of the principal means of achieving improved living standards in many regions of the developing world. Gastro-intestinal (GI) parasitic infections remain one of the major constraints to ruminant production. Insidious productivity losses through reduced feed intake and decreased efficiency in feed utilization, associated with subclinical or chronic conditions of parasitic infections are often the cause of large economic losses. The high incidence of parasitism of cows might have been due to the relative low influence of feeding behavior and a deworming program (Jittapalapong *et al*., 2011).

Livestock plays a crucial role both in national economies and the livelihood of rural communities. It provides milk, meat, input for crop production and soil fertility and raw material for industry. Diseases have numerous negative impacts on productivity and fertility of herds i.e. losses due to mortality and morbidity, loss of weight, depressed growth, poor fertility performance and decreased physical power (Bacha and Haftu, 2014)**.**

Gastro-intestinal (GI) parasitic infections may be either clinical or sub-clinical. Heavy infections can result in death before clinical signs appear such as progressive weight loss, weakness, anorexia and diarrhea. The prevalence of GIT parasite species and the severity of infection also vary considerably depending on local environmental conditions such as humidity, temperature, rainfall, vegetation and management practice **(**Bacha and Haftu, 2014**).**

The prevalence rates of bovine gastrointestinal parasitic infections in three states of northeast India bordering Myanmar and Bangladesh varied in different states. In that study, a total 50 faecal samples were examined by centrifugal flotation and sedimentation method. Samples collected from Tripura and Meghalaya showed that all the adult animals were infected with amphistomes species since the areas were favorable for propagation of snail intermediate host; considerable number (45.4 % in Meghalaya and 17 % in Tripura) were also infected with *Fasciola* sp. Samples were collected from Champhai district, area of Mizoram, bordering Myanmar but being hilly with no water logging, the occurrence of trematode infection was not found, in that

[1] Principal, Thingangyun Education College
[2] Associate Professor, Department of Microbiology, University of Medicine 2, Yangon
[3] Professor and Head, Department of Zoology, Panglong University

study but the animals were found to be infected with *Strongyloides* sp. parasites (52.9 % and 56.7 %) in young and adult animals respectively (Lalrinkima, 2016).

Basically there are two types of cattle farming in Myanmar. They are Dairy cattle and Zebu (Ploughing cattle). There is limited data on cow intestinal parasites in Myanmar. It is necessary to detect the incidence of intestinal parasites in cows since the high incidence of parasitism. Thus the present study was conducted with the aim to determine the occurrence of common GI parasites in Zebu and to assess seasonal prevalence rates of the intestinal parasites.

Material and Methods

Study site

The fresh faecal samples of Zebu were collected from Myin Hmwe Village 21˙55´ N, 95˙ 53´ E in Saging Township, Sagaing Region.

(Plate 1 and 2)

Study period

The study period was from January to December, 2019.

Plate 1 Satellite Image of Myin Hmwe Village (Google Earth, 2019)

Plate 2 Zebu Cattle

Sampling procedure

Samples were collected by simple random sampling method. In Myin Hmwe village, there are about 290 houses and total numbers of cattles were about 1200 and there were about 4 cattles in each household farm. A total of 50 faecal samples were collected for analysis of gastrointestinal parasites in each season (hot season, rainy season and cold season).

Preparation of samples for egg count

In a paper cup, 3 grams of faeces were mixed with 50 ml of sugar solution and stirred well. The mixes were poured through a strainer into another paper cup. The strained sample was poured from the paper cup into the test tubes that was secured in an upright position. The test tubes were filled up to the top so that when a cover slip was put onto the test tube, the fluid would be just in contact with the cover slip, but not spilled over and kept for 20 minutes. Subsequently the cover slip was carefully lifted straight up and gently placed onto the center of a microscope slide (WHO, 1991). The slides were then examined under the light microscope at 100X and 400X magnifications.

Identification of parasites eggs

The parasite ova were identified according to Soulsby, (1982) Chiodin *et* al., Cheesbrough and Monica, (1991), WHO (1991) and Zajac and Conboy (2012).

Intensity of infection

The number of helminths infecting individual cattle was measured directly by counting the number of eggs excreted in faeces.

Calculation of egg count per gram (EPG)

The following formula was used:

Eggs / g = (no. eggs counted x T/V)/F

T = total volume of faeces /flotation solution mixture

V = volume of aliquot examined in slide

F = grams of faeces

100 – 250 EPG – Not significant amount

250 -500 EPG – Low infection level

500 -1000 EPG – Moderate infection level

>1000 EPG - High infection level

(WHO, 2002; Zajac and Conboy, 2012)

Results

A total of 50 faecal samples were examined. Most Zebu cattles were found to be infected i.e. in the hot season 37 (74%), followed by cold season 30 (60%) and lowest in rainy season 26 (52%) (Table 1). A total of 15 males and 22 females were found to be infected in the hot season, 12 and 14 in the rainy season, and11 and 18 in the cold season respectively (Table2).

In the present study, intestinal parasites namely, eggs of *Strongyloides* sp., *Trichuris* sp. *Fasciola hepatica*, *Ascaris* sp. and oocysts of *Eimeria* sp. were detected in the feaces of zebu cattles (Table 3,4,5and Fig. 2,3, 4, Plate 3).

Only three types of warm parasites and one protozoan were observed in hot season i.e. *Strongyloides* sp., *Trichuris* sp., *Fasciola hepatica* and *Eimeria* sp. (Table 3, Fig.3). Moreover, many species of parasites consisting *Strongyloides* sp., *Trichuris* sp., *Ascaris* sp., *Fasciola hepatica* and *Eimeria* sp. were found in cold season (Table 5, Fig.4).

Eimeria sp. was higher in the rainy season than in the cold season. *Ascaris* sp. was found only in the cold season. *Trichuris* sp. was not found in the rainy season.

Strongyloides sp. and *Fasciola hepatica* were found in all ages of the cattle. *Eimeria* sp. and *Trichuris* sp. infections were detected in only young cattle.

In this study, regarding of the studied cattle, parasitism was high in Zebu and seasonal variation was also detected (Table 6 Fig.5).

Table 1 Seasonal prevalence of common parasites in the Zebu ploughing cattles of Myin Hmwe Village (January– December, 2019)

Number of cattles	Season	Infected (n)	% of total
n=50/season	Hot	37	74
	Rainy	26	52
	Cold	30	60

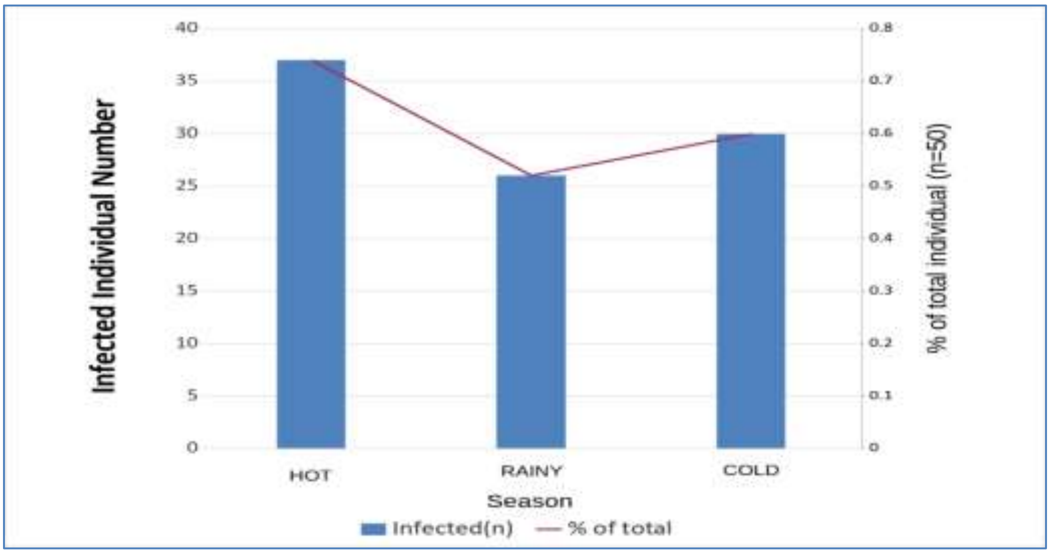

Figure 1 Seasonal prevalence of common parasites in faeces of Zebu cattles

Table 2 Seasonal prevalence of intestinal parasites in different sexes of Zebu (2019)

Types of cattle	Hot season		Rainy season		Cold season	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Infected cattle	15	22	12	14	11	18
Non-infected cattle	10	3	13	11	14	7
Total	**25**	**25**	**25**	**25**	**25**	**25**

Table 3 Distribution of parasitic eggs and oocysts in different ages of Zebu (Hot season, 2019)

Age (Yr) of cattle	Parasite species	Eggs/g
1½, 2,3, 4,5,6,7	*Strongyloides* sp.	8133
1½ ,2	*Trichuris* sp.	600
1½, 2 ,4 ,7	*Fasciola hepatica*	1333
1½ , 2 , 5	*Eimeria* sp.	43733

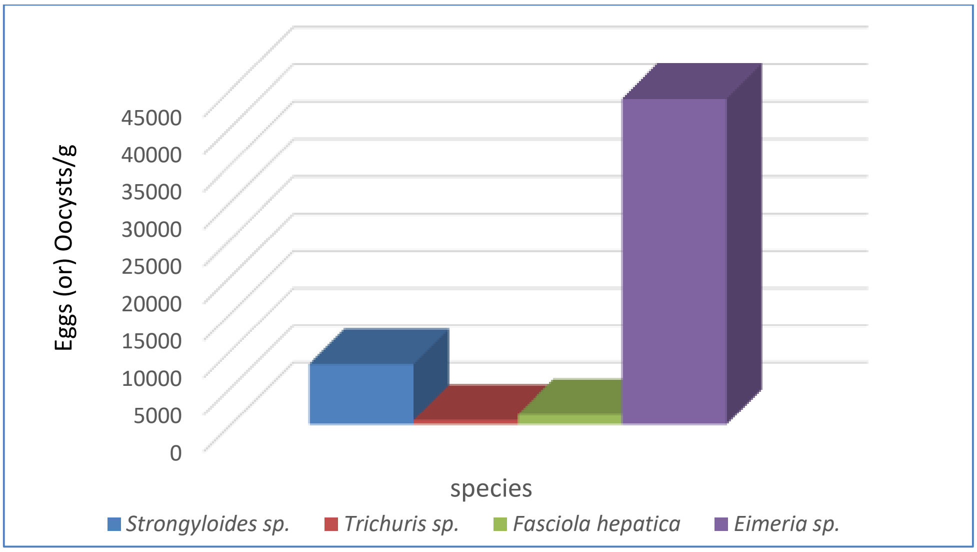

Figure 2 Number of parasitic eggs and oocysts in the faecal samples of Zebu (Hot season, 2019)

Table 4 Distribution of parasitic eggs and oocysts in the different ages of Zebu (Rainy season, 2019)

Age (Yr) of cattle	Parasite species	Eggs/g
1 ½, 2 ,3, 4 , 6	*Strongyloides* sp.	3200
1 ½, 2 , 4, 6 , 7	*Fasciola hepatica*	3467
1 ½,2 ,3 , 5	*Eimeria* sp.	6733

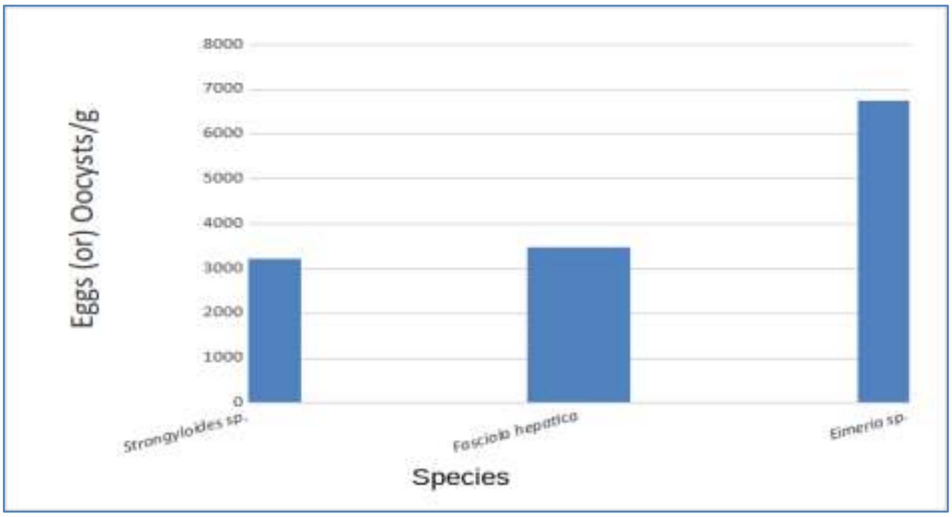

Figure 3 Number of parasitic eggs and oocysts in the faecal samples of Zebu (Rainy season, 2019)

Table 5 Distribution of parasitic eggs and oocysts among the different ages of Zebu (Cold season, 2019)

Age (Yr) of cattle	Types of parasites	ggs/g
1 ½, 3, 5	*Strongyloides* sp.	267
2, 7	*Trichuris* sp.	200
1 ½, 3, 5	*Ascaris* sp.	333
1 ½, 2, 3, 4, 5, 6, 7	*Fasciola hepatica*	1200
1 ½, 2	*Eimeria* sp.	133

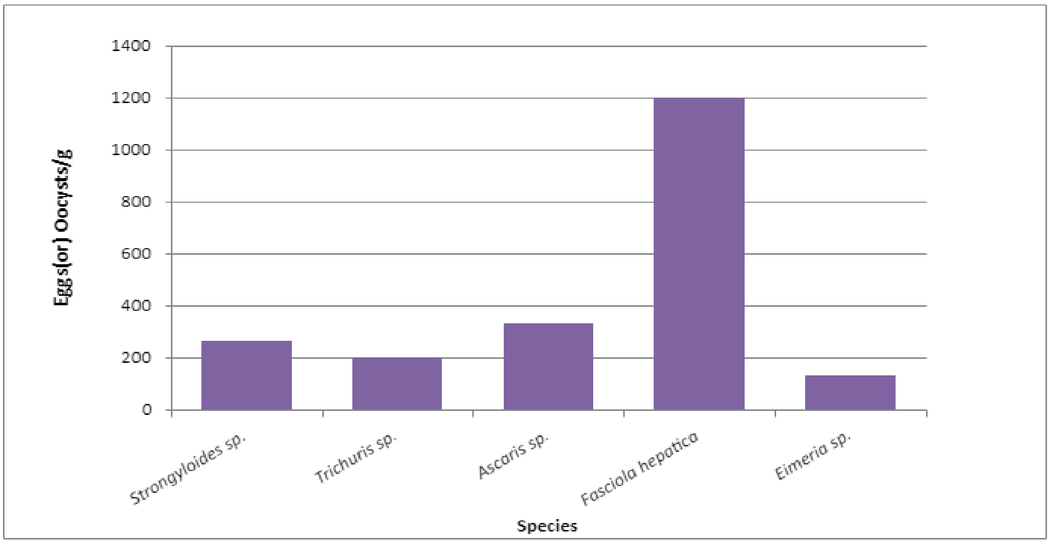

Figure 4 Counts of parasitic eggs and of oocysts among the Zebu (Cold season, 2019)

Table 6 Seasonal variations of common intestinal parasites among the Zebu (2019)

No	Parasite species	Hot season	Rainy season	Cold season
1	*Strongyloides* sp.	8133	3200	267
2	*Trichuris* sp.	600	0	200
3	*Ascaris* sp.	0	0	333
4	*Fasciola hepatica*	1333	3467	1200
5	*Eimeria* sp.	43733	6733	133

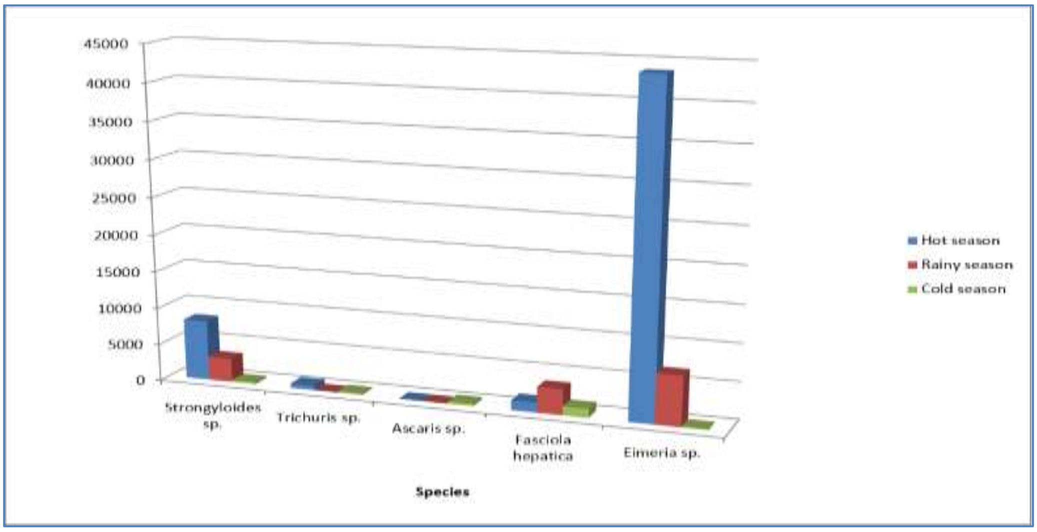

Figure 5 Seasonal variations of types of common intestinal parasitic eggs and oocysts among the Zebu

A. *Strongyloides* eggs (400X)

B. *Trichuris* eggs (400X)

C. *Ascaris* egg (400X)

D. *Eimeria* (400X)

E. *Fasciola hepatica* (400X)

Plate 3 Recorded parasitic eggs and oocysts

Discussion

The gastrointestinal parasites are one of the major health problems affecting the productivity of the cattle and sheep worldwide. In this study, prevalence of infection were high (52% -74%) in Zebu cattle at all season but was highest (74%) in the hot season.

In this study, cattle were infected mostly with nematodes including mostly *Strongyloides* sp.followed by *Trichuris* sp.,and,*Ascaris* sp. In addition, there was a common cestode, *Fasciola hepatica* and a protozoan parasite, *Eimeria* sp. among the parasites detected.

Cattle can acquire infections with any of several species of roundworms like Ascaris when grazing in pastures. The gastrointestinal tract of cattle is often infected with hairworms also called stomach worms and intestinal worms. These worms are transmitted when infected cattle pass eggs in manure onto the ground where the eggs hatch into larvae in the manure. After rain washes the larvae from the manure, the cattle then swallow larvae on wet grass in moderate temperatures.

Eberhard *et. al,* (2008) reported that in Southern Germany the predominant *Strongyloides* species were found in cattle and in sheep. The results of the present study for Zebu cattle were in agreement with their findings. *Strongyloides* sp. were most infected among the Zebu cattle in Myin Hmwe village. The recorded species of *Eimeria* had also high infection level.

Eimeria sp. cause Coccidiosis, a commonly disease of young cattle (1-2 months to1year of age) and usually is sporadic during the wet season of the year: coccidiosis typically results in diarrhea, weight loss and dehydration. A combination of these factors may result in poor growth and death of the animal (Thanmborg *et al*., 2017).

Interestingly, female cattle were found to be more infected in this study. However in the study of Algeria, except the *Emiera* sp, all prevalence rate of gastrointestinal parasites in male cattle were higher than female cattle (Moussouni , 2018).

High infection level of *Fasciola hepatica* was also observed in the study site. The Liver fluke is a widely spread parasite of ruminants which can have significant economic impact on cattle production (Schweizer *et al*, 2005).

Trichuris sp. infections are common in young calves and yearlings but the numbers of worms are seldom large. The eggs are resistant and infections are likely to persist in cattle premises. Clinical signs are unlikely but in occasional heavy infections, dark feces, anemia and anonexia may be seen (Merck manual, 1955).

Similar to the present study, the prevalence rates of Strongyloides sp., *Fasciola hepatica*, *Eimeria* sp.in young cattle were higher than old cattles (Moussouni, 2018)

In this study, Zebu (or) ploughing cattle in Myin Mhwe Village was found to be moderately to highly infected with common intestinal parasites. It is advisable to carry out regular deworming of cattle to reduce these infections. However, sanitary disposal of their faeces and proper hygiene of their feed are also essential to control the intestinal parasitic infections in Zebu cattle of the village in Sagaing Township.

Conclusion

In the present study, five species of intestinal parasites namely (i) *Strongyloides* sp. (ii) *Trichuris* sp. (iii) *Ascaris sp.* (iv) *Fasciola hepatica*, and (v) *Eimeria* sp. were observed in faecal sample of Zebu cattle. Female cattle were more infected than the males. Seasonally, hot(summer or dry) season showed the highest rate of intestinal parasitic infections.

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ICHTHYOFAUNAL DIVERSITY AND SPECIES RICHNESS IN MYIT DAUNT GWAE IN (LAKE), MYINGYAN TOWNSHIP, MANDALAY REGION

Su Mon Kyaw[1], Naw Dolly Wilbur[2], Thin Thin Khaing[3]

Abstract

Diversity of fish fauna in Myit Daunt Gwae In (Lake) was studied from September 2017 to August 2018 in two sampling sites (western part of the In Site I and eastern part of the In Site II). In the present study, a total of 42 species confined to 29 genera, 17 families and eight orders were recorded. In Site I total number of 189254 individuals with 42 species and in Site II 149786 individuals with 42 species were recorded. Diversity of fish species was assessed by calculating the various diversity indices such as Shannon-Wiener's index (H'), Simpson's index (D), Margalef's index of species richness and Hill's diversity number (N_1), (N_2) and (E). Diversity indices varied among the two sampling sites according to the catch in terms of both the number of species and the number of individuals. The values of species richness and diversity indices were found to be higher in Site I than in Site II. On the seasonal basis, the highest value of diversity indices was observed in cold season at Site I and in rainy season at Site II. According to the number of fish species, individuals and diversity indices, the study area Myint Daunt Gwae In may be related to the suitable ecology of water body. So there is a need to continuous maintain the In (Lake) in order to protect and conserve the fish fauna to thrive in the In (Lake).

Keywords: Fish fauna, Diversity indices, Species richness, Myint Daunt Gwae In.

Introduction

Fishes are important elements in the economy of many nations as they have been a stable item in the diet of many people. They constitute slightly more than one-half of total number of approximately 54,711 recognized living vertebrate species; there are descriptions of an estimated 27,977 valid species of fishes (Nelson, 2006). According to Ehrlich and Wilson (1991) biodiversity is essential for stabilization of ecosystem, protection of overall environmental quality for understanding intrinsic worth of all species on the earth. Fish diversity of river essentially represents the fish faunal diversity and their abundance. River conserves a rich variety of fish species which support to the commercial fisheries.

Many fish species have become highly endangered particularly in rivers where heavy demand is placed on freshwater. However, the impact of the anthropogenic activities, habitat degradation, exotic species introduction, water diversions, pollution and global climate change are the main causative agents for the aquatic species rapid decline (Basavaraja *et al*., 2014). Species diversity of aquatic organisms in flood plains connected with large rivers is always large, because they can easily recognize the floodplain from the main river channels and other permanently aquatic habitats (Junk, 1996).

The floodplain environment also promotes species diversity due to its dynamic habitat structure (Junk, 1989). In the present study, the study area Myit Daunt Gwae In is also floodplain Lake of Ayeyawady River and plays a significant role in supplying fish as a source of protein and providing livelihood to the local people. The biodiversity and its conservation are regarded as one of the major issues of enabling sustainable use of natural resources and are essential for the Myit

[1] Lecturer, Zoology Department, University of Meiktila
[2] Dr, Professor (Retd), Zoology Department, University of Mandalay
[3] Dr, Associate Professor , Zoology Department, University of Myingyan

Daunt Gwae In . Thus, the present study was undertaken to evaluate and assess the species richness, diversity and evenness of fish fauna in Myit Daunt Gwae In.

Materials and Methods

Study Area

The study area Myit Daunt Gwae In is situated in Myingyan Township, Mandalay Region. It lies about 2 km away from Myingyan and is located between 21°24'56" to 21°25'12" N Latitudes and 95°19'53" to 95°21'48" E Longitudes. The study area was divided into two sampling sites. The Site I is western part of the In situated near the Kuwe Gyan Village and Site II is eastern part of the In situated near the Lin Gyi Village (Fig 1).

Study Period

The study period was conducted from September 2017 to August 2018.

Specimen Collection and Preservation

The specimen collection was carried out at two sampling sites on monthly basis throughout the study period. Fish specimens were collected with the help of local fishermen using different types of fishing gears. The physical appearance of fish was noted down and photographs taken immediately after capturing the fish. At least five specimens were collected and preserved in 5% or 10% formalin depending on the size of specimens.

Identification and Classification of Fish Specimens

Collected fish specimens were identified and classified according to Talwar and Jhingran (1991) and Jayaram (2013).

Data Analysis

To measure the species richness, diversity and evenness of fish species, four methods of diversity were applied: Margalef (1958), Simpson (1949), Shannon-Wiener (1948) and Hill (1973) as given in Ludwing and Reynolds (1988).

Species richness of fish species were determined by using the formula of Margalef's index (1958) as follows:

For Margalef's species richness index (1958),

$$d = \frac{S-1}{\mathrm{Ln}(N)}$$

Where, d	=	Margalef's species richness index
S	=	number of species
N	=	total number of individuals in the sample

This method incorporates the total number of individuals and it is the measure of the number of species present for a given number of individuals.

Species diversity was determined by using two formulae of Simpson's index "D" and Shannon-Wiener's Information theory index H'.

For Simpson's index (1949),

$$D = \sum_{i=1}^{s} \frac{n_i(n_i - 1)}{n(n-1)}$$

Where, D = Simpson's index

n_i = total number of individuals in the i^{th} species

n = total number of individuals of all species

It ranges in value from 0 to 1. With this index, 0 represents infinite diversity and 1, no diversity. That is, the higher the value of D, the lower the diversity.

For Shannon-Wiener's index (1948),

$$H' = -\sum_{i=1}^{s} P_i (\mathrm{Ln} P_i)$$

Where, H' = Index of species diversity

S = number of species

P_i = the proportion of each species

ln = Natural Logarithm

$$P_i = \frac{n_i}{n}$$

ni = total number of individuals in the i^{th} species

n = total number of individuals of all species

A great number of species increase diversity, and a more even or equitable distribution among species will increase species diversity measured by Shannon- Wiener's function.

For Hill's diversity numbers (1973),

Number 0: N_0 = S

Where,

S = total number of species

N_0 = number of all species in the sample

Number 1: N_1 = $e^{H'}$

Where,

H' = Shannon's index

N_1 = number of abundant species in the sample

Number 2: N_2 = $\frac{1}{D}$

Where,

D = Simpson's index

N_2 = number of very abundant species in the sample

Note that N_1 is always being intermediate between N_0 and N_2.

The effective number of fish species is a measure of the number of species in the sample where each species is weighed by its abundance.

The measure of fish species evenness or equitability (or relative species abundances) was determined by using the evenness index of modified Hill's ratio (1973)

$$E = \frac{(1/D) - 1}{e^{H'-1}} = \frac{N_2 - 1}{N_1 - 1}$$

Where,

E	=	Hill's evenness index (which approaches zero)
D	=	Simpson's index
H'	=	Shannon's index
N_1	=	Number of abundant species in the sample
N_2	=	Number of very abundant species in the sample

Source: Google earth pro, 2018

Figure 1 Location map of study area

Results

Species Composition and Fish Species Recorded

In the present study, a total of 42 species confined to 29 genera distributed among 15 families and eight orders were recorded. Among eight orders, Osteoglossiformes, Beloniformes and Tetraodontiformes were each represented by a single species, genus and family. Clupeiformes was confined to two species, two genera and one family, while Cypriniformes was 15 species, ten genera and one family. Siluriformes was represented 12 species, seven genera and four families, whereas Perciformes was seven species, five genera and five families. The remaining order Synbranchiformes was three species, two genera and one family.

The percentage of fish species composition shown that order Cypriniformes was most dominant constituting 35.71% followed by order Siluriformes 28.57%, Perciformes 16.67%, Synbranchiformes 7.14%, Clupeiformes 4.76% and the remaining three orders namely, Osteoglossiformes, Beloniformes and Tetradontiformes were 2.38% each (Fig 2).

Species Richness, Diversity and Evenness of Fish Fauna

To get a better description of fish diversity, a measure of species richness and evenness of their distribution were undertaken during the study period. According to Margalef's species richness, in Site I the highest value of species richness (d = 3.735) was observed in November while the lowest value (d = 2.761) in April (Table 1, Fig 3). In Site II the highest value of species richness (d = 3.757) was recorded in October whereas the lowest value (d = 2.617) in April (Table 2, Fig 3).

During the study period, two diversity indices were calculated using Shannon-Wiener's diversity index (H') and Simpson's diversity index (D). At Site I, the highest value of Simpson's index (D = 0.113) and Shannon Wiener's index (H'= 2.549) was found in December while the lowest value of (D = 0.278) and (H'= 1.899) was observed in May. In Hill's diversity number, the highest value of abundant species (N_1 = 12.807) and very abundant species (N_2 = 8.815) were observed in December whereas the lowest value of (N_1= 6.679) and (N_2 = 3.601) were recorded in May. The highest value of evenness (E = 0.664) was recorded in September and the lowest value (E = 0.444) in June (Table 1, Fig 3 and 4).

At Site II, the highest value of Simpson's index (D = 0.114) and Shannon Wiener's index (H'= 2.567) was observed in August whereas the lowest value of (D = 0.205) and (H'= 2.084) in April. The highest value of abundant species (N_1 = 13.024) and very abundant species (N_2 = 8.775) were observed in August while the lowest value of (N_1 = 8.034) and (N_2 = 4.868) were recorded in April. The highest value of evenness (E = 0.705) was recorded in January and the lowest value (E = 0.524) was observed in June (Table 2, Fig 3 and 4).

During the study period, the value of species richness, diversity and evenness was varied according to different season at both study sites. The highest value of Margalef's species richness (d = 3.869) and (d = 3.911) were observed in hot season while the lowest value (d = 3.543) and (d = 3.513) were found in rainy season at both study sites. At Site I, the highest value of (D = 0.114) was observed in cold season and (D = 0.119) was recorded in rainy season at Site II. The lowest value of (D = 0.161) and (D = 0.159) were recorded in hot season at both study sites. The highest value of (H'= 2.576) was found in cold season at Site I and (H'= 2.565) was observed in rainy season at Site II. At both study sites the lowest values of (H'= 2.286) and (H'= 2.304) were recorded in hot season (Table 3 and 4, Fig 5 and 6).

Hill's diversity number, (N_1 = 13.150), (N_2 = 8.770) and (E = 0.639) were found to be highest in cold season at Site I while (N_1= 12.996) and (N_2 = 8.362) were to be highest in rainy season and (E = 0.619) in cold season at Site II. At Site I, the lowest value of (N_1= 9.833) and (N_2 = 6.221) and (E = 0.591) was observed in hot season and the lowest value of (N_1= 10.016), (N_2 = 6.286) and (E = 0.586) were also recorded in hot season at Site II (Table 3 and 4, Fig 5 and 6).

Table 1 Monthly diversity indices evaluated on the number of fish species and individuals recorded in Site I during September 2017 to August 2018

Diversity indices	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Total number of species	36	40	40	38	35	34	33	24	29	28	28	34
Total number of individuals	38522	38232	34282	20246	12322	9102	7109	4145	3487	1671	1484	18652
d	3.315	3.696	3.735	3.731	3.609	3.619	3.608	2.761	3.433	3.638	3.697	3.356
D	0.122	0.117	0.117	0.113	0.129	0.139	0.163	0.192	0.278	0.224	0.154	0.116
H'	2.469	2.522	2.541	2.549	2.472	2.347	2.246	2.117	1.899	2.173	2.467	2.531
N_1	11.814	12.449	12.692	12.807	11.84	10.456	9.446	8.304	6.679	8.781	11.787	12.565
N_2	8.175	8.542	8.565	8.815	7.769	7.158	6.135	5.215	3.601	4.454	6.473	8.624
E	0.664	0.659	0.647	0.662	0.624	0.651	0.608	0.577	0.458	0.444	0.507	0.659

Table 2 Monthly diversity indices evaluated on the number of fish species and individuals recorded in Site II during September 2017 to August 2018

Diversity indices	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Total number of species	35	40	37	36	31	30	29	22	29	25	27	34
Total number of individuals	30562	32212	27404	15985	7776	6342	4840	3053	2311	1181	1280	16835
d	3.292	3.757	3.523	3.615	3.349	3.312	3.300	2.617	3.615	3.393	3.634	3.391
D	0.128	0.119	0.126	0.126	0.137	0.155	0.164	0.205	0.159	0.189	0.131	0.114
H'	2.451	2.509	2.499	2.505	2.299	2.269	2.239	2.084	2.323	2.214	2.516	2.567
N_1	11.593	12.296	12.174	12.244	9.966	9.665	9.385	8.034	10.209	9.149	12.378	13.024
N_2	7.826	8.349	7.968	7.948	7.319	6.468	6.111	4.868	6.251	5.273	7.654	8.775
E	0.644	0.651	0.624	0.618	0.705	0.631	0.609	0.549	0.570	0.524	0.585	0.647

Table 3 Seasonal diversity indices of fish species recorded in Site I from Myit Daunt Gwae In (Lake) during September 2017 to August 2018

Diversity indices	Margalef's species richness	Simpson's diversity index (D)	Shannon-Wiener's diversity index (H')	Hill's diversity index (N_1)	Hill's diversity index (N_2)	Hill's evenness index (E)
Hot Season	3.869	0.161	2.286	9.833	6.221	0.591
Rainy Season	3.543	0.117	2.572	13.092	8.516	0.623
Cold Season	3.546	0.114	2.576	13.150	8.770	0.639

Table 4 Seasonal diversity indices of fish species recorded in Site II from Myit Daunt Gwae In (Lake) during September 2017 to August 2018

Diversity indices	Margalef's species richness	Simpson's diversity index (D)	Shannon-Wiener's diversity index (H')	Hill's diversity index (N_1)	Hill's diversity index (N_2)	Hill's evenness index (E)
Hot Season	3.911	0.159	2.304	10.016	6.286	0.586
Rainy Season	3.513	0.119	2.565	12.996	8.362	0.614
Cold Season	3.618	0.121	2.546	12.759	8.273	0.619

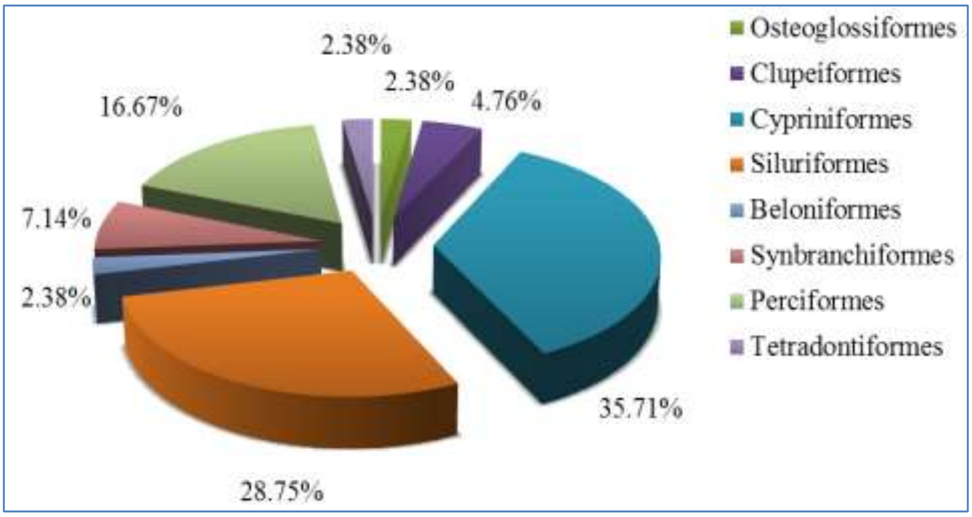

Figure 2 Percent composition of fish species in different orders in Myit Daunt Gwae In (Lake) during September 2017 to August 2018

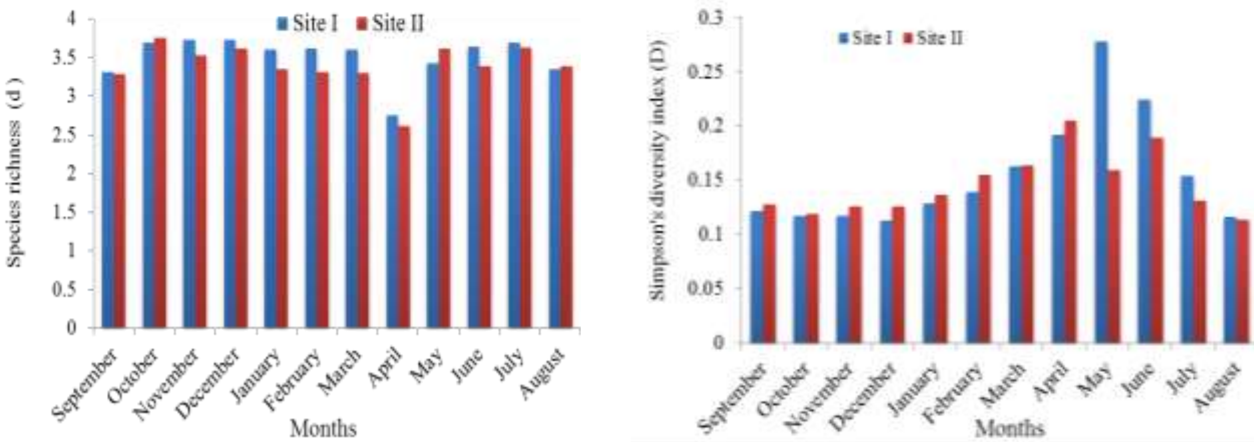

Figure 3 Comparison of the Margalef's species richness (d) and the Simpson's diversity index (D) between the two study sites during September 2017 to August 2018

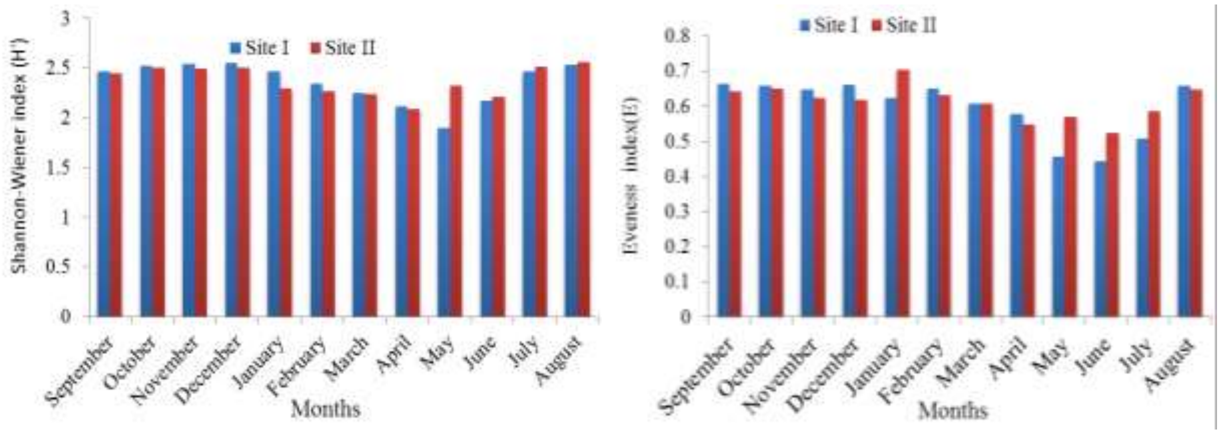

Figure 4 Comparison of the Shannon-Wiener's diversity index (H') and Hill's evenness index (E) between the two study sites during September 2017 to August 2018

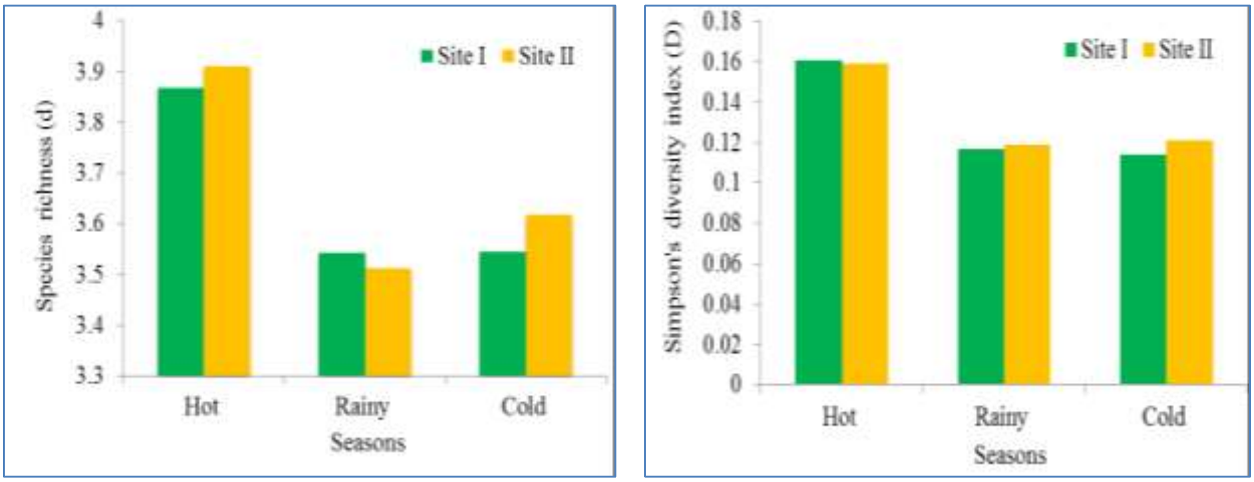

Figure 5 Seasonal comparison on Margalef's species richness (d) and Simpson's diversity index (D) of fish fauna in Myit Daunt Gwae In (Lake) during September 2017 to August 2018

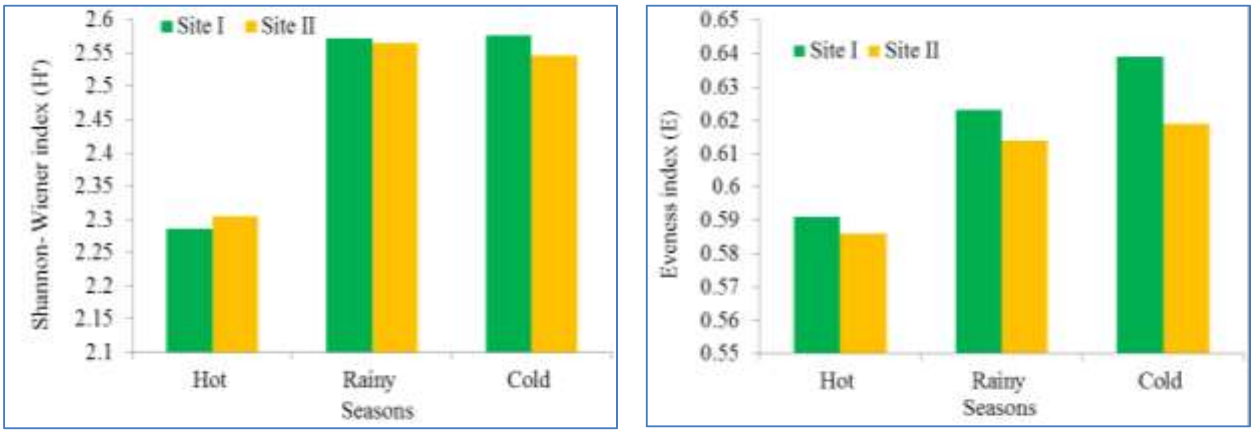

Figure 6 Seasonal comparison on Shannon-Wiener's index (H') and Hill's evenness index (E) of fish fauna in Myit Daunt Gwae In (Lake) during September 2017 to August 2018

Discussion

Biodiversity represents trophic status of an aquatic system. It is very essential to have a sufficient knowledge about fish faunal diversity and their present status because fish comprises the half of the total number of vertebrates in the world and they are the nutritional food source mainly the protein (Nath and Patra, 2017).

In the present study, a total of 42 species confined to 29 genera distributed among, 15 families and eight orders were recorded. In Site I total number of 189254 individuals with 42 species and in Site II 149786 individuals with 42 species were observed.

Magurran (2004) stated that biological diversity into two components; species richness and species evenness. Species richness measure that focus on the component of diversity. During the study period, Margalef's species richness (d) showed that it was highest in November at Site I and in October at Site II while lowest index was found in April at both study sites. A total of 40 species was observed in November and October at Site I and in October at Site II. The species richness was observed to be highest in November and October at Site I and Site II. It is assumed that this may be due to favorable condition such as sufficient water and ample food resources in these months for fish survival and aggregated in the In (Lake).

In the present study, species diversity was calculated by using two formulae indices, viz; Simpson's diversity index (D) and Shannon Wiener's index (H'). The highest value of (D), (H'), (N_1) and (N_2) were observed in December at Site I and in August at Site II. However, the lowest value of (D), (H'), (N_1) and (N_2) were recorded in May at Site I and in April at Site II. The highest value of (E) was observed in September at Site I and in January at Site II while the lowest in June at both study sites. When compare on the two sampling sites, species diversity was found to be higher in Site I than that of in Site II. This may be due to temporal variation and difference in their habitat types in the In (Lake).

According to Simpson's diversity index, the values range between 0 and 1 with this index 0 represents infinite diversity and 1, no diversity. This is the bigger the value of D, the lower the diversity. Hill's (1973) proposed a unification of several diversity measures in a single statistic while (N_1) is equivalent of Shannon diversity, (N_2) the reciprocal of Simpson's diversity. The value of Hill's evenness index (E) varied between 1 and 0. The closer to 1 the more even the population of fish that from the community.

In Myanmar, a clear defined hot season is from February to May, rainy season from June to September and cold season from October to January. On the seasonal basis, in the present study except Margalef's species richness (d), the values of (D), (H'), (N_1), (N_2) and (E) were observed to be highest in cold season at Site I. It may be due to healthy environment, water temperature, stable water environment and food available is optimal in this season for fish fauna. At Site II except Margalef's species richness (d), and Hill's evenness (E), the remaining diversity indices were found to be highest in rainy season. It is assumed that this may be due to during the rainy season, flood water of Ayeyawady River causes the entry of water with new stock of fish species from narrow channel near the Naung Bin village into the In where they utilize this habitat for their spawning and survival of larvae to adult. Thus the diversity of fish species may increase during the rainy season.

In the work of Htay Htay Sein (2010), described that the diversity was highest in rainy season in Lay- Eain- Su- Let- Kyar- In (Lake). Ni Ni Aye (2013) stated that diversity index was highest in cold season in the Synye In (Lake). Galib (2013), diversity and richness indices showed that diversity of fish fauna was higher in the winter months than other months. The maximum number of fish species was also recorded during this time. This is because, may be water depth reduced to minimum due to lack of sufficient rainfall this time allowing fishermen to employ their fishing gears more effectively.

According to Nath and Patra (2017), determination of biodiversity has become very essential aspect to understand and express the condition of an ecosystem. In the present study, the results revealed that the number of fish species, individuals, fish faunal diversity and their production in Myit Daunt Gwae In may be related to the suitable ecology of water body. In order to maintain the species diversity it is essential to preserve the habitat in which the living assets thrive.

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SCREENING THE EFFECTS OF PHOSPHATE SOLUBILIZING ACTERIA ON THE GERMINATION AND GROWTH RATE OF BLACK GRAM

Wai Zin Min[1] and Kyaw Myo Naing[2]

Abstract

Phosphorus is an important macronutrient in plant metabolism, ultimately reflected on crop yields. Phosphate solubilizing bacteria (PSB) play essential role in inorganic and organic soil P solubilization and mineralization for plant growth. This experiment was conducted to study the effect of phosphate solubilizing bacteria (PSB) on the growth of Black gram. Among 10 isolated phosphate solubilizing bacteria from the rhizosphere of *Vigna catjang* Walp (Cow pea) and *Phaseolus mungo* Lin (Black gram), six strains, PSBCPR-1, PSBCPR-2, PSBBGR-2, PSBBGR-6, PSBBGS-1 and PSBBGS-2 were selected and used as inoculums. Laboratory experiment with six treatments and one control (only diluted peptone water) with five replicates was carried out in Microbiology Laboratory, Zoology Department, Pathein University during June to December 2019. In this study, root length, shoot length and total seed germination rate of treated plants significantly increased ($p<0.05$) over control. The maximum root length on treatment 2 (PSBCPR.2) and maximum shoot length on treatment 3 (PSBBGR-2) and treatment 6 (PSBGBS-2) were observed. Fresh weight and dry weight of treated seedling were also significantly increased over control. The isolates obtained in this study showed a significant *in vitro* plant growth promoting activity onto black gram. The use of these bacteria as bioinoculants could be a sustainable practice to facilitate the nutrient supply to black gram plants and preventing negative side-effects.

Keywords: Phosphate solubilizing Bacteria, Rhizosphere, Black gram

Introduction

Phosphorus has been reported as one of the key elements in crop production which is associated with several vital functions and is responsible for many characteristics of plant growth such as nodule formation, cell division and organization, fat formation and transfer of heredity. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition (Qureshi *et al.,* 2012 and Saxena and Sharma, 2003).

Bacteria are more effective in phosphorus solubilization than fungi (Alam *et al.*, 2002). Phosphorus (P) is one of the essential macronutrients for plant growth and development. Plants acquire this element from soil solution as phosphate anions (Bhattacharyya and Jha, 2012).

Black gram (*Vigna mungo* L.) is one of the most important pulse crops next to chickpea, lentil and mungbean both in area and production (AIS, 2017). Black gram is important legume crop characterized by a relative high content of protein (25.67%), carbohydrates (5.4%), fat (1-3%), fibers (3.5-4.5%) and ash (4.5-5.5%), while calcium and phosphorus are 132 and 367 mg per 100 g of seed, respectively (Ahmad *et al*., 2008). Productivity of black gram is low in general due to poor management and low soil fertility. Chemical fertilizers are frequently used to achieve maximum crop production legume. These cost effective chemicals, however, when used roughly, have resulted in loss of soil fertility and consequently, the crop production. Due to these reasons, focus in recent times has been shifted towards the use of cost competitive biological resources such as Plant Growth Promoting Bacteria (Tiwari *et al.,* 2017).

[1]Assistant Lecturer, Department of Zoology, Hinthada University
[2]Lecturer, Department of Zoology, Pathein University

Plant growth promoting bacteria (PGPB) are a group of bacteria that actively colonize plant roots and increase plant growth and yield (Shaukat *et al.,* 2006). *Azospirillum, Pseudomonas* and *Azotobacter* strains could affect seed germination and seedling growth. Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* could increase root and shoot elongation in canola as well as wheat and potato (Glick *et al*., 1997).

Phosphate Solubilizing Bacteria (PSB) or Phosphobacteria (Muleta *et al.,* 2013), being an important member of PGPB family and are a viable substitute to phosphate fertilizers. Many mechanisms like lowering the pH by acid production, ion chelation, exchange reactions and polymeric substances formation in the growth environment have been reported to play a role in phosphate simultaneously P uptake by the plant and crop yields (Hameeda *et al*., 2008 and Shahab *et al.,* 2009).

Crop establishment depends on an interaction between seedbed environment and seed quality. Among the stages of the plant life cycle seed germination, seedling emergence and seed establishment are the key processes in the survival and growth of any plant species (Hadas, 2004).

Keeping in view the importance of low cost production of black gram using environmentally safe management approach, this research was carried out to investigate the effects of isolated bacteria on the germination rate and to compare the effects of isolated bacteria on the growth of Black gram seedlings as starter culture for biofertilizer.

Materials and Methods

Experimental site and study period

The experiment was conducted at Microbiology Laboratory, Department of Zoology, Pathein University during June to December 2019.

Inoculum preparation

Bacteria were grown in Pikovskaya's (PVK) agar medium. Selected isolates were grown in 10mL peptone water for 24hours at 37 ºC. Final concentrations of inoculums were made to 10^8CFU mL^{-1}. PSBCPR-1, PSBCPR-2, PSBBGR-2, PSBBGR-6, PSBBGS-1 and PSBBGS -2 were used as treatments and control (only diluted peptone water).

Sterilization of seeds

Seeds of Black gram were surface-sterilized with 0.02% sodium hypochlorite for 2 minutes and rinsed thoroughly in distilled water about five times.

Inoculation of seeds

Surface sterilized seeds (50 black gram seeds per selected isolate) were dipped into each suspension of bacteria (10^8 $CFUmL^{-1}$) for 5 hours. 0.5% of insoluble phosphate solution (gmL^{-1}) was also added in each treatment. Control seeds (50 black gram seeds) were immersed in sterile diluted peptone water for 5 hours.

Seed germination

Ten seeds per plate of inoculated and control Black gram were placed in Petri dishes with sterilized filter paper and five replicates were carried out. Petri dishes with treatments and control seeds were incubated at 30 ºC for 7 days. After 3 days the number of germinated seeds was counted. Then, root and shoot length of individual seedlings (50 seedlings from each treatments and control) were measured after 7 days.

Germination rate parameters

The seed germination was calculated by using the following formula (Gholami *et al.*, 2009).

$$\text{Germination rate (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Statistical analysis

Data of experiment was subjected to statistical analysis using IBM- SPSS software (version 25). The differences between the treatment and control means were determined by using One-way ANOVA with LSD, post-hoc test.

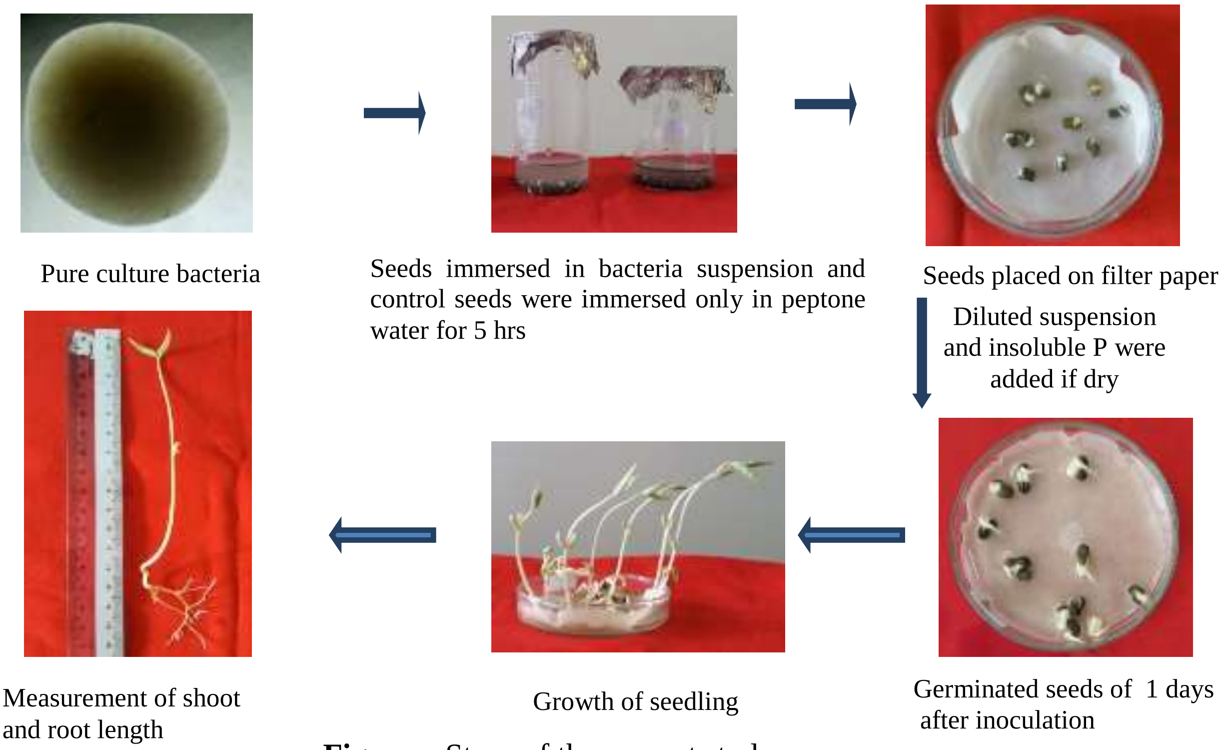

Figure Steps of the present study

Results

The seed inoculation significantly enhanced seed germination of Black gram. To evaluate the effects of selected PSB on the growth of Black gram, Laboratory experiment with six strains and Control (only diluted peptone water) with five replicates were carried out in Microbiology Laboratory, Zoology Department, Pathein University. The highest germination percentage (100%) was observed in T-4 (PSBGBR.6) and T-6 (PSBGBS.2) inoculated seedlings (Table 1, 2 and Fig 2). The maximum root length (5.0 ± 2.0 cm) was observed in T-2 (PSBCPR.2) and maximum shoot length (13.0 ± 3.1cm) was observed in T-3 (PSBBGR.2) inoculated seedlings (Table3-4 and Fig. 3). The maximum fresh root weight (0.186 ± 0.028 g) was observed in T-2 (PSBCPR.2) and maximum fresh shoot weight (0.379 ± 0.030 g) was observed in T-3 (PSBBGR.2). The maximum dry root weight (0.004 ± 0.001 g) was observed in T-2 (PSBCPR.2) and maximum fresh shoot weight (0.028 ± 0.006 g) was observed in T-3 (PSBBGR.2). In this study, total seed germination rate, root length and shoot length of treated seedlings significantly increased ($p<0.05$) over control (Table 1 to 4). Fresh shoot weight, dry shoot weight, fresh root weight and dry root weight of all treatment were also significantly increased ($p<0.05$) over control (Table 5 to 8 and Fig 4, 5).

Table 1 Effect of Phosphate solubilizing bacteria inoculation on the germination rate of Black gram

Treatment	Germination rate (Mean ± SD) (3Days)	Germinated seed (%)
Control	9.1 ± 1.0[a]	91.33
PSBCPR-1 (T-1)	9.6 ± 0.6[b]	97.33
PSBCPR-2 (T-2)	9.8 ± 0.4[b]	98.00
PSBBGR-2 (T-3)	9.9 ± 0.3[b]	98.67
PSBBGR-6 (T-4)	10.0 ± 0.0[b]	100.00
PSBBGs-1 (T-5)	9.9 ± 0.3[b]	98.67
PSBBGs-2 (T-6)	10.0 ± 0.0[b]	100.00

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 2 ANOVA result for the effect of isolated bacteria on germination rate of Black gram

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.981	6	1.330	5.313	.000
Within Groups	24.533	98	.250		
Total	**32.514**	**104**			

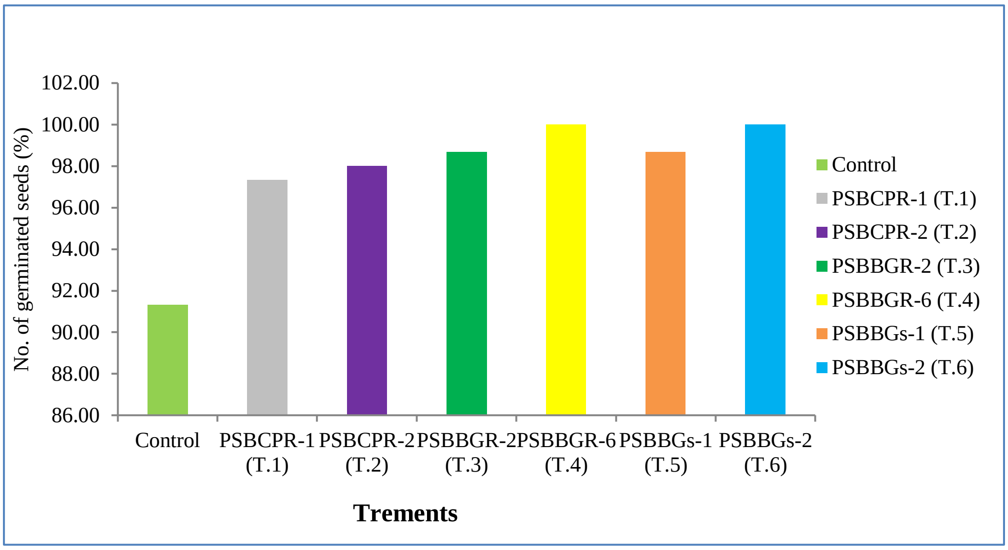

Figure 2 Effect of Phosphate solubilizing bacteria inoculation on the germination rate of Black gram

Table 3 Mean Root and Shoot length of Black gram seedlings

Treatment	Root Length (cm)(7Days)	Shoot Length (cm)(7Days)
Control	2.7 ± 1.0[a]	9.4 ± 2.8 [a]
PSBCPR-1 (T-1)	4.8 ± 2. 0 [c]	11.7 ± 2.7 [b]
PSBCPR-2 (T-2)	5.0 ± 2.0 [c]	12.3 ± 3.5 [c]
PSBBGR-2 (T-3)	4.6 ± 1.7 [b]	13.0 ± 3.1 [c]
PSBBGR-6 (T-4)	4.1 ± 1.5 [b]	12.0 ± 3.0 [b]
PSBBGs-1 (T-5)	4.0 ± 1.5 [b]	11.6 ± 2.3 [b]
PSBBGs-2 (T-6)	4.4 ± 1.8 [b]	12.9 ± 3.1 [c]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 4 ANOVA result for the effect of isolated bacteria on root and shoot length of Black gram

		Sum of Squares	df	Mean Square	F	Sig.
Root Length	Between Groups	151.418	6	25.236	8.909	.000
	Within Groups	971.590	343	2.833		
	Total	1123.009	349			
Shoot Length	Between Groups	487.833	6	81.305	9.192	.000
	Within Groups	3033.959	343	8.845		
	Total	3521.792	349			

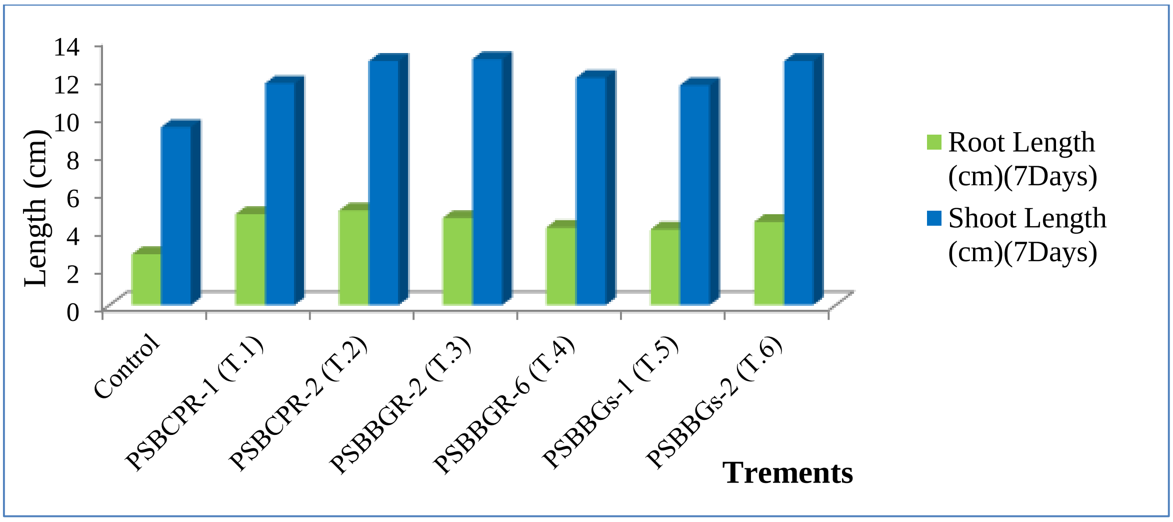

Figure 3 Effect of Phosphate solubilizing bacteria inoculation on the root length and shoot length of Black gram seedlings

Table 5 Mean fresh and dry weight of root of Black gram seedlings

Treatment	Fresh Root Weight (g)	Dry Root Weight (g)
Control	0.084 ± 0.013[a]	0.001 ± 0.001[a]
PSBCPR-1 (T-1)	0.132 ± 0.033[b]	0.003 ± 0.001[b]
PSBCPR-2 (T-2)	0.186 ± 0.028[c]	0.004 ± 0.001 [c]
PSBBGR-2 (T-3)	0.142 ± 0.043[b]	0.003 ± 0.001[b]
PSBBGR-6 (T-4)	0.134 ±0.036[b]	0.002 ±0.001[b]
PSBBGs-1 (T-5)	0.132 ± 0.030[b]	0.002 ± 0.001[b]
PSBBGs-2 (T-6)	0.136 ± 0.034[c]	0.003± 0.001[b]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 6 ANOVA result for the effect of isolated bacteria on fresh root weight and dry root weight of Black gram

ANOVA (Root and Shoot Lengths)						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Root Length	Between Groups	151.418	6	25.236	8.909	.000
	Within Groups	971.590	343	2.833		
	Total	1123.009	349			
Shoot Length	Between Groups	487.833	6	81.305	9.192	.000
	Within Groups	3033.959	343	8.845		
	Total	3521.792	349			

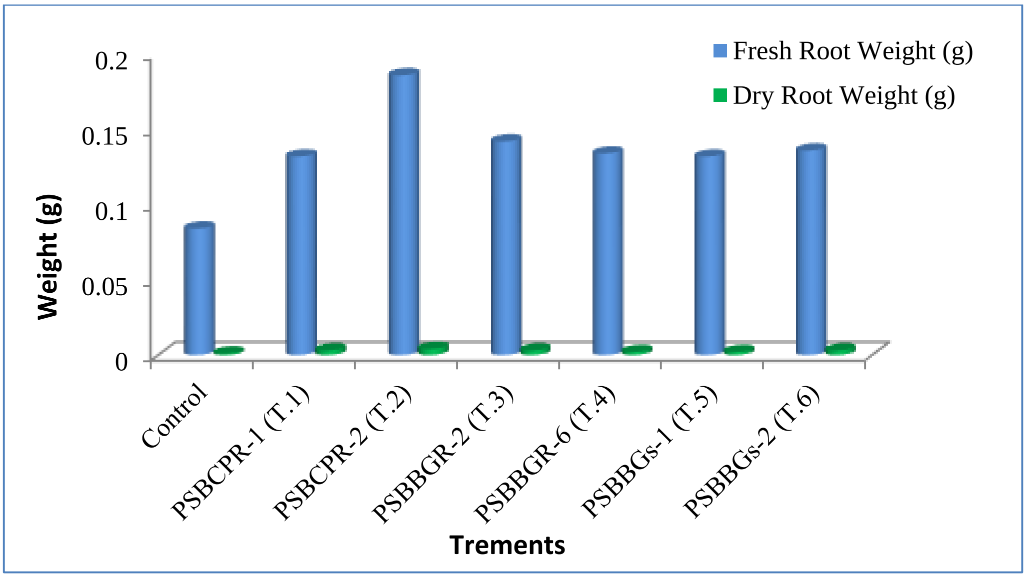

Figure 4 Fresh and dry root weight of Black gram seedlings

Table 7 Fresh and dry shoot weights of Black gram seedlings

Treatment	Fresh Shoot weight (g)	Dry Shoot weight (g)
Control	0.226 ± 0.027[a]	0.013 ± 0.004[a]
PSBCPR-1 (T-1)	0.327 ± 0.042[b]	0.019 ± 0.002[b]
PSBCPR-2 (T-2)	0.331 ± 0.035[b]	0.023 ± 0.004[b]
PSBBGR-2 (T-3)	0.379 ± 0.030[c]	0.028 ± 0.006[c]
PSBBGR-6 (T-4)	0.322 ±0.034[b]	0.021 ±0.004[b]
PSBBGs-1 (T-5)	0.310 ± 0.043[b]	0.019 ± 0.002[b]
PSBBGs-2 (T-6)	0.351 ± 0.037[c]	0.022± 0.005[c]

Means followed by a common letter in the same column are not significantly different at 5% level by LSD

Table 8 ANOVA result for the effect of isolated bacteria on fresh shoot weight and dry shoot weight of Black gram

ANOVA for Shoot Weight

		Sum of Squares	df	Mean Square	F	Sig.
Fresh Shoot Weight	Between Groups	.685	6	.114	89.396	.000
	Within Groups	.438	343	.001		
	Total	1.123	349			
Dry Shoot Weight	Between Groups	.007	6	.001	69.192	.000
	Within Groups	.005	343	.000		
	Total	.012	349			

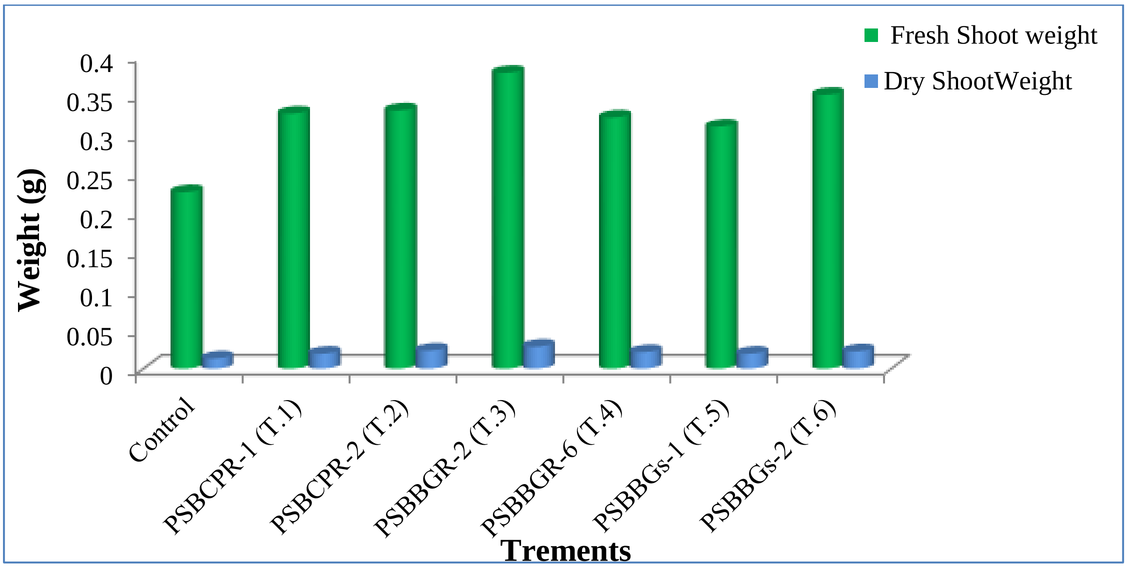

Figure 5 Fresh and Dry shoot weight of Black gram seedlings

A. Inoculated seeds (1 day)

B. Inoculated seeds (2 day)

Figure 6. Observation of seed germination

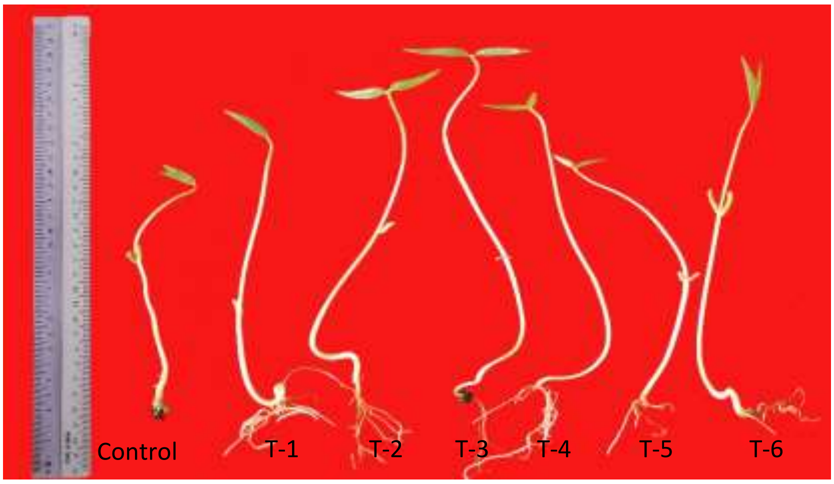

Figure 7 Root and Shoot Length of Control and Treatment after 7days inoculation

Discussion

In this research, the effect of isolated phosphate solubilizing bacteria on Black gram was observed in Laboratory. Gholami *et al.* (2009) used 0.02% sodium hypochlorite for 2 minutes to sterilize seeds of maize and rinsed thoroughly in sterile distilled water. Nwanyanwu *et al.* (2015) also made the sterilization of maize, beans and groundnut seeds by using 0.02% sodium hypochlorite for 2 minutes and rinsed thoroughly in sterile distilled water. In this study, seeds of Black gram were surface-sterilized with 0.02% sodium hypochlorite for 2 minutes and rinsed thoroughly in sterile distilled water.

Nonmavo *et al.* (2013) inoculated the seeds of maize with bacterial suspension of 10^8 CFU/ml^{-1}. Gholami *et al.* (2009) stated that maize seeds were dipped into the suspension of bacteria (10^8 CFU mL^{-1}). Demissie *et al.* (2013) also inoculated the seeds of Fava bean by dipping into the bacteria suspension of 10^8 CFU mL^{-1} for 5 hours. In this experiment, surface sterilized seeds were dipped into the suspension of bacteria 10^8 CFUmL^{-1} for 5 hours.

Gholami *et al.* (2009) observed the percent of geminated maize seeds for 1-3 days, root and shoot length of individual seedling was measured up to 7 days. Demissie *et al.* (2013) studied the percent of geminated Fava bean seeds for 1-3 days, root and shoot lengths of individual seedling were measured up to 7 days. In this observation, the percent of geminated seeds were observed at 1-3 days, root and shoot length of germinated seeds were taken up to 7 days.

Demissie *et al.* (2013) reported that Fava bean of seeds germination percentage, root and shoot length showed significant ($p<0.05$) increased over control. Nonmavo *et al.* (2013) described that the seeds of maize germination percentage, root and shoot length showed significant ($p<0.05$) increased over control. In this study, germination percentage, root and shoot length showed significantly ($p < 0.05$) increased over control.

Demissie *et al.* (2013) conducted the Fava bean plant dry weight after air dried and further oven dried at 72ºC. In the present study, Black gram plant dry weights were observed in the same manner of previous work.

Gholami *et al.* (2009) stated that fresh weight and dry weight of maize plant root and shoot increased over their respective controls. Demissie *et al.* (2013) observed fresh weight and dry weight of Fava bean plant root and shoot length and these weights increased over their respective controls. Shazia *et al.* (2016) stated that fresh weight and dry weight of mung bean root and shoot significantly increased over their respective controls. In this observation, fresh weight and dry weight of Black gram root and shoot increased over their respective controls.

Conclusion

In conclusion, microbial inoculation (biotechnological approach) could be a sustainable practice to facilitate germination rate and plant growth. In order to fulfill this biotechnological approach, different phosphate solubilizing bacteria from legume rhizosphere were isolated. Some strains showed a significant plant growth promoting activity onto black gram. As a final point, current investigation is in progress to evaluate the performance of these native strains and their relationship with native soil micro-organisms under field conditions.

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REPRODUCTIVE FEATURESOF*MYSTUS CAVASIUS* (HAMILTON-BUCHANAN, 1822) FROM AYEYAWADY RIVER SEGMENT, MAGWAY TOWNSHIP

Daw Win[1], Chaw Su Shwe[2], Win Ei Khaing[3]

Abstract

The study was conducted to elaborate the reproductive aspects, including sex ratio, condition factor, GSI, fecundity and correlation between fecundity and standard length, body weight and ovary weight in *Mystus cavasius* from Ayeyawady River Segment, Magway Township during December2018 to August 2019. A total of 78 males and 102 females contributing 1: 1.31 sexratios were presented. Mean standard length and mean body weight of collected fish samples were 10.39 to 11.86cm, 15.82 to 22.51 g and 10.06 to 12.68cm, 16.3 to 31.38g for male and female fish respectively. Condition factor (K) values ranged from 1.13 to 1.53 in male and 1.21 to 1.43 in female. The gonadosomatic index was calculated to be 0.059 to 0.22 and 0.57 to 12.33 respectively for male and female. The highest GSI 0.22 in May (male) and 12.33 in July (female) were observed. The absolute fecundity ranged from 4856 to 58889.5 eggs with the mean 27437.9±18193.4 for the corresponding length of 12.07±1.9cm, body weight of 27.57±11.28g and ovary weight of 3.86±2.5g. Relationships between standard length and body weight, were positively correlated in both sexes. Absolute fecundity was positively correlated with standard length and body weight whereas it was strong positively correlated with ovary weight.

Keywords: Sex ratio, Condition factor,GSI and fecundity, *Mystus cavasius*

Introduction

Cyclical or seasonal phenomenon of reproduction is commonly happened in most teleost species and is limited to a relatively brief time span (Misra, 1994 cited by Seetharaman, 2005).To manage appropriately for fishery conservation, a thorough knowledge of the cycles of gonad maturation is the most important (Seetharaman, 2005). The freshwater fishes are important and easily accessible source of protein. The amount and quality of protein is progressively degrading due to water pollution. In addition, the aquatic pollution has an effect upon fish reproduction. In recent past,many fish species population reduced drastically due to natural and anthropogenic stresses, so may lead to the extinction of some fish species.If any fish species is to be managed, conserved and exploited scientifically, various intricacies of reproduction should be deeply studied (Qasim, 1973 cited by Seetharaman, 2005). Small fish species are the biogenic sources of protein, micro-nutrients, vitamins and minerals which are not commonly available in many foods (INFS, 1977 cited by Islam and Das, 2006). In this area, small indigenous fish is important as a good resource of our poor and low income groups both in nutrition and economics. *Mystus cavasius*, a popular catfish is one of them. The length-weight relationship and condition factor of fish hadpositioned at the significant role in fishery management. The average weight of a given length group and the health status of the fish can estimate by the use of these senses (Bolger and Connoly, 1989 cited by Zargar *et al*., 2012). The sex-ratio of the population is analyzed to find out whether it deviates significantly from the hypothetical distribution of 1:1 or not (Siddique *et al*, 1976 cited by Seetharaman, 2005). In the absence of direct observation, gonadosomatic index canindicate the fluctuations in the breeding activity of a species all around the year (Kaul and Rishi, 1986 cited by Seetharaman, 2005).The fecundity of a fish is defined as the number of eggs that are likely to be laid during a spawning season (Bagenal, 1957 cited by Hossain *et al*., 2012). The fecundity estimation is important to understand its biology, population dynamics as well as evaluating the

[1] Dr, Associate Professor, Department of Zoology, University of Magway
[2] Dr, Lecturer, Department of Zoology, University of Magway
[3] Dr, Lecturer, Department of Zoology, University of Magway

commercial potentialities for scientific culture and management. The study of fecundity and its relationships with various body parameters such as body weight, body length, ovary weight advantage for effective fish culture, stock management and assessment in any water body (Chandanshive, 2016).

The objectives of present study were:

- to observe length-weight relationship and condition factor of *Mystus cavasius* in the study area
- to investigate the reproductive performance of the studied species
- to analyze relationships between fecundity and the standard length, body weight and ovary weight

Materials and Methods

Ayeyawady River Segment, Magway Township situated between 20º 08′ to 20º 09′ N and 94º 54′ to 94º 55′ E was the study site (Fig1).

Figure 1 Map of the study site, Ayeyawady River Segment, Magway Township

Data collection was conducted from December 2018 to August 2019.Randomly 20 fishes were purchased from fishermen who fished in the study area. Collected fishes were identified according to Talwar and Jhingran 1991. After specimen collection, the standard length and total length were recorded to the nearest centimeter scale. The weight of the specimen was taken by an electric balance and recorded to the nearest gram. The gonad of each fish was taken out very carefully and preserved in 10% formalin with labeled vials for further study.

The condition factor (K), sex ratio, gonadosomatic index (GSI)of studied species were calculated by:

$$K = \frac{\text{Eviscerated body weight}}{\text{length}^3} \times 100 \text{ (Htun-Han, 1978)}$$

$$\text{Sex ratio} = \frac{\text{Number of female}}{\text{Number of male}}$$

To confirm the sex ratio of studied species, χ^2 test was done.

$$GSI = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100 \text{ (Nikolsky, 1963)}$$

Gravimetric method or weight method (Lagler, 1956) was used to estimate the fecundity of *Mystus cavasius*. In using this method, the external connective tissues were removed from the surface of the ovaries. Moisture of ovaries was removed with the help of blotting paper. Then the gonad weight was measured. Three subsamples, each from anterior, middle and posterior parts of the ovary, were taken and weighed (0.22 g), theneggs were counted carefully. Counting of eggs from each subsample was done under the sliding magnifier (10x). Then, calculated by:

$$\text{Absolute fecundity} = \frac{\text{the numbers of eggs in sample} \times \text{gonad weight}}{\text{sample weight}}$$

From the above data, the relationships of different parameters such as standard body length and body weight, fecundity and standard length, body weight and ovary weight were determined as simple linear relationship with the help of Excel programme.

Results

A total of 180 *M.cavasius* were examined, of which 78 (43.33%) were males(Plate 1)and 102(56.67%) were females (plate 1) giving an overall ratio (M:F) of 1:1.31 (Table 1). According to χ^2 test, there was no significant difference between male and female sex ratio ($P< 0.05$).The testes were paired, elongated structures located dorsally in the body cavity and the right testis was usually longer than the left one. The testes had short finger like projections forming a fringe along their length and whitish in color (Plate 2). The weight of testis in studied species ranged from 0.01 to 0.04 g (Table 2).The paired ovary situated along the body cavity and ventrally beneath the air bladder closely allied to the body wall was separated into two lobes. Spindle-shaped ovary was large at the middle than the extremities. The color of the ovary was deep yellow to yellow and sometimes whitish depending on the maturity of the ovary. The mature ovary was found yellow and immature one was found whitish in color (Plate 3). The ovary weight varied with the size and maturity of the females. The weight of the ovary ranged from 0.14 to 3.73 g (Table 2)

Mean standard body length and body weight in male ranged from10.39 ± 0.36 cm to 11.86 ± 1.49 cm and from 15.82 ± 1.64 g to 22.51 ± 4.09 g (Table 2). With respect to female, mean standard body length and body weight ranged from 10.06 ± 1.65 cm to 12.68 ± 2.25 cm and, from 16.3 ± 2.09 g to 31.38 ± 11.99 g (Table 3). The coefficient of determination (r^2) between standard length and body weight was 0.2805 for maleand 0.7582 for female*M.cavasius* were observed (Fig. 2).

The lowest and highest condition factor (K) of male studied fishes were 1.13 ± 0.32 at the eviscerated body weight 15.5 ± 4.37 g and the standard length 11.23 ± 1.25 cm and 1.53 ± 0.14 when the eviscerated body weight 20.91 ± 3.76 g and the standard length 11.08 ± 0.55 cm (Table 2). In the females, the lowest and highest K values were 1.21 ± 0.18 at the eviscerated weight of 24.5 ± 5.7g and the standard length 12.63 ± 1.12 cm and 1.43 ± 0.15 when the eviscerated weight 22.14 ± 6.22 g and the standard length 10.06±1.65cm respectively (Table 3).The values of GSI in males ranged between 0.059 ± 0.07 in January 2019 and 0.22 ± 0.07 in May 2019 (Table 2). In females, this value was between 0.57 ± 0.14 in January 2019 and 12.33 ± 4.73 in July 2019 (Table 3). Relation of GSI with body length was presented in Fig 3.The estimate of fecundity was based on 20 ripe females (Table 3). Their length and weight ranged from 10.2 to 17.5 cm and 13.76 to 54.67 g respectively. Estimation of total ova ranged from 4856 eggs for fish of standard length 10.2 cm, body weight 14.56 g and ovary weight 0.92g to 58889 eggs for fish of standard length 15.5 cm, body weight 54.67 g and ovary weight 8.89 g. The r^2 values were found as 0.6033between absolute fecundity and standard body length, 0.7258 between absolute fecundity and body weight, and 0.9406in relation absolute fecundity with ovary weight (Fig 4).

Table 1 The sample size and sex ratio of *Mystus cavasius* from studied area

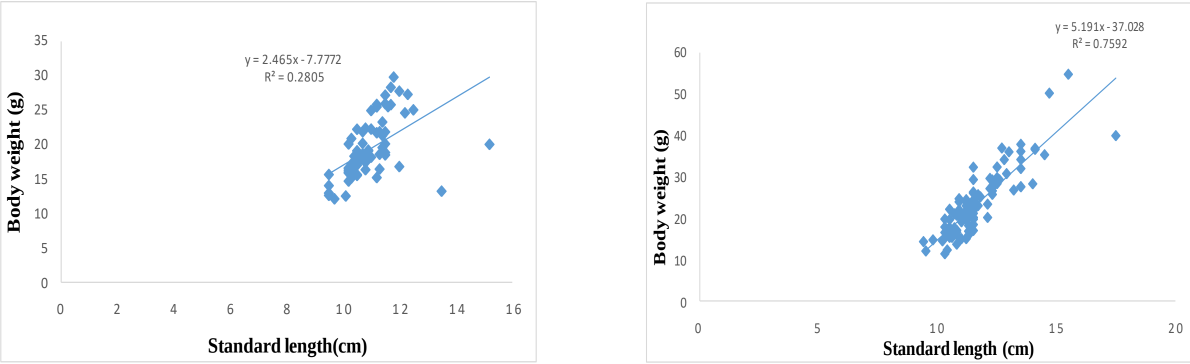
Month	Sample size	Male	Female	Sex ratio Male : Female
December, 2018	20	8	12	1:1.5
January, 2019	20	7	13	1:1.86
February, 2019	20	7	13	1:1.86
March, 2019	20	8	12	1:1.5
April, 2019	20	9	11	1.1.2
May, 2019	20	8	12	1:1.5
June, 2019	20	11	9	1:0.82
July, 2019	20	12	8	1:0.67
August, 2019	20	8	12	1:1.5
Total	180	78	102	1:1.31

Table 2 Monthly mean reproductive parameters of male *Mystus cavasius* in study area

Parameters	Month								
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Standard length (cm)	11.23 ± 1.25	11.1 ± 0.64	11.06 ± 0.73	11.86 ± 1.49	10.39 ± 0.49	10.39 ± 0.36	10.54 ± 0.55	11.08 ± 0.55	10.51 ± 0.59
Body weight (g)	17.37 ± 5.27	19 ± 3.35	21.46 ± 3.89	22.14 ± 3.59	16.05 ± 1.77	15.82 ± 1.64	17.55 ± 1.99	22.51 ± 4.09	19.1 ± 3.99
Gonad weight (g)	0.013 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Condition factor (K)	1.13 ± 0.32	1.29 ± 0.22	1.43 ± 0.13	1.28 ± 0.36	1.32 ± 0.08	1.15 ± 0.41	1.39 ± 0.17	1.53 ± 0.14	1.52 ± 0.22
GSI	0.075 ± 0.03	0.06 ± 0.07	0.09 ± 0.06	0.08 ± 0.02	0.13 ± 0.04	0.22 ± 0.07	0.15 ± 0.04	0.12 ± 0.03	0.1 ± 0.04

Table 3 Monthly mean reproductive parameters of female *Mystus cavasius* in study area

Parameters	Month								
	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Standard length (cm)	12.23 ± 1.26	12.6 ± 1.12	11.79 ± 0.55	11.47 ± 0.71	10.7 ± 0.58	10.48 ± 0.77	10.82 ± 0.64	10.06 ± 1.65	12.68 ± 2.25
Body weight (g)	26.27 ± 9.01	27.2 ± 6.23	24.26 ± 3.68	21.33 ± 3.12	16.30 ± 2.09	18.09 ± 4.42	20.28 ± 3.55	25.65 ± 7.21	31.38 ± 12
Gonad weight (g)	0.15 ± 0.06	0.16 ± 0.06	0.15 ± 0.03	0.14 ± 0.04	0.14 ± 0.04	1.12 ± 0.59	2.34 ± 0.54	3.73 ± 1.71	3.24 ± 3.9
Condition factor (K)	1.26 ± 0.26	1.21 ± 0.18	1.33 ± 0.07	1.28 ± 0.02	1.22 ± 0.12	1.24 ± 0.12	1.36 ± 0.13	1.43 ± 0.15	1.34 ± 0.28
GSI	0.61 ± 0.24	0.57 ± 0.14	0.63 ± 0.15	0.68 ± 0.17	0.86 ± 0.27	6.15 ± 3.25	11.69 ± 3.44	12.33 ± 4.73	7.67 ± 8.93
Absolute fecundity	0 ± 0.0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.0	9135 ± 3278.0	13704 ± 7156.8	31597 ± 12002.3	52496.0 ± 6191



A. Male B. Female

Figure 2 Relationships between body weight and standard length of male and female *Mystus cavasius*

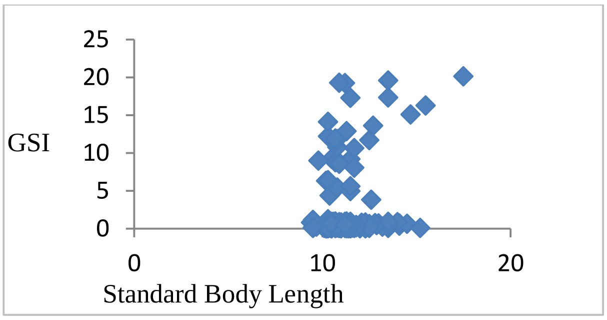
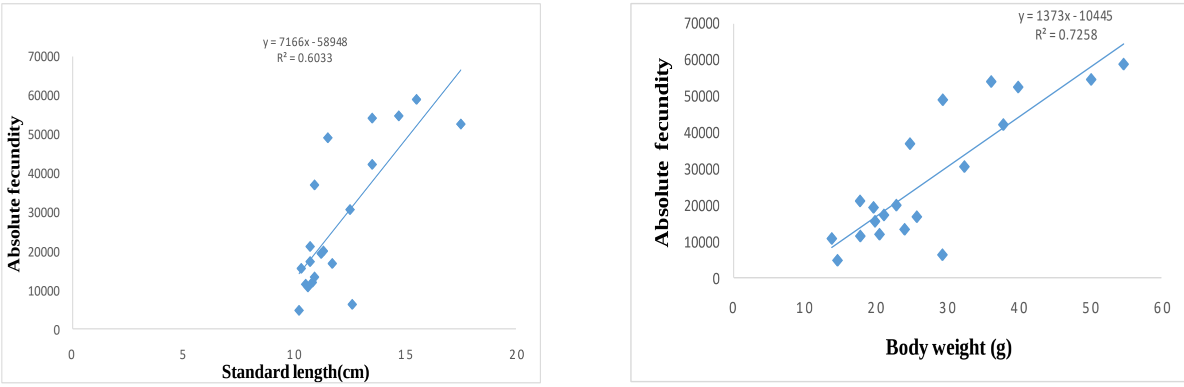
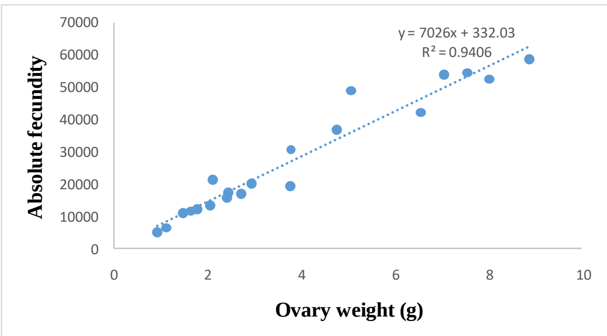


Figure 3 Relationship between GSI and standard body length of *Mystus cavasius*



A. Relationship between absolute fecundity and standard length

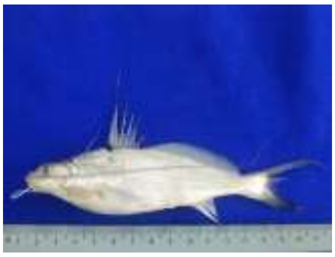
B. Relationship between absolute fecundity and body weight



C. Relationship between absolute fecundity and ovary weight

Figure 4 Relationships between absolute fecundity and standard body length, body weight and ovary weight of female *Mystus cavasius*

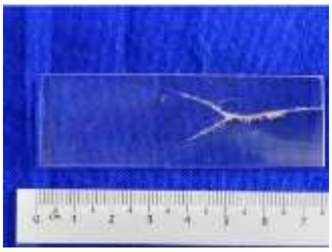
A. Male *Mystus cavasius*



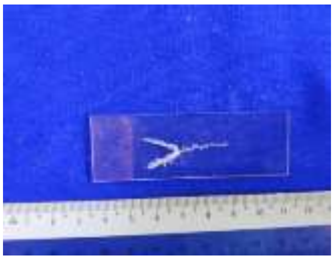
B. Female *Mystus cavasius*

C. Mature eggs (12X) of *Mystus cavasius*

Plate 1 *Mystus cavasius* and mature eggs



A. December, 2018



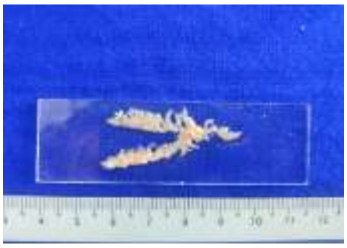
B. January, 2019

C. February, 2019

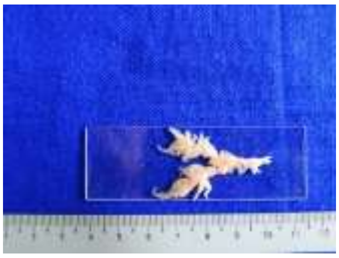
D. March, 2019

E. April, 2019

F. May, 2019



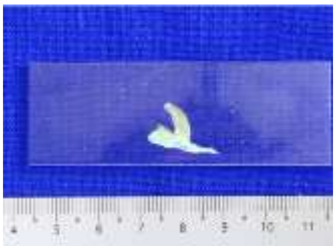
G. June, 2019



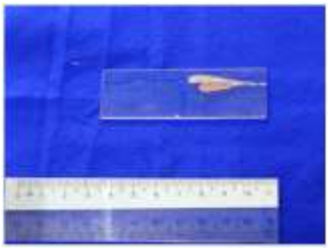
H. July, 2019

I. August, 2019

Plate 2 Monthly variations of testis

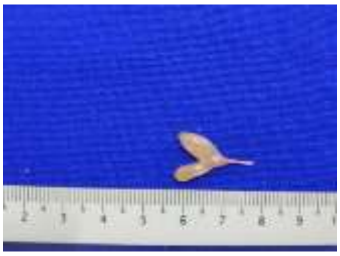


A. December, 2018

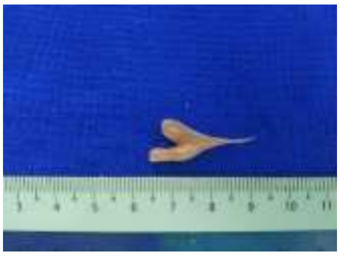


B. January, 2019

C. February, 2019



D. March, 2019



E. April, 2019

F. May, 2019

G. June, 2019

H. July, 2019

I. August, 2019

Plate 3 Monthly variations of ovary

Discussion and Conclusion

A total of 180 fish samples were analyzed during the study period. The sex ratio was 1:1.31 (male:female) not departing significantly from the expected sex ratio of 1:1 (P <0.05). Most researchers have reported sex ratios in favor of female populations. Soomro *etal*., 2015 stated the dominance of female numbers in their studied fish *Mystus cavasius* from lower Indus River. They also revealed that previously female population dominating over male *M.cavasius* is reported by different authors (Rao and Sharma, 1984; Roy and Hossain, 2006; Musa and Bhuiyan, 2007;Gupta and Banerjee, 2013) cited by Soomro et al., 2015. According to monthly calculated mean values, testis weight ranged from 0.01 (January 2019) to 0.04 (May 2019), whereas the range of ovary weight was from 0.14 (March 2019) to 3.73 (July 2019). In most of teleostean species, gonad weight depends on the part of body weight (Mahboob and Sheri, 1997 cited by Mahboob and Sheri, 2002). However, in this study, gonad weight does not usually depend on the body weight. Monthly calculated mean values in standard length and body weight of male ranged from 10.39 cm to 11.86 cm and 15.82 g to 22.51 g in male. This data in female, 10.06 cm to 12.68 cm and from 16.3 g to 31.38 g were observed. According to mean values, present results also indicate that the females *M.cavasius* were larger than the males. Different finding was observed for *M. cavasius* from Indus river (Soomro *et al*., 2015). Male *M.cavasius* ranged in standard length from 9.5 cm to 13.5 cm with a mean of 10.88 ± 0.88 cm while female ranged from 9.4 cm to 17.5 cm with the mean value of 11.67 ± 1.3 cm. With respect to weight of studied fish showed the lowest 12.09 g and the highest 29.71 g with the mean value of 19.05 ± 4.10 in male, and the lowest 11.46 g and the highest 54.67 g with the average of 23.54 ± 7.75 in female. Length-weight relationship is considered to be one of the important biological information in order to describe mathematical relationship between the two variables, length and weight. If one is known, the othercan be determined easily. The coefficient determination r^2 is closed to (1), suggesting a good adjustment (Costa and Araujo, 2003 cited by Cho Cho Win, 2009). Regard with length-weight relationships, the correlation coefficient in studied species r = 0.5296 showed moderate correlation for male and r= 0.871 with high correlation for female *M.cavasius*. The length-weight relationship in fishes can be affected by several factors including habitat, area, seasonal effect, degree of stomach fullness, gonad maturity, sex, health preservation techniques and differences (Tesch 1971 cited by Hossain *et al*., 2009). The condition factor (K) gives information on the physiological condition of fish in relation to its welfare (Getso *et al*., 2017). Perry *et al*. (1996) reported that fishes experienced with adverse physical environment or insufficient nutrition showed low condition index. Monthly mean K values of studied male species ranged from 1.13 to 1.53 and in female from 1.21 to 1.43. Ujjania *et al*. (2012) stated that condition factor greater or equal to one is good, indicating a good level of feeding and proper environmental condition. According to Maguire and Mace (1993) cited by Getso *et al*. (2017), increase in K values indicates the accumulation of fat and sometimes gonadal development. Angelescu (1958) cited by Getso *et al*. (2017) reported that the highest K values are reached in species if the fish is fully mature, and have higher reproductive potentiality. Generally the present results agreed with their findings. Gonadosomatic index (GSI) has been considered as reliable estimation method for gonad maturity and spawning of any species. GSI has increased according to the fish maturation and reaches to its maximum at the peak period of maturity (Nandikeswari and Anandan, 2013). The highest mean GSI calculated based on monthly for male was 0.22 in May 2019 and the lowest 0.059 in January 2019.The highest 12.33 in July 2019 and the lowest 0.57 in January, 2019 were calculated for females. The highest GSI value of testis in May and that of ovary in July indicates that male mature earlier than females. Maya *et al*. (2018) found that the GSI in *M.cavasius* for male was found highest in early July and lowest in late September, and for female it was highest in late July and the lowest in October. Farqu (1995) and Saha *et al*. (2014) described that four catfish species (*M.cavasius, M.vittatus, Heteropneustes fossilis and Clarias batrachus*) breeds from June to July. In this studied species, eggs were clearly visible in May and commenced egg count from this month. With scattered diagram that show relation between GSI and standard body length, this studied species mature and spawn at the size of 9-13 cm. So somewhat smaller or equal to this size should not catch to conserve this species. Fecundity is an index which measures the number of eggs carried by a gravid female fish. It is one of the various reproductive characteristics of fish species, thus fecundity estimate is of great importance for fisheries science (Hunter and Goldberg, 1980 cited by Eyo *et al*., 2013). Fecundity estimation was made based on the 20 gravid female *M.cavasius* with the range of standard length 10.2 cm to 17.5 cm and body weight from 13.76 g to 54.67 g. The largest fecund female with 58889.5 eggs measured 15.5 cm standard length, 54.67 g body weight and ovary weight 8.89 g. Least number of eggs 4856 was found in a female measuring 10.2 cm in length, 14.56 g body weight and 0.92 g ovary weight. The results showed that the largest number of egg was found in the largest female while the smallest number of egg count was not found in the smallest female. During the study, it was observed that the ovaries of the same size of fishes contained different numbers of eggs. Similar results were obtained in the works of Hossain (2014). He also stated that this variation may be due to the variations in environmental conditions and food intake by individuals. The fecundity and its relation to female size make it possible to estimate the potential egg output (Chondar 1977 cited by Hossain *et al.,* 2012) and the potential number of offspring in a season and reproductive capacity of fish stocks (Qusim and Quyyum 1963 cited by Hossain *et al.,* 2012). The relationships with absolute fecundity and standard length, body weight and ovary weight were highly correlated in studied species. Thus the present work would be useful for the future research with the fish *M.cavasius* and to develop an appropriate culture technology for the species and subsequently for the better management of the fishery resources and proper conservation of the species.

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DIGESTIVE TRACT AND STOMACH CONTENTS OF THREE FISH SPECIES FROM AYEYAWADY RIVER SEGMENT AT LEKONE QUARTER IN MYITKYINA TOWNSHIP, KACHIN STATE

Aung Lu[1], Lazat Lum Nyoi[1], M Roi Lum[2]

Abstract

The study was conducted from the Ayeyawady River Segment at Lekone Quarter in Myitkyina Township, Kachin State to investigate the digestive tract and stomach contents of three fish species (*Channa striata*, *Cirrhinus latia* and *Barilius barna*) from December 2018 to August 2019. The gut index of *C. striata* (0.59), *C. latia* (9.25) and *B. barna* (0.71) were recorded. According to the stomach content analysis, the value of frequency occurrence of *C. striata* showed earthworm (37.5%), insect parts (25%), mud (25%) and fish (12.5%). The species *C. latia* showed each 80% for plant materials and mud. *B. barna* showed mud (75%), insect parts (62.5%), plant materials (12.5%) and earthworm (25%). The vacuity index in *C. striata* and *B. barna* showed 20% in each and 0% in *C. latia*.

Keywords: Ayeyawady River, fishes, gut index, stomach contents, frequency, vacuity index

Introduction

Myanmar is rich in natural resources of both marine and freshwater, such as rivers, streams and lakes providing a wide range of commercially valuable fish species. Human livelihoods especially in many developing countries depend on aquatic resources mainly fish. The Kachin State has also a variety of rivers, streams and lakes that support rich fish diversity. Moreover, Ayeyawady River starts its origin near Myitkyina, Kachin State.

Fish are generally divisible into herbivores, carnivores and omnivores. The different types of feeding habits could be determined by their morphology of digestive tracts (Kalaya-murthy and Rao, 1970; Ramakrishniah, 1983; Santhanakuma and Job, 1983; Devi *et al*., 1992; Yusuf and Majumdar 1993; Raj, 2002).

Hickman *et al*. (2001) reported most fishes are carnivores that prey on a myriad of animal food from zooplankton and insect larvae to large vertebrates. A second group of fishes are herbivores that eat plants and alga but that are few number and crucial intermediates in the food chain, especially freshwater rivers, lakes, and ponds that contain very little planktons. Omnivores feed on both plants and animals food.

Thandar Aye (2010) stated that determination of food and feeding habit has been made on the basic of the occurrence of food items in the stomach contents, relative length of the alimentary canal, variation among the digestive system.

Hyslop (1980) described that study of diet based upon analysis of stomach contents is now a standard practice in fish ecology works. Pillay (1952) also reported that the analysis of stomach contents of fish could provide information about the niche of a particular of fish in its ecosystem.

The present study is focused on the analysis of stomach contents of three fish species such as *Channa striata*, *Cirrhinus latia* and *Barilius barna* according to feeding habits from the upper most part of Ayeyawady River Segment at Lekone Quarter in Myitkyina Township, Kachin State. Thus, the main objectives of the study were;

- to access the length ratio of standard length and digestive tract length of each study species

[1] Dr, Associate Professor, Department of Zoology, University of Myitkyina
[2] Dr, Lecturer, Department of Zoology, University of Myitkyina

- to examine the types of stomach, intestine and stomach contents
- to record the intensity of feeding, vacuity and gut index of the fish species based on the stomach contents

Materials and Methods

Study site

The present study was conducted to investigate the digestive tract and stomach contents of three fish species from the Ayeyawady River at Lekone Quarter in Myitkyina Township, Kachin State. It is located at 25° 20´50.492" N and 97° 24´ 30.063" E (Fig. 1 and Plate 1).

Study period

The study was conducted from December 2018 to August 2019.

Specimen collection and identification

Specimens were randomly collected from study site, emphasize on *Channa striata*, *Cirrhinus latia* and *Barillus barna*. Three species were approximately collected from three feeding categories, carnivores, harvivores and omnivores. Each specimen was taken as photo for records. These species were preserved in 10 percent formaldehyde solution for further investigation. The collected specimens were identified according to Talwar and Jhingram (1991) and Rainboth (1996).

Data collection

Ten individuals of each studied fish species were observed to investigate the feeding habits. Collected fishes were measured individually for total body length (cm), standard length (cm) and length of alimentary canal (cm) were taken by in situ and by removing of its body. The stomach contents of each species were examined and identified by nacked eyes, dissecting microscope and stereo microscope. Then recorded contents from stomach were photographed.

The ratio between length of digestive tract and standard length

The ratio between length of digestive tract and standard length (L ratio) was calculated using the following formula according to Taki, 1978.

$$\text{Length ratio} = \frac{\text{Digestive tract length}}{\text{Standard length}}$$

Frequency occurrence of foods in the stomach

The food items in the stomach were evaluated by calculating the frequency of occurrence method of Hyslop, 1980.

$$Fp = Ns \times 100/Nf$$

Where, Fp = the percentage frequency of occurrence of one prey item or prey occurrence index

Ns = the number of stomachs containning the prey item

Nf = the number of stomach that contained food

Intensity of feeding

The intensity of feeding was determined based on the degree of distention of stomach wall and amount of contained food in it and classified as full (gorged), 3/4full, 1/2full, ¼ full, trace and empty (Pillay, 1952).

Vacuity index

The vacuity index or index of emptiness was estimated from the equation

$$CV = ES\times100/TS \text{ (Euzen, 1987).}$$

Where, CV = the vacuity index

ES = the number of empty stomachs

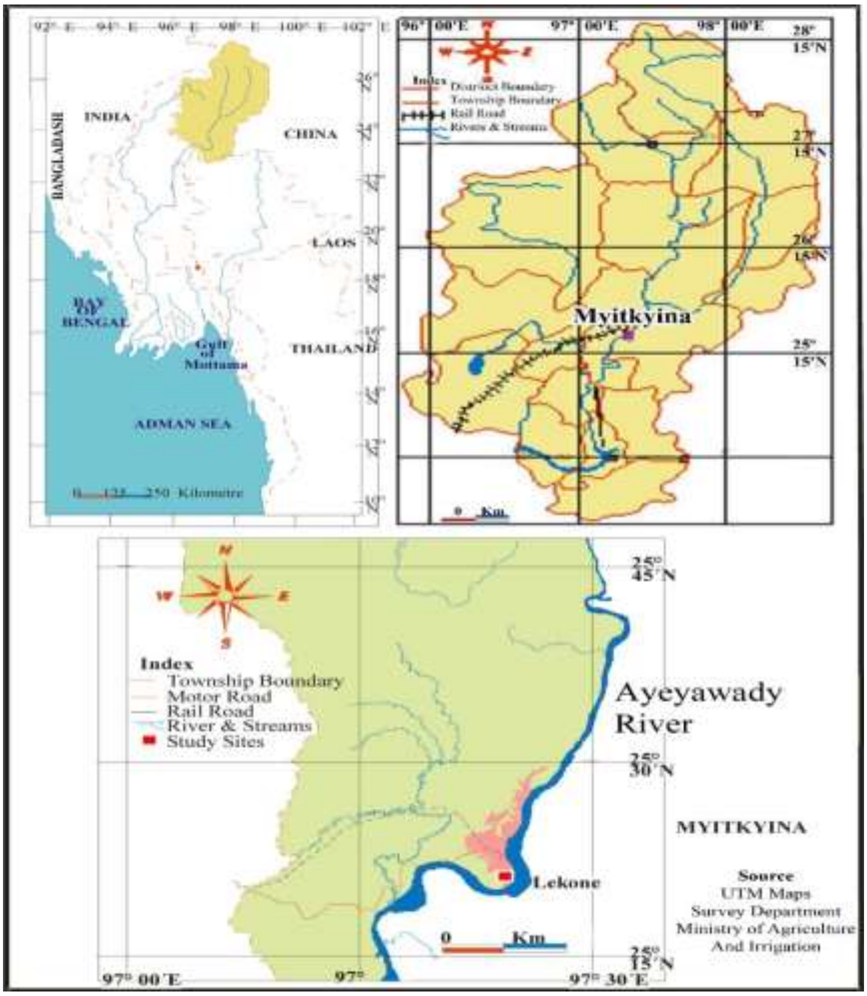
TS = the total number of stomachs analysed

Gut index (L ratio)

Gut index was calculated according to Smith, 1980.

Data analysis

Data were analysed using descriptive statistic (in percentage, mean, standard deviation). Graphics were performed by Microsoft Excel Programme.



Source: UTM Maps, Survey Department, Ministry of Agriculture and Irrigation

Figure 1 Location map of study area showing study site

A. Fishing boat in the study site

B. Fishing in the study site

C. Fish collected by local fisherman

Plate 1 The Ayeyawady River Segment in Lekone Quarter showing the study site

Results

In the study period, the total of three species of fishes belonging to three genera, two families including Channidae (Order Anabantiformes) and Cyprinidae (Order Cypriniformes) were collected from the Ayeyawady River Segment at Lekone Quarter in Myitkyina Township, Kachin State shown in Table 1 and Plate 2.

Morphology of stomach and intestine of selected fish species

Stomach of *Channa striata* is elongated bag like (J shaped) and slightly swollen muscular stomach is present. A pair of short tubular pyloric caeca hangs from the anterior part of intestine. Intestine is originated from the fore part of the stomach and positioned at the right side of the stomach. The intestine is tubular in structure. *Cirrhinus latia* has a long tube like and coiled gastrointestinal tract while *Barilius barna* also tube like stomach and moderate elongated intestine recorded. (Table 2 and Plate 2)

Table 1 Selected study species recorded from the study site

Sr. No	Order	Family	Scientific name	Common name	Local name	IUCN Red List
1.	Anabantiformes	Channidae	*Channa striata*	Snakehead Murrel	Nga-Yant	LC
2.	Cypriniformes	Cyprinidae	*Cirrhinus latia*	Carp	Nga-Lu	VU
3.	Cypriniformes	Cyprinidae	*Barilius barna*	Barna Baril	Nga-La-War	LC

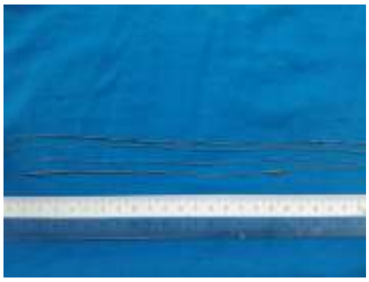
Table 2 Stomach shape and intestine type of fish species recorded from the study site

Sr. No	Name of species	Shape of stomach	Type of intestine	Type of consumer
1.	*Channa striata*	J Shaped	Short	Carnivore
2.	*Cirrhinus latia*	Tube Liked	Long	Herbivore
3.	*Barilius barna*	Tube Liked	Moderate	Omnivore



A. *Channa striata* B. Gastrointestinal tract in situ C. Extracted gastrointestinal tract





D. *Cirrhinus latia* E. Gastrointestinal tract in situ F. Extracted gastrointestinal tract



G. *Barilius barna* H. Gastrointestinal tract in situ I. Extracted gastrointestinal tract

Plate 2 Gastrointestinal tract of studied species

Comparison of standard length and gastrointestinal tract length

Ten fish samples in *C. striata* (carnivore), mean of total length was 15.54 cm (9.8-19.3 cm), standard length 13.29 cm (7.9-17.7 cm) and gastrointestinal length 7.81 cm (3.4-9.2 cm) showed respectively. The gut index was recorded to be 0.59.

In recorded species *C. latia* (herbivore) mean of body length 14. 4 cm (10.5- 18.7 cm), standard length 12.17 cm (9.3-15.5 cm) and digestive tract length 110.65 cm (72.0-136 cm) mean selected for this study. The gut index was calculated to be 9.25.

The species *B. barna* (omnivore) mean of body length 14.4 cm (10.5-14.9), standard length and digestive tract length showed 11.29 cm (8.7-13.9 cm) and 7.91 cm (6.0-11.5 cm) respectively. The gut index was found to be 0.71. (Table 3 and Fig.2)

Stomach contents of fish species recorded

The food items in fish species recorded were examined into animal sources (insect parts, earthworm and fish), plant materials, algae and mud. Among the studied fish species, *C. striata* was designated as carnivore and its stomach involved animal source only. *C. latia* confined as herbivores and its stomach contained plant materials and mud. The stomach of *B. barna* species was found animal parts, plant parts and mud as omnivore in habit. (Table 4 and Plate 3)

Table 3 Relationship between standard length and digestive tract length of studied fishes

Fish species (N=10)	Mean (±SD)(cm)			L ratio
	Total length	Standard length	Digestive tract length	
Channa striata	15.54±2.8 (9.8-19.3)	13.29±2.77 (7.9-17.7)	7.81±1.68 (3.4-9.2)	0.59±0.1
Cirrhinus latia	14.4±11.64 (10.5-18.7)	12.17±2.05 (9.3-15.5)	110.65±19.09 (72-136)	9.25±1.87
Barilius barna	14.4±2.9 (10.5-14.9)	11.29±1.49 (8.7-13.9)	7.91±1.47 (6.0-11.5)	0.71±0.13

Table 4 Stomach contents of fish species recorded from the study site

Sr. No	Name of species	Animals source	Plant materials	Mud
1.	*Channa striata*	+		+
2.	*Cirrhinus latia*		+	+
3.	*Barilius barna*	+	+	+

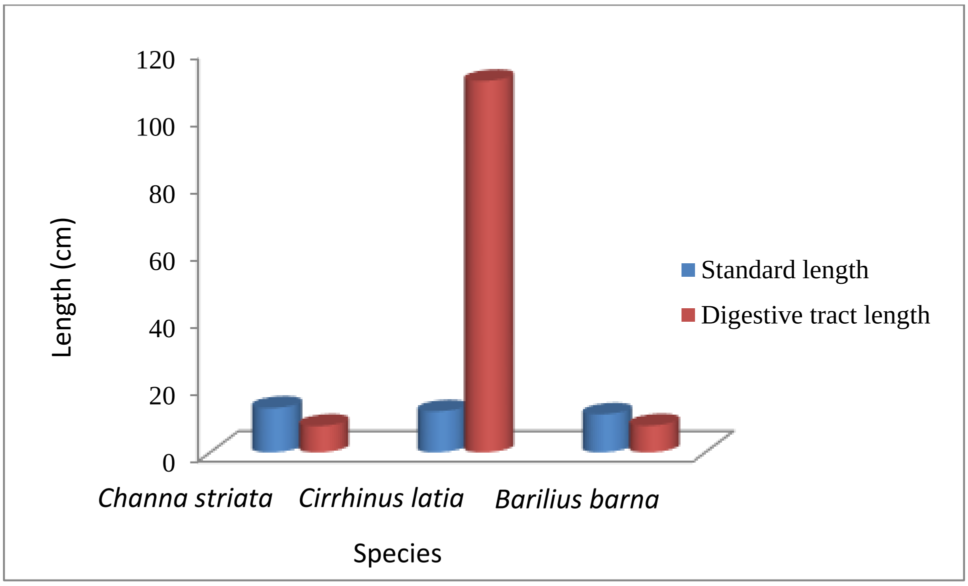
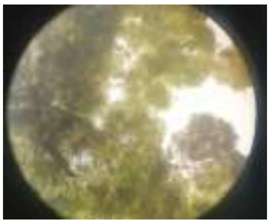


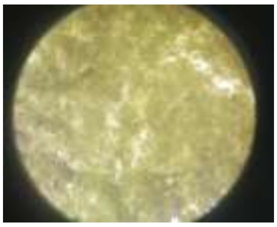
Figure 2 Comparison of standard length and gastrointestinal tract length of studied species

A. Insect part B. Insect part C. Insect part D. Earthworm


E. Fish part


F. Mud

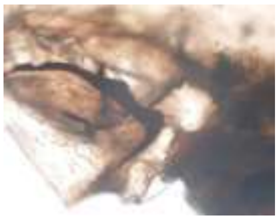

G. Algae


H. Algae


I. Plant material

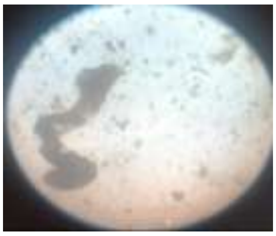

J. Mud


K. Mud


L. Insect part


M. Insect part


N. Earthworm


O. Earthworm

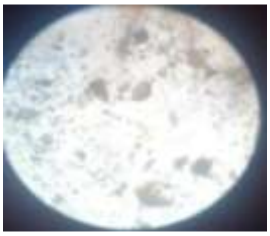

P. Mud

Plate 3 Food items of studied fish species *Channa striata* (A-F), *Cirrhinus latia* (G-K), *Barilius barna* (L-P)

Stomach content analysis

According to the stomach content analysis, out of the stomachs of 10 individuals analyzed, eight individuals contained (trace-full). Stomach while two individuals with empty stomach in *C. striata*. The value of Fp index showed 37.5% for earthworm followed by 25% insect parts, 25% mud and 12.5% fish respectively shown in Fig. 3.

In *C. latia*, the stomach of 10 individuals contained (trace-full). The value of Fp index showed 80% each for plant materials and mud shown in Fig.4.

The stomach of *B. barna* out of 10 individuals analyzed, eight individuals contained (trace-full) although two individuals with empty stomach. The value of Fp index showed 75% for mud, 62.5% for insect parts and 25% for earthworm and for plant materials 12.5% respectively shown in Fig.5.

Intensity of feeding

The intensity of feeding in *C. straita* was found to be 37.5% in full and ¾ full, 12.5% in ½ full and ¼ full, 0% in trace and 20% in empty. The intensity of feeding in *C. latia* was calculated to be 100% in full however 0% in ¾ full, ½ full, ¼ full, trace and empty. The intensity of feeding in, *B. barna* was examined to be 0% in full, 25% in ¾ full, 50% in ½ full, 12.5% in ¼ full and trace and 20% in empty. (Table 5)

Vacuity index

The result of vacuity index in *C. striata* and *B. barna* showed 20% in each and 0% in *C. latia* (Table 5).

Table 5 Intensity of feeding in studied fish species recorded

Fish species	Intensity of feeding (%)					
	100 (full)	75 (¾ full)	50 (½ full)	25 (¼ full)	<25 (trace)	Empty
Channa striata	37.5	37.5	12.5	12.5	0	20
Cirrhinus latia	100	0	0	0	0	0
Barilus barna	0	25	50	12.5	12.5	20

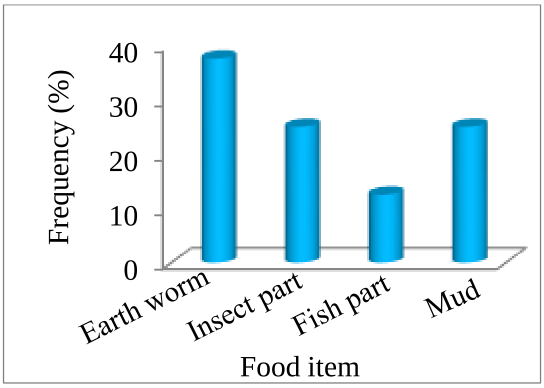


Figure 3 Frequency occurrence of food items of *Channa straita*

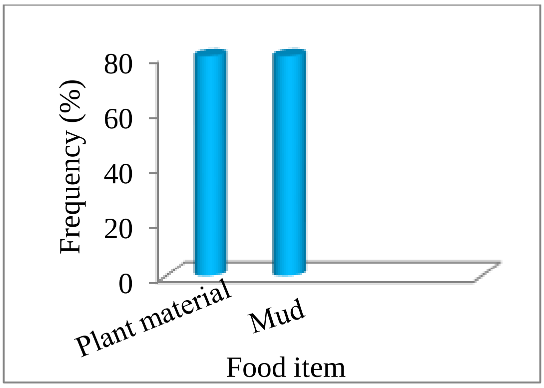


Figure 4 Frequency occurrence of food items of *Cirrhinus latia*

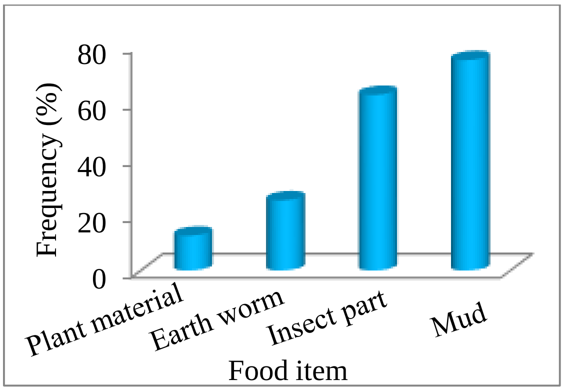


Figure 5 Frequency occurrence of food items of *Barilius barna*

Discussion

Three species of fish, *Channa striata*, *Cirrhinus latia* and *Barilius barna* belonging to three genera, two families of order Anabantiformes and Cypriniformes were selected from the Ayeyawady River Segment at Lekone Quarter in Myitkyina Township, Kachin State for investigating analysis of stomach contents according to their feeding habits.

Lagler *et al*. (1977) reported that the stomach shows various adaptations. In carnivorous fishes, the stomach is typically quite elongate than omnivorous species. In the present results, J shaped stomach was found in *C. striata* and tube-liked stomach in *C. latia* and *B. barna*. The different shapes of the stomach may be due to their various feeding habits.

In the previous recorded, the intestine has many variations. It is shortened in essential carnivores. In the opposite fashion it is often elongated and arranged in many folds in predominantly herbivorous species (Lagler *et al*., 1977). Zar Phyu Win (2015) stated that three intestinal types were found such as short, moderate and long looped. In the present study agree to that the above record. Short intestine type is found in *C. striata* while moderate intestine in *B. barna* and long looped intestine in *C. latia*.

According to the gut index of *C. striata* was calculated to be 0.59 indicating the length of digestive tract was 0.59 time shorter than that of fish total length. Thus, the gut index of studied fish species was fall in the category of carnivorous fish. The gut index of *C. latia* was found to be 9.25 indicating the length of digestive tract was 9.25 times longer than that of fish length. Thus, the gut index of studied fish species was fall in the category of herbivorous fish. The gut index of *B. barna* was found to be 0.71 indicating the length of digestive tract was 0.71 time shorter than that of fish length. Thus, the gut index of studied fish species was fall in the category of omnivorous fish. The current result agrees with Smith, 1980 listed that the gut length as 0.2-2.5, 0.6-8.0 and 0.8-15.0 times of body length in carnivores, omnivores and herbivores, respectively. Thidar Aung *et al.* (2012) also observed that the gut index was 1.35 indicating *Epinephelus coioides* as carnivore.

Zar Phyu Win (2015) observed that the food items consist of fish, fish bone, shrimp, snail, frog, insect, plant materials, algae and mud. The adult fish of *C. striata* was expected to consume on fish, frogs, snakes, insects, earthworms, tadpoles, crustaceans and molluscs (Allen, 1991). However, the present study was recorded animal sources such as fish, earthworms and insects in *C. striata*, plant parts such as algae and mud in *C. latia*. Insect parts, plant materials and mud were found in *B. barna*.

In the present result of the stomach contents was observed from ten individuals of each species, the highest value of Fp index of *C. striata* showed for earthworm (37.5%), insect parts and mud (25%) and fish (12.5%). In the species *C. latia* was found the high value for plant materials and mud (80%). Moreover, the highest value of Fp index of *B. barna* showed 75% for mud and followed 62.5% for insect parts and (25%) for earthworm and (12.5%) for plant materials. In the previous study, stomach of *Epinephelus coioides* contained fish (73%), crab (11%), shrimp (8.8%), squid (3.9%), gastropod (1.7%) and bivalves (0.4%) were analyzed (Thidar Aung *et al*., 2012). These may be due to the different species and study sites.

Mohammadi *et al*. (2007) stated that the CV index gives an estimate of the voracity of the predator fish; the more voracious fish species, the lower percentage of empty stomachs. This previous statement is similar to the present result of vacuity index, *C. striata* and *B. barna* showed 20% in each and 0% in *C. latia*. The fluctuations of vacuity index probably due to the occurrence or abundance of predation in the habitats where they lived during the study months. The finding in the present study and previous workers might be different.

Conclusion

The study area is considered as an important resource of fish community. The result may also important to evaluate the ecological role and the food web of the ecosystems. Therefore, the study area is need to study more fish species for stomach contents analyze seasonally different of feeding habits in various species and to investigate the histology of the digestive tracts from the Ayeyawady River Segment in Myitkyina Environs.

Acknowledgements

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NEST CHARACTERISTICS OF SOME BIRD SPECIES IN MAGWAY TOWNSHIP

Chaw Su Shwe[1], Win Ei Khaing[2], Daw Win[3]

Abstract

Nest has important effects upon bird's growth and breeding. Nest characteristics of some bird species were studied in Magway Township during December, 2016 to November, 2017. Nests of 14 bird species were observed. Five different nesting types were observed; globular or dome nest, platform nest, cup-shaped nest, pendent nest and irregular-shaped nest. Birds used different plant species as nesting substrate. They preferred spiny tree for prevention from predators. They used a variety of nesting materials, mostly straw, grasses, sticks and feathers. The highest mean nesting height 18.54±8.85 m was found in *Columba livia* and the lowest mean nesting height 0.53±0.07 m was found in *Chrysomma sinense.* Cup nest type in *Aegithina tiphia, Orthotomus sutorius*, *Chrysomma sinense*, *Pycnonotus cafer* and *Pycnonotus blandfordi*, and pendant nest type in *Prinia inornata* and *Ploceus phillipinus* were recorded. Dome nest were found in *Lonchura punctulata* and *Passer flaveolus*. Two platform nests were observed in *Columba livia* and *Streptopelia chinensis*. Nest of *Passer domesticus*, *Passer montanus* and *Acridotheres tristis* were irregular shaped. The major aim of this study was to provide the basis information about bird's nest, placement, other key factors for nest construction which can be helpful as reference for such studies in other areas.

Keywords: species, predators, *Columba livia*, *Chrysomma sinense*

Introduction

Birds vary a great deal from one another in most of their life pattern like habitat, food, colorations, beaks, feet, size, plumage pattern, distribution and so on, likewise nests of birds are also having huge variations in their size, shape, structure, construction material used, construction pattern, selection of site for nest, nest architecture etc. Nest building in birds require extreme skills of engineering. In this regard birds have proved themselves expert engineers. Nest construction skills can be considered as a part of parental care or Instincts. Birds provide care to their young ones before birth by constructing nests. (Raval, 2011)

Birds build nest to protect themselves their eggs and their young from predators and from adverse weather. Other animals also build nests, but birds do so in a great variety of forms, from a great variety of materials and on a great variety its sites (Gill, 2001).

All birds construct nests in which to lay eggs and/or raise offspring. Traditionally, it was thought that natural selection and the requirement to minimize the risk of predation determined the design of completed nests. (Mainwaring, *et al*., 2014)

The nest is built in roofs of houses, holes of walls, trees, railway station and wells. Nesting materials were categorized and identified into different groups such as twigs of *Azadirachta indica, Delonix regia*, *Cocos nucifera*, grass, feathers of birds, plastic, cloth, flowers of Acacia, Rubber rings, matal wire and snake slough, which were found in nest cavity (Dhandhukia and Patel, 2012). Different environments are likely to favor the positioning of nests in different places, whenever nest predators, microclimates, nest parasites, or competitors differ (Yeh *et al*., 2007).

Magway Township is situated in Magway District within the Dry Zone of Central Myanmar, which has dry and hot climate. It has an area of 1098.37 km^2. It is situated on the eastern bank of the Ayeyawady River. It stands at the height of 54.86 m above sea-level. The temperature

[1] Lecturer, Department of Zoology, University of Magway
[2] Lecturer, Department of Zoology, University of Magway
[3] Associate Professor, Department of Zoology, University of Magway

ranges from 10˚ to 45˚C. It is surrounded by dry forests, cultivated lands, some hilly regions and human habitations. Agriculture is important for the people of Magway.

Although great research effort has been focused in study of nest-site selection and nest-site characteristics of birds, the information from this study is largely lacking. Therefore, this study was conducted to investigate the occurrence of nests in some areas of Magway Township and to assess the nest characteristics of some nesting bird species.

Materials and Methods

Study area

Magway Township is situated in Dry Zone of Myanmar, which has dry and hot climate. It is located at the eastern bank of the Ayeyawady River. It lies between 20˚ 6′ 44.38″ and 20˚ 10′ 30.22″ N and between 94˚ 54′ 42.06″ and 94˚ 57′ 57.26″ E.

Three study sites were allocated in Magway Township. These three study sites were (i) Magway University Campus (Site I), (ii) Wa-taw-chaung Village (Site II) and (iii) Kyit- Sone-Pwe village (Site III). (Fig.1)

Figure 1 Location map of study area and study sites

Study period

This study was carried out from December, 2016 to November, 2017.

Data collection

Field survey was conducted for regular day of every week. A few nests were located by watching birds carrying nesting material. Nesting bird species were firstly viewed, and immediately taken on photographs by using Cannon SX 430 IS45x.

Identification of species

The bird species were identified referring to the taxonomic descriptions given by Smythies (2001), Robson (2011) and Robson (2015). The nesting plants were checked according to Kress *et al.* (2003).

Results

Number of nesting birds and nests

A total of 14 species of nesting birds and their 105 nests were recorded from three study sites during December, 2016 to November, 2017. It consists of 11 genera, nine families and two orders.

Characteristics of nests of some breeding bird species

Columba livia

A total of four nests were recorded. The shape of nest is platform type. Nest height from the ground ranged from 9.1 to 27.43 m (18.54±8.85 m). The length of nest ranged from 23 to 28 cm (26.13 ± 1.88cm) and the height of nest ranged from 2.5 to 3.2 cm (2.93 ± 0.26 cm). They built their nests with sticks and twigs (Table 3, Plate 1). The nests were observed on the window ledge of buildings.

Streptopelia chinensis

A total of five nests were observed. The shape of nest is platform type. Nest height from the ground ranged from 1.98 to 4.8 m (3.27±1.05 m). The length of nest ranged from 12.6 to 15.2 cm (13.66±0.9 cm) and the height of nest ranged from 2.5 to 3 cm (2.76±0.22cm). They built their nests with sticks and twigs (Table 3, Plate 1). Nests were encountered on the trees such as *Dypsis lastellina* (Ohn) and *Senegalic catechu* (Shar).

Aegithina tiphia

A total of two nests were recorded. The shape of nest is cup-shaped type. Nest height from the ground ranged from 1.8 to 2.13 m (1.97±0.17 m). The length of nest ranged from 5 to 5.5 cm (5.27±0.25 cm) and the height of nest ranged from 3.8 to 4 cm (3.9±0.1 cm). They built their nests with soft grass, root fiber and wool (Table 3, Plate 1). The nests were found on the tree *Senegalia catechu* (Shar).

Acridotheres tristis

A total of three nests were recorded. The shape of nest is irregular-shaped type. Nest height from the ground ranged from 6.8 to 7 m (6.9 ± 0.1 m). The length of nest ranged from 17.4 to 18 cm (17.7 ± 0.3 cm) and the height of nest ranged from 8.6 to 9.3 cm (8.95 ± 0.35 cm). They built their nests using twigs, grass, leaves and sticks. (Table 3, Plate 1). The nests were found in the hole of the wall and in the crevice of building.

Pycnonotus blanfordi

A total of eight nests were encountered. The shape of nest is cup-shaped type. Nest height from the ground ranged from 1.6 to 2.9 m (2.28±0.43 m). The length of nest ranged from 6.7 to 10.3 cm (8.26±0.98 cm) and the height of nest ranged from 2.8 to 5.4 cm (4.73±0.77 cm). They built their nests with grass, straw, sticks, string and wool (Table 3, Plate 1). Nests were encountered on the trees such as *Vachellia nilotica* (Sue- Phyu), *Citrus limon* (Shauk) and *Ziziphus jujube* (Zi).

Pycnonotus cafer

A total of five nests were encountered. The shape of nest is cup-shaped type. Nest height from the ground was 0.08 to 2 m (1.4±0.6 m). The length of nest was 11.3 to12 cm (11.65± 0.35 cm) and the height of nest was 4.5 to 5.3 cm (4.9±0.4 cm). They built their nests with twigs and sticks. (Table 3, Plate 1). Nests were encountered on the tree *Cedrela febrifuga* (Yay-Tamar) and *Vachellia nilotica* (Sue- Phyu).

Prinia inornata

A total of three nests were recorded. The shape of nest is pendant type. Nest height from the ground ranged from 0.02 to 7.3 m (2.45±3.43 m). The length of nest ranged from 6 to 7 cm (6.33±0.47cm) and the height of nest ranged from 9 to 12 cm (10±1.41 cm). They built their nests with used twigs and grass (Table 3, Plate 1). The nests were found on the tree *Ricinus communis* (Kyat-su).

Orthotomus sutorius

A total of two nests were observed. The shape of nest is cup-shaped type. Nest height from the ground was 1 to 2.4 m (1.7±0.7 m). The length of nest was 6 to 7 cm (6.5±0.5 cm) and the height of nest was 10.6 to 12 cm (11.3±0.7 cm). They built their nests with thread and wool (Table 3, Plate 1). The nest was found on the trees of *Mangifera indica* (Tha-yet) and *Terminalia catappa* (Bar-dan). They stitch leaves together to make a nest, using its needle-shaped beak and fine thread.

Chrysomma sinense

A total of two nests were recorded. The shape of nest is cup-shaped type. Nest height from the ground ranged from 0.46 to 0.6 m (0.53±0.07 m). The length of nest ranged from 7 to 7.3 cm (7.15±0.15 cm) and the height of nest ranged from 5 to 5.2 cm (5.1±0.1 cm). They built their nests with twigs and sticks (Table 3, Plate 1). The nests were found on the tree *Senegalia catechu* (Shar).

Passer domesticus

A total of 26 nests were found during the study. The shape of nest is irregular. Nest height from the ground ranged from 1.98 to 15.24 m (5.59±2.74 m). The length of nest ranged from 7 to 29 cm (17.8±7.21 cm) and the height of nest ranged from 15 to 31 cm (18.6±2.96 cm). They built their nests with feathers, string, paper, grass inflorescences, stalks, threads, straw, wool, leaves and any other available materials (Table 3, Plate 1). The nests were found on the ceiling, in the fan and under the electric light, in the artificial nest house, on the top of pagoda, on the ceiling and in the pipe, on the shelf of air-conditioner and on the lamppost.

Passer flaveolus

A total of four nests were found. The shape of nest is dome type. Nest height from the ground ranged from 2.1 to 3.5 m (2.58 ± 0.54 m). The length of nest ranged from 13 to 16.3 cm (14.9 ± 1.2 cm) and the height of nest ranged from16 to 18.7 cm (17.18 ± 0.97 cm). They built their nests with used feathers, human hair, wool, grass and straw (Table 3, Plate 1). The nests were found on the trees *Senegalia catechu* (Shar) and *Ziziphus jujube* (Zi).

Passer montanus

A total of five nests were recorded. The shape of nest is irregular-shaped type. Nest height from the ground ranged from 4.14 to 5.03 m (4.56±0.32 m). The length of nest ranged from 15 to 20 cm (17.52±2.11 cm) and the height of nest ranged from 20 to 25.3 cm (22.62±2.2 cm). They built their nests with grass, straw, twigs and wool (Table 3, Plate 1). Nests were encountered on the trees such as *Azadirachta indica* (Ta- mar), *Cedrela febrifuga* (Yay-Tamar) and *Mimusops elengi* (Kha-yay).

Ploceus phillippinus

A total of 18 complete nests and 7 incomplete nests were found. The shape of nest is pendant type. In complete nests, nest height from the ground ranged from 4.14 to 22.1 m (17.33±5.44 m). The length of nest ranged from 13 to 19 cm (16.61±1.53cm) and the height of nest ranged from 23 to 32 cm (27.39±2.63 cm). They built their nests with used twigs and grass (Table 3, Plate 1). Nests of *Ploceus phillippinus* were encountered on the trees such as *Cocos nucifera* (Ohn) and *Albizia saman* (Kote-ko).

Lonchura punculata

A total of 22 nests were found. The shape of nest is dome type. Nest height from the ground ranged from 1.5 to 27.43 m (4.05±5.33 m). The length of nest ranged from 9.6 to 19.5 cm (13.56±2.16 cm) and the height of nest ranged from 15.9 to 23 cm (18.05±1.91 cm). They built their nests with used feathers, hair, wool, grass and straw (Table 3, Plate 1). Nests were encountered on the trees such as *Dypsis lastellina* (Ohn), *Vachellia nilotica* (Sue- Phyu) and on the window ledge of buildings, *Azadirachta indica* (Ta- mar), *Ziziphus jujube* (Zi), *Acacia leucophloea* (Hta-naung), *Senegalic catechu* (Shar) and *Citrus limon* (Shauk).

Occurrence of nests in study sites

Three nests of *Columba livia*, were found two nests in each of Site II and III. In the case of *Streptopelia chinensis*, two nests in each of Site I and II and one nest in Site III were recorded. Regarding *Lonchura punculata*, eight nests in Site I, five nests in Site II, and nine nests in Site III were observed. Among the species of genus *Passer*, five nests of *Passer domesticus* were recorded in Site I, nine nests in Site II and 12 nests in Site III respectively. Two nests of *Passer flaveolus* was observed in each of Site II and III. Two nests of *Passer montanus* were observed in Site II and three nests in Site III. Two nests of *Aegithina tiphia* were observed in only Site III. One nest of *Chrysomma sinense* was found in each of Site II and III. Two nests of *Acridotheres tristis*, were found only in Site I. 18 nests of *Ploceus phillipinus* was observed in only Site I. Three nests of *Prinia inornata* was recorded in only Site III. One nest of *Pycnonotus cafer* was found in each of Site I and III. Four nests of *Pycnonotus blanfordi* were recorded in Site I, three nests in Site II and one nest in Site III. One nest of *Orthotomus sutorius* was found in each of Site II and III (Table 2 and Fig 2).

Altogether 40 nests in Site I, 27 nests in Site II and 38 nests in Site III were recorded (Table 2 and Fig 2).

Occurrence of nests in study months

During the study period, one nest of *Columba livia* was observed in each month of December, April, July and October. One nest in each month of January, March, July, August and October were recorded for *Streptopelia chinensis*. Five nests in June, six nests in each month of July and August, four nests in September and one nest in October were represented by *Lonchura punculata*. *Passer domesticus* revealed three nests in each of December, August and October, one nest in each of January, March and April, two nests in each month of Feburary, May, June, September and November and four nests only in July. Two nests of *Passer flaveolus* were observed in month of May and one nest in each of June and July. Two nests of *Aegithina tiphia* was found only in July. Two nests in April and one nest in each month of May, June and July were observed in *Passer montanus*. One nest of *Chrysomma sinense*, *Acridotheres tristis* and *Pycnonotus cafer* were recorded in each month of June and July. Six nests in June, eight nests in July and four nests in August were that of *Ploceus phillipinus*. Two nests of *Prinia inornata* were observed in July and one nest in August. *Pycnonotus blanfordi* revealed two nests in each month of May and July, one nest in June and three nests in August. One nest in each month of July and August were recorded in Orthotomus *sutorius* (Table 4).

Table 1 List of recorded bird species

Sr No	Order	Family	Scientific Name	Common Name
1	Columbiformes	Columbidae	*Columba livia* Gmelin, 1789	Pigeon
2			*Streptopelia chinensis* (Scopoli, 1768)	Spotted Dove
3	Passeriformes	Aegithinidae	*Aegithina tiphia* (Linnaeus, 1758)	Common iora
4		Sturnidae	*Acridotheres tristis* (Linnaeus, 1766)	Common myna
5		Pycnonotidae	*Pycnonotus blanfordi* Jerdon, 1862	Streak-eared Bulbul
6			*Pycnonotus cafer* (Linnaeus, 1766)	Red- vented bulbul
7		Cisticolidae	*Prinia inornata* (Sykes, 1832)	Plain Prinia
8			*Orthotomus sutorius* Horsfield, 1821	Tailor Bird
9		Paradoxornithidae	*Chrysomma sinense* (Gmelin, 1789)	Yellow-eyed babbler
10		Passeridae	*Passer domesticus* (Linnaeus, 1758)	House Sparrow
11			*Passer flaveolus* Blyth, 1844	Plain- backed Sparrow
12			*Passer montanus* (Linnaeus, 1758)	Tree Sparrow
13			*Ploceus philippinus* (Linnaeus, 1766)	Baya Weaver
14		Estrildidae	*Loncura punculata* (Linnaeus, 1758)	Scaly-breasted Maunia

Table 2 Number of nests of nested bird species recorded in different study sites

Sr No	Study species	Site			Total
		I	II	III	
1	*Columba livia*	-	2	2	4
2	*Streptopelia chinensis*	2	2	1	5
3	*Aegithina tiphia*	-	-	2	2
4	*Acridotheres tristis*	2	-	-	2
5	*Pycnonotus blandfordi*	4	3	1	8
6	*Pycnonotus cafer*	1	-	1	2
7	*Prinia inornata*	-	-	3	3
8	*Orthotomus sutorius*	-	1	1	2
9	*Chrysomma sinense*	-	1	1	2
10	*Passer domesticus*	5	9	12	26
11	*Passer flaveolus*	-	2	2	4
12	*Passer montanus*	-	2	3	5
13	*Ploceus phillipinus*	18	-	-	18
14	*Lonchura punctulata*	8	5	9	22
	Total	**40**	**27**	**38**	**105**

Table 3 Mean values of nest characteristics of nested bird species

Sr. no	Species	Nest height from ground (m)	Nest length (cm)	Nest height (cm)
1	*Columba livia*	18.54 ± 8.85	26.13 ± 1.88	2.93 ± 0.26
2	*Streptopelia chinensis*	3.27 ± 1.05	13.66 ± 0.9	2.76 ± 0.22
3	*Aegithina tiphia*	1.97 ± 0.17	5.25 ± 0.25	3.90 ± 0.10
4	*Acridotheres tristis*	6.9 ± 0.1	17.7 ± 0.3	8.95 ± 0.35
5	*Pycnonotus blandfordi*	2.28 ± 0.43	8.26 ± 0.98	4.73 ± 0.77
6	*Pycnonotus cafer*	1.4 ± 0.6	11.65 ± 0.35	4.9 ± 0.4
7	*Prinia inornata*	2.45 ± 3.43	6.33 ± 0.47	10 ± 1.41
8	*Orthotomus sutorius*	1.7 ± 0.7	6.5 ± 0.5	11.3 ± 0.7
9	*Chrysomma sinense*	0.53 ± 0.07	7.15 ± 0.15	5.1 ± 0.1
10	*Passer domesticus*	5.59 ± 2.74	17.80 ± 7.21	18.60 ± 2.96
11	*Passer flaveolus*	2.58 ± 0.54	14.90 ± 1.20	17.18 ± 0.97
12	*Passer montanus*	4.56 ± 0.32	17.52 ± 2.11	22.62 ± 2.20
13	*Ploceus phillipinus*	17.33 ± 5.44	16.61 ± 1.53	27.39 ± 2.63
14	*Lonchura punctulata*	4.05 ± 5.33	13.56 ± 2.16	18.05 ± 1.91

Table 4 Monthly number of nests of different species encountered during the study period

Sr.no	Study species	Dec	Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
1	*Columba livia*	1	-	-	-	1	-	-	1	-	-	1	-	4
2	*Streptopelia chinensis*	-	1	-	1	-	-	-	1	1	-	1	-	5
3	*Aegithina tiphia*	-	-	-	-	-	-	-	2	-	-	-	-	2
4	*Acridotheres tristis*	-	-	-	-	-	-	1	1	-	-	-	-	2
5	*Pycnonotus blandfordi*	-	-	-	-	-	2	1	2	3	-	-	-	8
6	*Pycnonotus cafer*	-	-	-	-	-	-	1	1	-	-	-	-	2
7	*Prinia inornata*	-	-	-	-	-	-	-	2	1	-	-	-	3
8	*Orthotomus sutorius*	-	-	-	-	-	-	-	1	1	-	-	-	2
9	*Chrysomma sinense*	-	-	-	-	-	-	1	1	-	-	-	-	2
10	*Passer domesticus*	3	1	2	1	1	2	2	4	3	2	3	2	26
11	*Passer flaveolus*	-	-	-	-	-	2	1	1	-	-	-	-	4
12	*Passer montanus*	-	-	-	-	2	1	1	1	-	-	-	-	5
13	*Ploceus phillipinus*	-	-	-	-	-	-	6	8	4	-	-	-	18
14	*Lonchura punctulata*	-	-	-	-	-	-	5	6	6	4	1	-	22
	Total	4	2	2	2	4	7	19	32	19	6	6	2	105

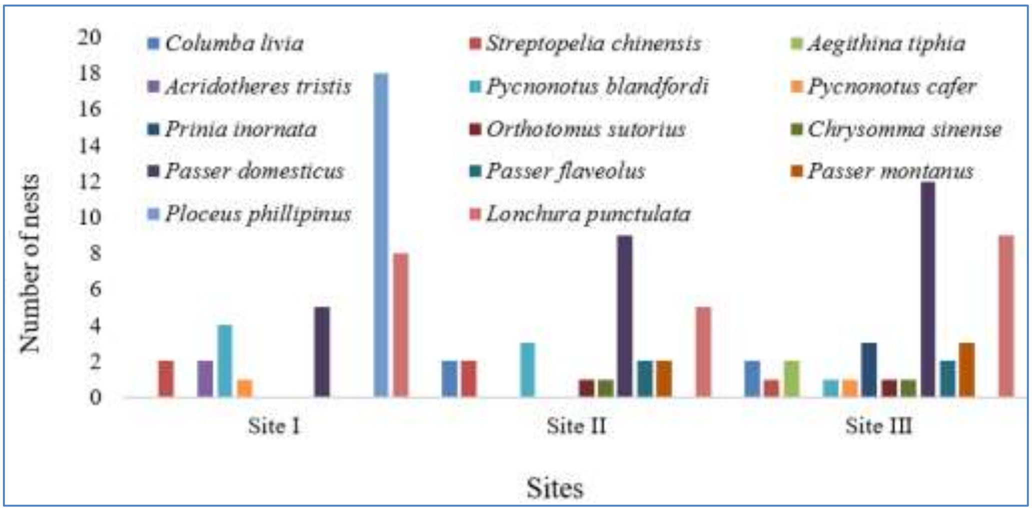

Figure 2 Relative numbers of nests of bird species recorded in different study sites

Columba livia and its

Streptopelia chinensis and its nest

Aegithina tiphia

Nest of *Aegithina tiphia*

Acridotheres tristis and its

Pycnonotus blandfordi

Nest of *Pycnonotus blandfordi*

Pycnonotus cafer

Nest of *Pycnonotus*

Prinia inornata

Nest of *Prinia inornata*

Orthotomus sutorius

Nest of *Orthotomus sutorius*

Chrysomma sinense

Nest of *Chrysomma*

Passer domesticus

Nest of *Passer domesticus*

Passer flaveolus

Nest of *Passer flaveolus*

Passer montanus

Nest of *Passer montanus*

Ploceus phillipinus and its nest

Lonchura punctulata

Nest of *Lonchura punctulata*

Plate. 1 Bird species studied and their nests

Discussion

A total of 14 species of nesting birds representing 105 nests was recorded from three study sites of Magway Township during December, 2016 to November, 2017. The 14 species of birds are distributed among 11 genera, nine families and two orders and all are terrestrial birds.

Collias and Collias (1984) observed that birds construct nests in a variety of different shapes, such as cups, domes, scrapes, or burrows and these act as an extended phenotype. Different types of nests were found in the present study such as platform type, dome type, irregular-shaped type, pendant type, cavity type and cup-shaped type respectively.

Hansell (2000) stated that the majority of nests are differentiated structures that are constructed from a variety of materials which can generally be classified as being either structural materials or lining materials. While structural materials make up the general shape of the nest and provide structural support for the parents and offspring. The lining materials generally create a suitable microclimate in which parents can raise their offspring. During the present study, 14 bird species used materials to construct the nests such as sticks, twigs, feathers, hair, wool, grass, straw, string, paper, grass inflorescences, stalks, threads, wool, leaves, human hair, spider silk and any other available materials.

Ashpole, *et al.* (2015) revealed that breeding season of *Columba livia* varies between regions. It breeds year-round in the U.K. and Ireland with peak breeding in April. In the Mediterranean it breeds between March and July and in Cyprus from March to May. The nest is a cup loosely fashioned from stems, leaves, roots, driftwood, seaweed and feathers. In this research, new nests of *Columba livia* were observed in each month of April, July, October and December. But they stayed in these nests the whole year.

Rajashekara and Venkatesha (2013) observed that the nest of *Streptopelia chinensis* was constructed on three to four ramified branches of a Croton plant, at a height of 1.52 m from ground level. The simple and fragile saucer nest was built with a loose platform of thread-like sticks and a few dried Croton leaves. During the present study, nest of *Streptopelia chinensis* is platform type. Nest height from the ground ranged from 9.1 to 27.43 m. The length of nest ranged from 23 to 28 cm and the height of nest ranged from 2.5 to 3.2 cm. They built their nests with sticks and twigs.

In the present study, the nest of *Aegithina tiphia* used sticky spider silk for nest building. Nest height from the ground is 1.8 m (5.91 ft) to 2.13 m (7 ft). The nest is a compact little cup, soft grass and root fibers neatly rounded off at the bottom.

Dhandhukia and Patel (2012) stated that the myna may also construct nest in holes on the wall of houses even in city area and old forts. Its colony size is often delimited by the availability of holes in manmade structures like bridge. Nest materials were grass, feathers of birds, plastic, cloth and flowers of Acacia. In this study, the common myna built their nests in the hole of the wall and in the crevice of building with twigs, grass, leaves and sticks.

Smythies (1953) observed that the breeding season of *Pycnonotus blanfordi* extends from March to August, but an odd nest may be found in almost any month, as with most of the common bulbuls. In the present study, three nests of *Pycnonotus blanfordi* revealed two nests in each May and July, one nest in June and three nests in August.

Rao *et al*. (2012) found that Red-vented Bulbul mostly preferred the small leafy, dense bushes and small leafy trees for nesting. *Pycnonotus cafer* constructs the nest at the junction of the bifuracated or trifurcated branch or on some similar substrate so as to get a firm support at bottom. It prefers the material for built up the nest like as small and smooth dry branches of stems and roots of herbs and grasses. Bulbul generally selects 2.0 to 3.5 height for nest construction. In the present study, nest height from the ground was 0.08 to 2 m. They built their nests with twigs and sticks. Nests were encountered on the tree *Cedrela febrifuga* (Yay-Tamar) and *Vachellia nilotica* (Sue- Phyu).

Gajera *et al.* (2009) stated that nest of Plain prinia and black-breasted weaver were preferably constructed among grasses and sedges, *Pennisetum typhoides* and *Typha angustifolia.* During this study, *Prinia inornata* selects their nest site marshy grasses especially *Lolium perenne* (Myat).

Tiwari and Anupana (2006) observed that nesting season of *Orthotomus sutorius* is from June to September. The usual nest type is a pouch formed by stitching together two leaves. The nest was a cup of soft material, slung from a small shelter of dry and dead date-palm leaves, 1.2 m above the ground. During the present study, nest of *O. sutorius* was found in the month of June and August. The nest was found on the trees of *Mangifera indica* (Tha-yet) and *Terminalia catappa* (Bar-dan) with thread and wool. Nest height from the ground was 1 to 2.4 m (1.7±0.7 m). They stitch leaves together to make a nest, using its needle-shaped beak and fine thread.

Nirmala (2015) stated that Yellow-eyed Babbler placed the nest at the junction of the main bifurcated branches so as to get a firm support at the bottom. The nests are easily distinguished with a definite deep statant cup, and were built with grass, rootlet and fully lined by cobweb outside, which gives the appearance of cemented outer layer. Leaves softened the inner base. In this study, the nest of *Chrysomma sinense* is deep cup shaped. They built their nests with twigs and sticks and the outside is covered by cobwebs.

House Sparrow built nest in small cavities like caves of houses holes in brick walls, dense trees and bushes and other natural and man-made nest boxes. Nest were made of coarse material on the outside such as, straw, twigs, paper, leaves, grasses and any other available material. Inside the nest was lined with feathers or fine grasses. House sparrow nest often in enclosed place and may expand to fill available volume. Nest in trees are usually globular structure with a side entrance. Dail (2003) reported that nest construction and placement are correlated with breeding season, suitable nest sites, nesting materials availability, food availability and predator's interaction. This study is in conformity with the finding of Dail, 2003.

Summers-Smith (1981) observed that the sites of *Passer flaveolus* included the outer branches of trees, where the nests were lodged in the forks and well concealed by the leaves; the crowns of coconut and sugar palms; a hole in a tree. During the present study, the nest of *Passer flaveolus* is dome type. They built their nests on the tree of *Senegalia catechu* (Shar) and *Ziziphus jujube* (Zi) used with feathers, human hair, wool, grass and straw.

Achegawe *et al.* (2016) stated that nesting material was used to construct the nest proper that was 1-2 ft by *Ploceus phillipinus.* When the monsoon starts during month of July, August each year in this part of the world, the grasslands flourish and grow well till end of August to September. In the present study, pendant nests were constructed by the Baya Weaver. Pendant nests are elongated sacs woven of pliable materials such as grasses and soft plant fibers. The Baya Weaver prepared Palm and Coconut trees for nest construction. Many males may construct their nests on *Cocos nucifera* (Ohn) and *Albizia saman* (Kote-ko) and nest height from the ground ranged from 4.14 m (13.58 ft) to 22.1 m (72.507 ft).

In the present study, Scaly-breasted Munia built globular and dome-shaped nest with a lateral entrance hole. Grass blades were used to build the nest. Nest was largely built on the twigs present inside the canopy. Nests were encountered on the trees such as *Dypsis lastellina* (Ohn), *Vachellia nilotica* (Sue- Phyu) and on the window ledge of buildings, *Azadirachta indica* (Ta-mar), *Ziziphus jujube* (Zi), *Acacia leucophloea* (Hta-naung), *Senegalic catechu* (Shar) and *Citrus limon* (Shauk). The present study is in agreement with early finding of Kumari, *et al.* (2016) who suggested that breeding season of Spotted Maunia was initiated in rainy season from end of June and continued till November. However, its peak was observed to be during August and September. Maunia was globular or dome shaped made of grass blades and leaves with a lateral entrance hole oriental mainly along the most frequent wind direction. The majorities of the nests was located on the thorny plants species as well as on ornamental trees and shrubs and were placed on twigs towards the centre covered with thick tree canopy.

A total of 105 nests of birds were recorded during study period. In study Site I, Magway University Campus, a total of 40 nests in seven species were recorded. During the present study, two nests of *Streptopelia chinensis,* eight nests of *Lonchura punculata,* five nests of *Passer domesticus,* two nests of *Acridotheres tristis*, 18 nests of *Ploceus phillipinus*, one nest of *Pycnonotus cafer* and four nests of *Pycnonotus blanfordi* were recorded in Site I. Site I is located in the center of city with median and large trees, some crops plants and bushes.

In study Site II, Wa-taw-chaung Village, a total of 27 nests in nine bird species were recorded. It has cultivated crops and large and small trees. In this Site II, two nests of *Columba livia,* two nests of *Streptopelia chinensis,* five nests of *Lonchura punculata,* nine nests of *Passer domesticus,* two nests of *Passer flaveolus,* two nests of *Passer montanus,* three nests of *Pycnonotus blanfordi*, one nest of *Chrysomma sinense* and one nest of *Orthotomus sutorius* were found.

In study site III, Kyit- Sone Pwe Village, a total of 38 nests but twelve species of bird's nest were recorded. It is located beside of Magway- Naypyitaw highway and it is rural area with thick vegetation, standing or flowing water and ponds, large and median size trees. This site provides various kinds of bird species. Two nests of *Columba livia,* one nest of *Streptopelia chinensis,* nine nests of *Lonchura punculata*, 12 nests of *Passer domesticus,* two nests of *Passer flaveolus,* three nests of *Passer montanus,* two nests of *Aegithina tiphia*, one nest of *Chrysomma sinense,* three nests of *Prinia anorata,* one nest of *Pycnonotus cafer* and one nest of *Pycnonotus blanfordi* were recorded in Site III. This site provides various kinds of bird species.

The occurrence of bird nest in different habitats may be due to availability of suitable breeding site and local environmental condition. These kinds of habitat provide concealment to the nest from higher risk of predation and anthropogenic disturbance.

In the present study bird species showed denser canopy of trees and thorny plant species as nesting substrate. The denser canopy of the nesting tree could provide safe nesting place from the predators as well as weather related problems. This present study was conducted very short period and future long-term studies covering all aspects to understand the bird nest-site selection are needed.

Conclusion

In conclusion, Magway Township is situated in a central dry zone of ecosystem, and the habitat coverage of Magway Environ is sound for birds. Bird population, richness, and density are depended on habitat condition of their habitat area. Thus, habitat conservation is needed to more variety of birds for shelter, food and breeding. During the study period, the habitat was destroyed by people. Therefore, the bird population was reduced conspicuously. Hence, conservation should be done not to destroy the habitats for the breeding of birds. This is not only to protect the habitat but also to maintain the population status.

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ASSESSMENT ON THE DIVERSITY OF BIRD FAUNA FROM THILAWA SPECIAL ECONOMIC ZONE AND IMPACTS OF THE PROJECTS IN THANLYIN TOWNSHIP, YANGON, MYANMAR

San San Oo[1], Nyo Nyo Aung[2], Myo Sandar Win[3], Aung Pe Lwin[4],
Nyunt Nyunt Oo[5], Theingi Thein[6], Khin Mar Lwin[7]

Abstracts

This study is to assess the diversity of birds in Thilawa Special Economic Zone, outskart of Yangon, Myanmar.The bird species diversity was investigated in theproject area, Thilawa Special Economic Zone (SEZ) to assess the environmental impact of the project. The point count method was used and the data were monthly collected during June, 2015 to May 2016. The project area is 2400 hectares covering mostly paddy field with some human inhabitations. A total of 91 bird species representing 55 genera, 40 families and 8 orders were recorded from the Thilawa SEZ. Among them, 32 species from paddy field habitats, 58 species from shrubs and bushes, 32 species from wetland habitat and 55 species from scattered trees habitats wer observed. They represented 61 species of terrestrial birds (51 species of resident birds and 10 species of migratory birds), and 30 species of water birds (20 species of resident birds and 10 species of migratory birds) could be categorized. 73 species (1439 individuals of birds) in cool season, 36 species (644 individuals of birds) in wet season and 50 species (733 individuals of birds) in dry season were assessed. According to abundance category, two species of abundance, two species of frequent species, nine species of uncommon and 78 rare species were examined. The birds were moderately diverse according to Shannon Index value (H"= 8.566) with the evenness value (-8.577). According to IUCN Redlist category, 90 species are under least concern species and only one species Ploceus phillipinus was least concern species. Hence, the bird fauna in the project area is still diverse.

Keywords: Bird diversity, Impact Assessment, Special Economic Zone.

Introduction

Withmore than 5,000 special economic zones (SEZ) across 140 economics today, the world Investment Report 2019 says SEZs perform as a new wave of industrial policyand as response to an increasing competition for internationally mobile investments (Lynn, 2019). Accelerated foreign direct investments through new settings of special economic zones have becoun in recent years. The government commited to development two more SEZs worth billions of dollars namely Dawei SEZ and Kyauk Phyu SEZ: in addition to Thilawa SEZ, which is alrearly completed and start running. The Environmental Impact Assessments (EIA) on the aspects of social, hydrological, health status and biological in which floral and fauna (fishes, herpets, birds and insects) surveys were conducted. This paper is species diversity of the birds in the project area and potential impacts of the Thilawa SEZ project on the birds (Thilawa SEZ).

An Environmental Impact Assessment (EIA) is a legal requirement in Myanmar for all development projects. It was initited since the 2005-06, when the Htamanthi Hydropower Dam construction was implemented. After wards, EIA is playing the major roles for all projects to be legaledimplemented in Myanmar. In some projects, the EIA was started after initiation of the

[1] Professor, Department of Zoology, University of Yangon
[2] Associate Professor, Department of Zoology, University ofDawei
[3] Lecturer, Department of Zoology, Yangon University
[4] Lecturer, Department of Zoology, Dagon University
[5] Professor, Department of Zoology, DaweiUniversity
[6] Associate Professor, Department of Zoology, Maubin University,
[7] Lecturer, Department of Zoology, Yangon University

projects and running condition. The Thilawa SEZ was conducted the EIA assessment well before the stated of the project (Thilawa SEZ).

Creation of environemtal impact assessment (EIA) system is vital to confirmscio-economic development project to environmental safety and there by ensure sustainable economic development. It helps the planning and management to take long time measures for effective management as well as environmental conservation. EIA thus ensure that the potential problem are foreseen and addressed at early stage in project planning and design. In any country, the environmental impact assessments (EIA) are inevitable conducted before project implementation. Any project usually makes more or less land cover changes that might affect negatively on the ecosystem. Actually the project is essential for the development of the country, but any project will effect more or less on the environment and sustainable ecosystem. There are also many ways of impacts, such as social impact, physical impacts, the impacts on the air and water and biological impacts that cause the air pollution and water pollution. Every aspect needs to assess the duration and magnitude of impacts. In biological study, what plant and animal species are present and what are their eological roles in the project area first, and assess the kinds of impacts on the eosystem. Mitigation measure during the project implementation and continue monitoring the impacts.

Birds are one of the important indicators of environmental condition, highest species numbers and bird numbers were observed in healthy environment and lowest of bird diversity in poor habitat (Krebs, 2001). Species diversity representing species richness and individual numbers of particular species can assess the condition of the habitats of any ecosystem. Myanmar is one of the heighest biodiversity areas in Southeast Asia including richness in avifauna. The current classification of living birds is a hierarchical arrangement of roughly 29 orders, 187 families, over 2000 genera, and over 9,600 species. The total number of bird species today has been estimated more than 400 in the Europe, 645 in North America and 1300 in the Indian Sub-Continent comprising India, Pakistan, Bangladesh, Bhutan etc, Malaysia has 725, Singapore has 335 and Thailand has 915 (Gill, 1994).

Myanmar has about 1100 species, an estimate based on records by Ministry of Forestry and bird guidebooks of Southeast Asia and the Indian Sub-Continent (Gill, 2001). Animal distribution is mainly dependent on at least six factors; means of dispersal, habitat selection, interaction with other species, temperature, moisture, and other physical-chemical factors and the relationship between distribution and abundance (Krebs, 2001). Habitat selection in bird is partly a genetic trait although it can be modified by learning and experience. The original habitat selected by a bird is often reinforced by tenacity of individuals to their sites. Many old birds return year after the same nesting sites, even if the habitat at that sites is in deterioration. Most terrestrial environments undergo seasonal changes in habitat structure and food abundance and these changes are likely to influence diversity. Seasonal variations in the diversity of the bird species in several habitats decreased with increasing vegetation complexity (Haslem and Bennett, 2008).

Ecosystems with scattered trees occur throughout the world. Scattered trees are prominent features in many landscapes worldwide, including natural landscapes, cultural landscapes, and recently modified landscapes. The ecological importance of scattered tree is widely acknowledged in natural landscapes, but has not been sufficiently appreciated in human-modified landscapes.

Wetland habitat are commonly used by fish-eating birds, such as Egrets and Pelicans, but a lot of birds can feed together by eating different kinds of food at different levels in the water. Wetlands are often refuges for rare birds, as large mammals predators cannot easily hunt there. These habitats are also used by home to a rich variety of birds, such as Ducks, Coots, Rails, Herons and Storks. Many birds rest and feed on lakes, marshes and swamps during migration. But drainage schemes, dams, acid rain and pollution from farms and factories threaten these habitats.

Forest habitats provide birds with plenty of food and safe nesting places. A greater variety of birds live in the deciduous and eucalyptus woodland than in the dark conifer forests, because of the warmer, wetter conditions. Similar threats from habitat alteration face birds of moorland and wetland. Afforestation of moorland may diversely affect relatively common birds, such as meadow pipit and skylark, as a consequence of changes in the farming landscape. Habitat fragmentations not only reduce the area available to species but extend the amount of edge and increase the changes of population isolation. Habitat heterogeneity is also an important variable, through often a larger area is actually associated with greater habitat diversity (Jones, 1998).

The followings are the objectives for this research:

- To record the bird species and their relative abundance
- To categorize the bird status in the Thilawa SEZ project area
- To access the utilization of different habitat types
- To examine the seasonal variation and
- To calculate species diversity and its evenness

Materials and Methods

Study area and study period

The study area of Thilawa Special Economic Zone located in (N 16˚ 67′ 80. 47" and E 96˚ 27′ 24. 5") was classified into four habitat types depending on the plant species composition and water sources, (1) Paddy field habitat types, (2) shrubs and bush habitat type (3) wetland habitat type (4) and scattered trees during June 2015 to May 2016. Most of the study area was convered with paddy field, and several human habitations (villages and Buddhist moenstries) where tall and median sized pants are growing. Shrubs and bushes are covered unused land area, near vicinity of human habitations. Lakes and ponds in the study area are also filled with water throughout the year. Hmaw-iwn River is running southern to western sites of the study area.

Bird watching and data collection

Bird watching was weekly conducted in the morning from 6:00 am-11:00 am and in the afternoon from 3:00 pm - 6:00 pm. Number of birds, and bird activity patterns (flying, feeding, perching, resting and single or couple or groups) and their habits were observed andnoted down in field data form. Point count method was used in this study. A binoculars and digital camera power shot (5 × 50 Hs) were used for observing the plumage colours and patterns of the birds and for taking bird photographs to confirm the species for further investigation. Identifications followed after Robson (2008) and Smythies (1986).

Data analysis

The recorded data were calculated into five catogeries, (1) habitat preference, (2) seasonal variation, (3) the status of bird species (resident birds, migratory birds, terrestrial birds, water birds), (4) abundance categories and (5) species diversity. Habitat preference was grossly assessed on the basis on the habitat types the bird species observed as habitat type (1) paddy field habitat type (2) scater trees and bushes habitat type (3) grass land habitat type and (4) water habitat type (5) wetland habitat type including lake, stream and river side. Actually, the habitat types are usually changes with seasons due to the growing plants and trees. The status of bird species were also classified as terrestrial birds and water bird, resident birds, migratory birds. The different status of bird plays and used as different feeding strategy. The abundance category indicating individual numbers of birds of each species was followed after Kumar and Sivaperuman (2005)and was calculated as the formula mentioned below:

$$\text{Relative abundance} \quad = \quad \frac{\text{Total numbers of particular species}}{\text{Total species numbers}}$$

Abundance categories were determined based on index values as follows:

Rare Species = (0.1 – 2.0) Common = (6.1- 8.0)

Uncommon = (2.1 -4.0) Abundant = (8.1 – above)

Frequent = (4.1 – 6.0)

$$\text{Dominance index} \quad = \frac{\text{Total numbers of each species}}{\text{Total individual numbers of all species}}$$

For the assessment of diversity of bird species in the study area, Shannon index and Evenness formula were used (Stilling, 1999).

Shannon Index $= H' = \Sigma \text{ Pi LnPi}$

Where

$$\text{Pi} \;=\; \frac{\text{No.of bird species}}{\text{Total No.of all bird species}}$$

Identification

Identification was followed after Robson (2008) and KyawNyuntLwin (2003) and Khin Ma MaThwin (2003), and Bird life international (2013).

Source: Google Earth2019

Figure 1 Map of ThilawaSpecial Economic Zone of Thanlyin Township. Yangon, Myanmar

Results and Discussion

In the project area of Thilawa Special Economic Zone (SEZ), Thanlyin area, Yangon, Myanmar, 91 species, under 40 families of eight orders of the birds were recorded during the surveyed period. In this project area of Thilawa (SEZ), the majority was resident bird species (78%) and the minority was migratory species (22%), that represent 51 resident species and 20 species of water birds.according to the combined data, terrestrial resident species was 51 species of birds. Among the 91 bird species, 61 species of terrestrial birds 51 resident birds, 10 species of migratory birds) and 30 species of water birds representing 20 resident birds and 10 migratory bird spcies were recorded. Hence the status of observed bird species from this area showed that the different ecological functioning are diverse in their habitat types (Table 1Figure 2 and Table 2).

There were 58 species representing 440 individuals from shrub and bush habitat types. This type was the boundariesofbetween the paddy fields, vicinity of humanhabitations such as villages and monestries. In this area, no crops were planted, the plant types of small plants, bushes, shrubs and harbs grow. Their habitat was changes a little during different seasons. The insects and small grains as food and also as shelther in the hot times and also hiding places. The shurbs and bushes are smallin sizes and this is main causes of small population size with higher numbers of species (Table 4). In the wetland representing lakes, ponds, streams and Hmaw-win River, 32 species including both miratory and resident species were observed. The numbers of birds was highest (1412 individuls) (Table 2). Tall trees were found in the villages, around the lakes and ponds, in which 55 bird species found. The birds were found during the day time with perching on the trees. The nests of some birds were found in this stuy site. Hence, this is roosting sites of the terristral birds. The habitat types are always changes through times and palces. The birds are also changes with seasons due to their habitats supported of different kinds of foods. The weather conditions such as temperature, humidity, rain fall etc. are aso found to vary withof cool season and breed in the hot season. Some migratory birds were not going back their northern parts of their home. The lowest diverse bird species was in wet season (raining season). The reason of the low numbers and low species was that the encountered rate (so call observability) was low in this season (Table 5).

According to the habitat utilization of bird species 32 bird species with 548 individual numbers of birds were examined from paddy field. Actually, this habitat types is seasonally changes, paddy plants usually growin the wet season and it was changes after harvest time. This habitat type was changes onlyweathercondition but also human affect. At the beginning time of growing season, the insectivorous bird species were dominately observed in this habitat type. At the ripening stage of paddy plants, the granivorous birds were dominated. This habitat type supports the bird species for food as foraging ground. Hence, this habitat type was foragingground of both insectivorous and carnivorous birds (Table 2).

According to the abundance categories, only two species, Lesserwhisting duck and Brown shrike were observed. The most important thing is rare species (78 species) and they were very small in numbers, consecuently they are very vulnearable to natural selection and might be easily extinct locally.Assessment of species diversity of Shannon index was measured by two parameters, species richness and individual numbers. This index vaule was representing the common species and also rare species. Conclusively, the calculated value of diversity assessment, both values showed that the study area was still diverse in avifauna meaning that this environment and ecosystem were good in healthy condition. No fauna species under the IUCN Red List and Cites appendices were recorded in SEZproject area at the survey time. According to IUCN Redlist, only one species *Ploceus phillipinus* was least concern species.The population of each species in the project area varied greatly from one individual number to over 1000 birds.

Table 1 Status of the bird species recorded from Thilawa Industry Zones

Bird status	Terrestrial birds	Water birds	Total No. of species
Resident birds	51	20	71
Migratory birds	10	10	20
Total No. of species	61	30	91

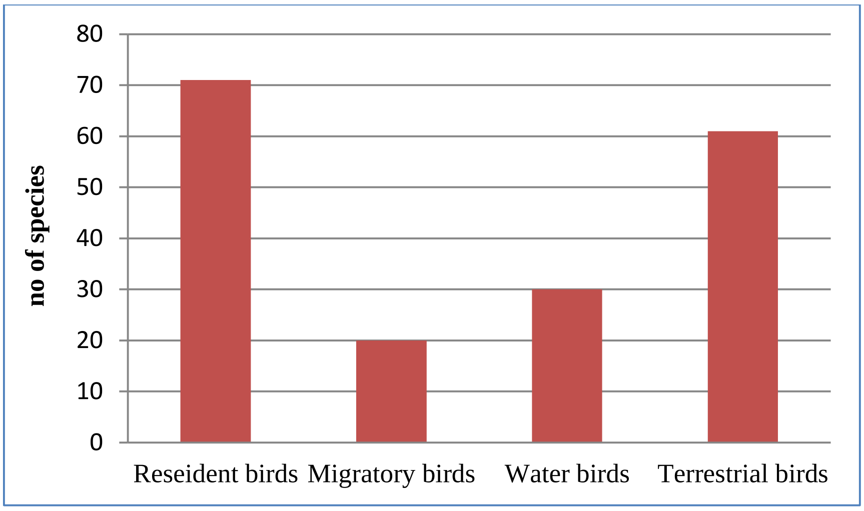

Figure 2 Number of species of different bird status collected from Thilawa SEZ project area

Table 2 Abundant categories of bird species and status of bird species

No.	Scientific Name	Common Name	Bird Status	Total No	AC	No.
1	*Tadornaferruginea*	Ruddy Shelduck	M	W	4	Rare
2	*Nettapuscoromandelianus*	Cottom Pygmy Goose	R	W	11	Rare
3	*Dendrocygnajavanica*	Lesser Whishing Duck	R	W	1030	Abun
4	*Tachybaptus ruficollis*	Little Grebe	R	W	2	Rare
5	*Amaurornisphoenicurus*	White breasted water hen	R	W	3	Rare
6	*Porzanafusca*	Ruddy breasted crake	R	W	1	Rare
7	*Gallinula chloropus*	Common Moorhen	R	W	1	Rare
8	*Metopidius indicus*	Bronze-winged Jacana	R	W	1	Rare
9	*Tringa tetanus*	Common Redshank	M	W	136	Freq
10	*T. nebularia*	CommonGreenshank	M	W	2	Rare
11	*T. ochropus*	Green Sandpiper	M	W	1	Rare
12	*T. stagnatitis*	Marsh Sandpiper	M	W	4	Rare
13	*Actitishypoleucos*	Common Sandpiper	M	W	12	Rare
14	*Charadrius dubius*	Little-ringed Plover	M	W	6	Rare
15	*Himantopus himantopus*	Black-winged stilt	M	W	7	Rare
16	*Chlidoniasleucopterus*	White-winged Term	M	W	1	Rare
17	*Egretta garzetta*	Little Egret	R	W	104	Unco
18	*Mesophoyx intermedia*	Intermediate Egret	R	W	4	Rare
19	*Bubulcuscoromandus*	Cattle Egret	R	W	17	Rare
20	*Ardeolagrayii*	Indian Pond Heron	R	W	58	Unco
21	*Butoridesstriatus*	Little Heron	R	W	4	Rare
22	*Ardeoabacchus*	Chinese Pond Heron	R	W	1	Rare
23	*Ardeapurpurea*	Purple Heron	R	W	7	Rare
24	*A alba*	Great Egret	R	W	13	Rare
25	*A cinerea*	Grey Heron	R	W	4	Rare
26	*Nycticoraxnycticorax*	Black-crowned Night Heron	R	W	4	Rare
27	*Ixobrychus flavicollis*	Black Bittern	M	W	3	Rare
28	*I. sinensis*	Yellow Bittern	R	W	1	Rare
29	*Anastomusoscitans*	Asian –Open bill	R	W	4	Rare

No.	Scientific Name	Common Name	Bird Status	Total No	AC	No.
30	*Phalacrocorax niger*	Little Cormorant	R	W	31	Rare
31	*Tyto longimembris*	Grass Owl	R	T	1	Rare
32	*Milvus migrans*	Black Kite	M	T	65	Unco
33	*Elanus caeruleus*	Black shouldered kite	R	T	3	Rare
34	*Accipiter badius*	Shikra	R	T	1	Rare
35	*Falco tinnunculus*	Common Kestrel	M	T	2	Rare
36	*Coracias benghalensis*	Indian Roller	R	T	2	Rare
37	*Megalaimahaemancephala*	Coppersmith Barbet	R	T	2	Rare
38	*Meropsorientalis*	LittlegreenBeeeater	R	T	15	Rare
39	*M. philippinus*	Blue-Tailed Beeater	R	T	5	Rare
40	*Alcedoatthis*	Common King fisher	R	T	2	Rare
41	*Halcyon smyrnensis*	White throated Kinfisher	R	T	3	Rare
42	*H. pileata*	Black copped Kingfisher	M	T	2	Rare
43	*Cacomantismerulinus*	Plaintive cuckoo	R	T	6	Rare
44	*Centropus sinensis*	Greater Coucol	R	T	147	Freq
45	*Cypsiurusbalasiensis*	Asian Palm Swift	R	T	7	Rare
46	*Hemiprone coronate*	Crested Tree Swift	R	T	1	Rare
47	*Iynxtorquilla*	Eurasian wryneck	R	T	1	Rare
48	*Artamusfuscus*	Ashy Wood Swallow	R	T	2	Rare
49	*Aegithinatiphia*	Common Iora	R	T	2	Rare
50	*Rhipiduraalbicollis*	White throated Fantail	R	T	13	Rare
51	*Columba livia*	Rock-Pigeon	R	T	67	Unco
52	*Streptopelia chinensis*	Spotted Dove	R	T	1	Rare
53	*S. tranquebarica*	Red turtle Dove	R	T	66	Unco
54	*Laniuscristatus*	Brown shrike	M	T	228	Abun
55	*Corvus splendens*	House Crow	R	T	7	Rare
56	*C. macrobrynchos*	Large –billed Crow	R	T	76	Unco
57	*Dicrurusmacrocercus*	Black Drongo	M	T	7	Rare
58	*Rhipiduraalbicollis*	White-throated Fantail	R	T	10	Rare
59	*Copsychussaularis*	Oriental magpie-Robin	R	T	8	Rare
60	*Saxicola Maura*	Eastern Stone cat	M	T	17	Rare
61	*S. capratastar*	Pied bush cat	R	T	70	Unco
62	*Acridotheres tristis*	Common Myna	R	T	47	Rare
63	*A. fuscus*	Jungle Myna	R	T	7	Rare
64	*A. burmannicus*	Vinaus breasted starling	R	T	2	Rare
65	*Gracupica contra*	Asian pied staring	R	T	1	Rare
66	*Sturnus malabaricus*	Chestrut tail Starling	R	T	1	Rare
67	*Alauda gulaula*	Oriental skylark	R	T	1	Rare
68	*Riparia paludicola*	Sand Martin	M	W	8	Rare
69	*Hirundorustica*	Barn Swallow	R	W	86	Unco
70	*Pycnonotuscafer*	Red vented Bulbul	R	W	11	Rare
71	*P. jocosus*	Red whiskered bulbul	R	T	7	Rare
72	*P.blafordi*	Streak eared bulbul	R	T	2	Rare
73	*Cisticola juncidis*	Zitting cist cola	R	T	6	Rare
74	*Orthotomussutorius*	Common tailor bird	R	T	5	Rare
75	*Priniainornata*	Plain prinia	R	T	36	Rare

No.	Scientific Name	Common Name	Bird Status	Total No	AC	No.
76	*P.hodgsonii*	Grey breasted prinia	R	T	1	Rare
77	*P. flaviventris*	Yellowbellied prinia	R	T	10	Rare
78	*Acrocephalusorientalis*	Oriental reed Warbler	R	T	15	Rare
79	*Phylloscopusfuscatus*	Dusky Warber	M	T	6	Rare
80	*Timaliapileata*	Chestnut Capped babbler	R	T	1	Rare
81	*Turdoidesgularis*	White throated babbler	R	T	1	Rare
82	*Chrysommasinense*	Yellow –eyed babbler	R	T	8	Rare
83	*Motacilla alba*	White Wagtail	M	T	86	Unco
84	*M. flava*	Yellow Wagtail	M	T	11	Rare
85	*Passer domesticus*	House Sparrow	R	T	7	Rare
86	*P. montanus*	Eurasian tree Sparrow	R	T	2	Rare
87	*Ploceusphilippinus*	Baya Weaver	R	T	6	Rare
88	*P.hypoxanthus*	Asian Golden Weaver	R	T	5	Rare
89	*Lonchuapunctulata*	Scaly breasted Munia	R	T	36	Rare
90	*L. atricapilla*	Chestnut Munia	R	T	1	Rare
91	*L. striata*	White rumped Munia	R	T	10	Rare

T=terrestrial birds, W= water birds, R=Resident birds, M= Migratory Birds

Table 3 Abundance category of birdpecies in the present study area

Abundance categories	Abundance	Common	Frequent	Uncommon	Rare
No of species	2	-	2	9	78

Table 4 Numbers of birds and bird speciesrecorded from different habitat types

Current status	Paddy field	Shrubs and bushes	Wetland	Scattered trees	Total
No of species	32	58	32	55	50
No of birds	548	440	1412	416	2816

Table 5 Seasonal variation of birds and bird species in the study area

Birds and seasons	Cool season	Wet season	Dry season	Total
No. of species	73	36	50	50
No. of birds	1439	644	733	2816

Table 6 Diversity index value of birds of the study area

No.	Diversity index	Index value
1.	Shannan Weiner H' = ΣPiLnPi	8.566E-02
2.	EvenessE = H'/S	8.557

The project area of Thilawa Special Economic Zone located at Kyauktan Township was assessed as high diversity of avifauan assessing 91 bird species including 61 terrestrial bird species (51 resident species and 10 migratory species) and 30 species of water bird species (20 resident species and 10 migratory species). In the project area, resident birds were dominant species (71 species). The most diverse species was Lesser whishing Duck *Dendrocygna*javenicaand the second highest species was the Brown Shrike *Lanituscristatus*. They were observed in three seasons the highest numbers compare with other speices. In the project area, 78 species are rare species, 9 species are uncommon, 2 frequent and 2 species are abundance categories, and no common specie is not observed. According to the concept of population ecology, this may be assumed as balance ecosystem.

In the project area, four habitat types as paddy field, shrubs and bushes habitat, wetland land habitat and scattered trees habitats were utilized by the bird fauna. The species was highest in the shrubs and bushes habitat while the highest numbers of birds was observed in wetland habitat. Here, two permant lakes, three small streams and the large Hmaw-win River were included in this habitat type. This is due to the migratory birds, they all have large number of group in each species.

Accoding to seasonal observation, largest species numbers and populations size was examined cool season (73 species and 1439 individual numbers), while second highest was observed in dry season. The least number was observed in wet season. This is due to availability of the food source. They definitely use these habitat types for the foraging although they may use as roosting. In the survey time, the very few bird nests were observed. Conclusively, there was high bird species diversity in this area, and we can conclude this project area is suitable for the bird fauna and can good provide. After implementation on the project, the bird fauna have to move to another place due to the habitat loss.

Conclusion

In the project, 91 bird species representing 71 resident species and 20 migratory species were recorded. According to habitat preference, highest species number was observed in shrubs and bushes habitat types, although individual numbers was highest in wetland field. The seasonal variation was examined and the highest species diversity was in cool season and lowest was in wet season. The project area of Thilawa Special Economic Zone located at Kyauktan Township was concluded as high diversity of avifauan. There was no adverse impact in this area.

Acknowledgements

We would like to thank Dr.NilarAung, Pro-Rector of Yangon University, Dr. Aye Mi San, Head and Professor of Zoology Department, University of Yangon for their kind permission and NEPS International Non-Government Organisation for financial support this project assessment. The last but not least Dr. Khin Maung Swe, Retired Professor of Zoology Department, Dagon University, the Project leader of Environmental Impact Assessemnt (Fauna Specialist) to permit the research paper.

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LEARNING EXPERIENCE AND AGONISTIC PLASTICITY IN *BETTA SPLENDENS*

San San Myint[1], Omar Myint[2]

Abstract

Learning behaviour in a population evolves when it improves fitness of the individual. Interactions within the members of population may require the cognitive abilities which gives advantages in their lifetime reproductive fitness. Individuals may gather information and use the experience later in their life. In this study the learning behaviour of *Betta splendens* and its advantages in the social environment was investigated. It was hypothetized that organism will take advantages by learning from others, especially from the conspecific, to promote their lifetime reproductive fitness, in changing environment. Aggressive behaviour of two males *Betta splendens* were observed by the learners from separate tank (experiment 1). The social interaction between the winner from the first experiment and the learners were also observed (experiment 2). The learners received information through visual learning took advantages from prior experience. When learners encounters with the winners, they rarely compete to fight with them. In contrast, when they met with the losers, they always try to compete with them. The non-experienced individuals usually try to compete with each other unless they were injured during combat between them. These results clearly show that learners took advantages from prior social experience by showing behavioural plasticity in the changing environment. It was concluded that learning behaviour was simply evolved during the individual lifetime since adoption of learning gives some benefits to their life time reproductive fitness.

Keywords: learning, aggressiveness, evolutionary fitness, experience

Introduction

Learning simply defined as the acquisition of a new behavior or change in behaviour through interaction with the stimulus. Learning behavior is a common phenomenon among vertebrates especially found in birds, fish and mammals. It provides individual fitness, effective foraging, access to mates and predator avoidance. Knowledge on learning behavior is essential to the understanding of individual adaptation through which an organism responds to its changing environment.

The interaction among members of the same species reflects the sociality of the species. Different sensory cues such as vision and olfaction might be utilized by different species for communication. Members from a population might use different sensory cues to recognize each other in some animals. Thus, learning behaviour plays an important role in the evolution of animal behavior.

Baldwin (1896) proposed that a trait, which is evolutionary beneficial, is selected in one generation and passed it on to later generations successfully, then the trait will be genetically specified and appear in subsequent generations. Maynard-Smith (1972) developed an evolutionary stable strategy (ESS) which is a strategy once adopted by a majority of the members of population, it cannot be replaced with any alternative strategy through natural selection.

Learning ability depends on memory of the individuals and the memory reflects the individual's neuroplasticity. In recent decades, many researchers had been attempted to clarify the complexity between the learning behavior and the brain function in many different species.

[1] Lecture, Department of Zoology, University of Yangon
[2] Lecture, Department of Zoology, University of Yangon

However, the understanding on the mechanism of learning behaviour is still a challenging problem for neuroscientists.

Many researchers have been used game theory to analyze animal behaviour such as predator-prey interactions, learning, communication, habitat selection etc. Game theory also provides researchers to assess the potential benefits of novel strategies upon introduction into a population. In this study, learning behaviour of *B. splendens* was investigated in order to understand their cognitive ability and the payoff towards the fitness of the individual through learning experience.

B. splendens is well known for its aggressive behaviour, showing sequences of threats (display) and attacks to opponents, and it is one of the most suitable fish for observing agonistic plasticity (Baenninger, 1984; Bronstein, 1985; Evans, 1985; Galizio et al., 1985; Halperin et al., 1992; Robertson, 1979; Wallen and Wojciechowski Metzlar, 1985). The differences in individual aggressiveness of *B. splendens* might be the result of prior social experience and the individual physiological condition. In their natural habitat, they may receive information from various sources such as encountering with conspecific and visual learning (Cain, 1980). We hypothesized that individuals experienced with visual learning may avoid physical damage from intensive aggressiveness and allow fostering pay-offs to invest in their lifetime reproductive fitness. In this study, we will investigate the learning ability of *B. splendens* and their agonistic plasticity with the two continuous experiments.

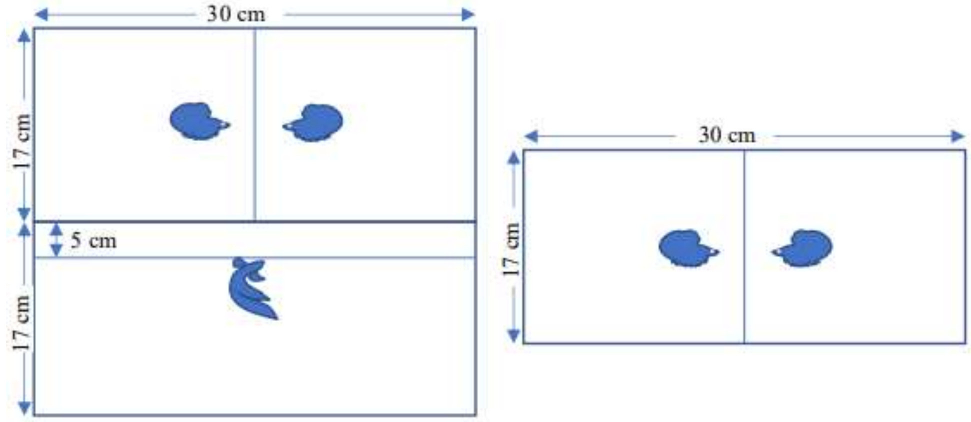
Materials and Methods

Learning ability of *B. splendens* and their plasticity in aggressive behaviour were investigated from May 2019 to July 2020. A total of ninety adult males Siamese fighting fish were obtained from a local supplier and kept separately in 1 liter glass bowls. They were visually

isolated from one another before starting of the experiment. All fish were fed food pallet twice a day and kept under a 12:12 light dark cycle. Water in the plastic bottles was refreshed once a week in order to maintain the health of the fish and if there was infection of disease, fish were treated with medicine. Individual fish were housed in the experimental tank 48 hr prior to the experiment to acclimatize with the new environment. The following experiments were conducted to understand the learning behaviour of *B. splendens* and their behaviour plasticity. Experiment 1 (Observing the learning behaviour of *B. splendens*)

Two matched size males *B. splendens* were randomly assigned into the two compartment tank (30x22x17cm), which is separated with a transparent plastic sheet in the middle of the tank (Fig.1), 48 hr prior to the experiment. An opaque glass was placed between the two compartments to prevent fish seeing from each other before the experiment and it was removed once experiment start. Another one compartment tank (30x22x17cm), which was designed to see from one side using the sun visor thus the fish from outside of the tank could not see inside of the tank, was placed next to the experimental tank (Fig. 1). A similar sized male was placed into one compartment tank in order to allow the fish to observe the behaviour of fish from the two compartment tank. Hence, learner fish could see both of the two males from two compartment tank but the encounters could not see learner fish. The experiment was lasted for fifteen minutes and all fish behaviour were recorded with a digital video camera (Canon EOS 7D Mark II. Experiment 2 (observing the agonistic plasticity)

To observe the learning experience, an experimental tank was equally divided into two compartments with a transparent plastic plate. Both learner and winner fish were placed into each compartment, and were observed the frequency of mutual attack between them for 15 minutes (N=15). If one individual defeats the other within the observation period, the experiment was finished and recorded the time. The observed fish from the two experiments were not repeated. The same experimental design was conducted using with observer and loser fish from the first experiment (N=10). The following behavior were recorded during the experiment: (a) frontal display: duration of fish showing frontal display within 5cm of the wall, (b) attack: number of biting and tail beats (c) escape: duration of fish outside of 5cm of the wall, (d) threat: duration of gill erection, and (e) learning: duration of fish learning. Experiment was lasted 15 minutes and testing was terminated when one fish fled at the approach of the other.



(experiment 1) (experiment 2)

Figure 1 Top view of the experimental tank designed to observe the learning behaviour of male *B. splendens* (experiment 1) and agonistic plasticity (experiment 2)

Statistical analysis

All data were checked for normality test using the Kolmogorov-Smirnov test. Some of the distributions were significantly deviating from the normal distribution then we used non-parametric test. When the data were normally distributed, we used parametric test. All statistics were analyzed using the SPSS 24.0 package.

Results

In experiment 1, the learning behaviour of B. splendens was observed (N=30). The competition between paired males was also recorded (N=30). Twenty five out of 30 pairs of male *B. splendens* established clear dominant/subordinate relationship during the experiments. Two pairs of males never attacked and avoided each other and 3 pairs of males were convinced to get draw in the competition during the trial. The duration of frontal display of paired males were significantly different (t-test: t= 3.281, p = 0.002; dominant = 364.0 $\pm$ 59.84 seconds; subordinate = 306. 0 $\pm$ 65.06 seconds; Fig. 2). Dominant males performed more biting and tail beats (t = 4.725, p = 0.001; dominant = 7.80 $\pm$ 2.79; subordinate = 4.56 $\pm$ 1.98; Fig. 3) and more gill cover erection (t= 8.421, p = 0.001; dominant = 42.72 $\pm$ 8.08; subordinate = 25.56 $\pm$ 6.19; Fig. 4). Escape behavior was found in subordinate male 183.6 $\pm$ 58 but this behavior was not found in dominant males. All the learners participated in learning and the duration of time spent on this behaviour was ranged from 300 to 800 seconds. The percentage of time spent by the learners during the experiment was highest in learning (58%) followed by display (24%), gill cover erection (17%) and biting and tail beats (1%), respectively (Fig. 5).

In experiment 2, the agonistic plasticity of learner males was observed. When learner males encountered with dominant male from the experiment 1, a significant difference in frontal display was found (U=11, p<0.001; dominant = 324.67 $\pm$52.63; learner = 172.0$\pm$ 70.02). Number of attack between paired males (t = 7.491, p<0.001; dominant = 9.47 ± 2.59; learner = 2.87 ± 2.36), and number of gill erection (t = 14.823, p<0.001; dominant = 37.93 ± 3.43; learner =12.20 ± 9.85) were significantly different (Fig. 6 and Fig, 7). Highly significant difference was found in frontal display (Mann-Whitney U Test: U=6, p<0.001; learners = 260.0 ±39.44 ; subordinate = 334.0 ± 34.38), escape behaviour (t = -7.791, p<0.001, learners = 335.0 ±91.56; subordinate = 96.0 ± 32.04), number of biting and tail beat (t =7.793, p<0.001; learners = 2.1 ± 0.99 ; subordinate = 10.50 ± 2.46), number of gill erection (t = 10.770, p<0.001; learners = 23.80 ± 2.52 ; subordinate = 41.10 ± 2.56) respectively (Fig. 8 and Fig, 9). Different display patterns between learner males who combat with dominant and subordinate males was found in the experiment 2. Number of biting and tail beating (U= 35.0, p<0.05; D_learner = 2.87 ± 2.36; S_learner = 2.1 ± 0.99) and number of gill erection (U= 12.0, p<0.001; D_learner = 12.20 ± 9.85; S_learner =23.80 ± 2.52)

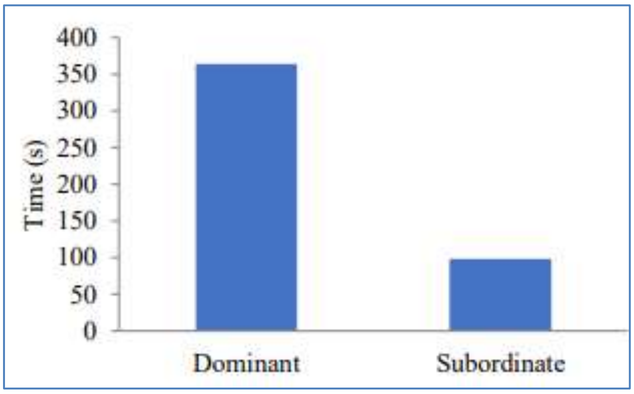


Figure 2 Interaction (display) between dominant and subordinate males

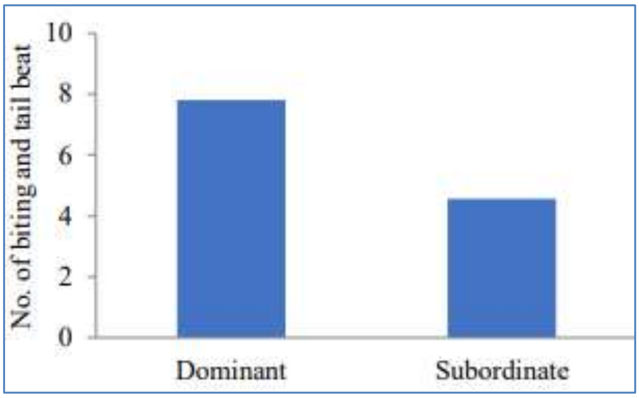


Figure 3 Number of biting and tail beat between dominant and subordinate males

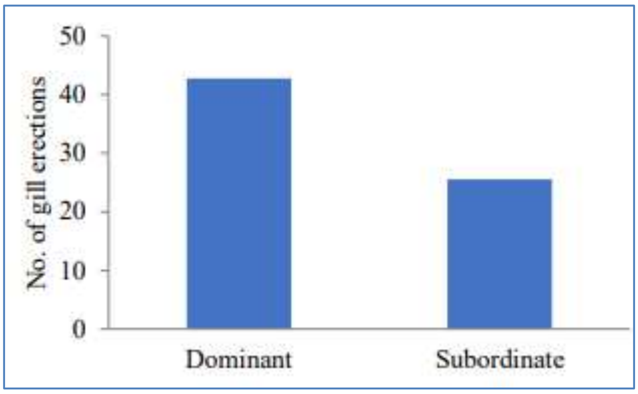


Figure 4 Number of gill erections between dominant and subordinate males

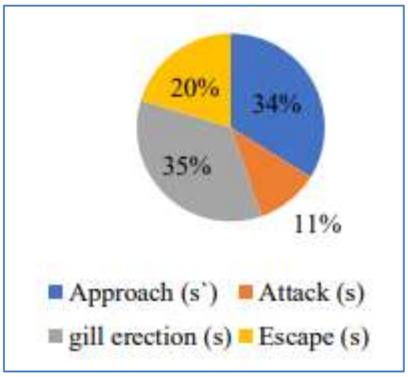


Figure 5 Percentage of display pattern of learner fish

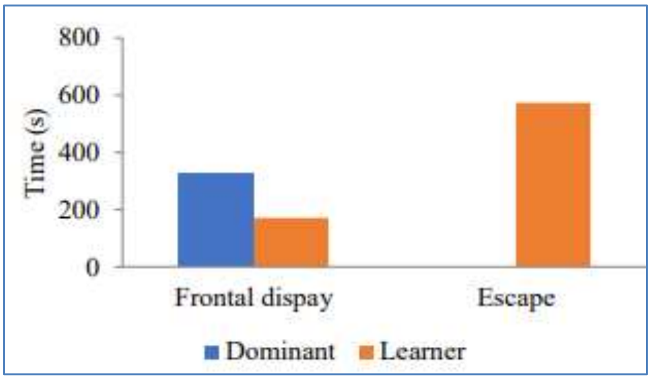


Figure 6 Interaction (display and escape) between dominant and learner males

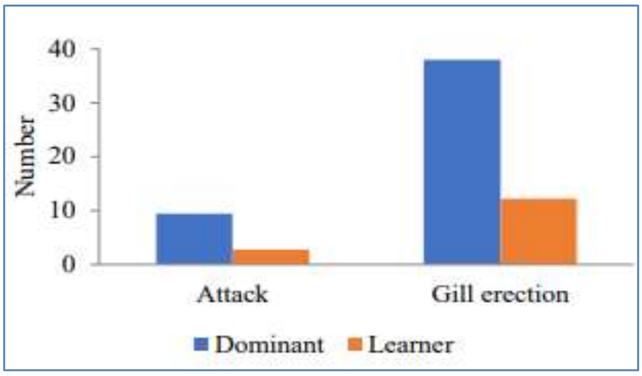


Figure 7 Interaction (attack and gill erection) between dominant and learner males

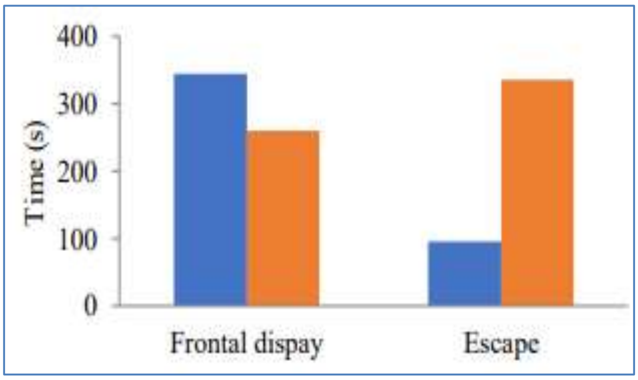


Figure 8 Interaction (display and escape) between subordinate and learner males

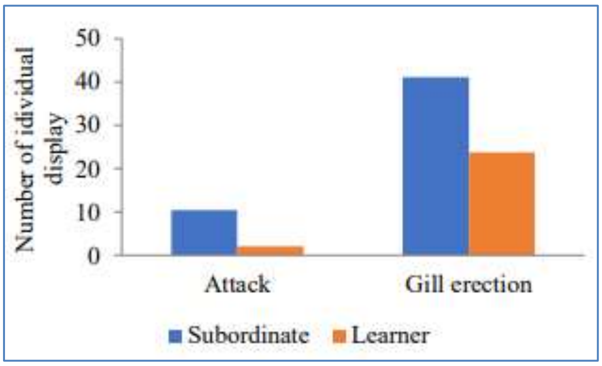


Figure 9 Interaction (attack and gill erection) between subordinate and learner males

Discussion

Learning behaviour of *B. splendens* was investigated with two continuous experiments, observing the learning behaviour of male *B. splendesn* and observing its agonistic plasticity. The results from these two studies clarify the effect of learning or prior visual experience on *Betta* agnoistic plasticity. When two males encounters, both males showed agonistic behaviour to the opponents until they reach to clear social hierarchy. We found that non-experience individuals (paired males) showed more aggressiveness and this finding is consistent with the results of others (Bronstein, 1984; Cain, 1980; Haller, 1994). This result explains that non-social- experience males spend high energetic cost in social combat which may potentially result injury or death. Dominant males showed more gill cover erections than the losers since gill cover erection is correlated with ability of males and its dominancy (Even, 1985). Number of bites and tail beats were also significantly different between two paired-males. Halperin et al., (1998) reported that biting and tail beating have been shown to be an honest indicator of energetic and male quality. Animals who do not directly involve in an interaction have been called audiences and they might receive various information from visual learning (Evans and Marler, 1984). In this experiment, most of the learners pay attention to social combat between two paired males indicating that learning behaviour is very common in *B. splendens* and/or in their natural habitats.

When we investigated the plasticity of agonistic behaviour of male *B. splendens* (experiment 2), we found that learners usually avoid dominant or winner males which indicated that learner males may take advantages from the prior experience to mitigate high energetic cost in fighting. In contrast, learners tried to compete with subordinate or loser males since submissive displays of loser in the prior experiment might be an honest indicator of loser condition and/or motivation (Evans, 1985; Halperin *et a*l., 1998; Simpson, 1968). Prior social experience such as dominant and subordinate roles resulting from paired encounters (Baenninger, 1970; Lobb & McCain, 1976; Meliska et al., 1975), prior visual experience with a conspecific (Meliska and Meliska, 1976), and visual and combat experience with responsive or passive conspecifics and non-conspecifics (Johnson and Johnson, 1973) might operate on agonistic plasticity of male *B. splendens*. The results from this study clearly explain that there was a positive correlation between learning experience/prior social experience and agonistic plasticity in male *B. splendens.*

Conclusion

The effect of learning experience on agonistic plasticity was investigated. The non-experienced males (paired males) actively involved in fighting display and males with prior visual experience took advantages from previous learning to mitigate high energetic cost from fighting. From this research it was concluded that learning behaviour is common in *B. splendens* and learning experience might affect on the agonistic plasticity of *B. splendens*.

Acknowledgements

We are very grateful to Professor Dr Aye Mi San, Head of Professor, Department of Zoology, University of Yangon for her encouragement to conduct this research.

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ASSESSMENT OF *POECILIA RETICULATA* (PETERS, 1859) ON *CULEX* (LINNAEUS, 1758) MOSQUITO LARVAE IN MAGWAY TOWNSHIP

Win Ei Khaing[1], Daw Win[2], Chaw Su Shwe[3]

Abstract

Larvivorous fishes feeding on immature stages of mosquito form an efficient bio-control agent. Observation on predation activities of *Poecilia reticulata* on mosquito larvae was carried out under laboratory conditions at 21.5±1.3˚C and 80.78±5.7% RH, during October 2018 to October 2019. Male and female guppies were used as predators for predation experiments on the 3rd and 4th instars of mosquito larvae. The female guppies consumed more mosquito larvae than male guppies did. The data indicated that *P.reticulata* have different mean consumption rates on 3rd 100 mosquito larvae with 1 liter of water volume (64 ± 17.1 by female, 28 ± 6.7 by male, with an average of 56 ± 8.8 (female) and 26 ± 4.8 (male). The data indicated that *P.reticulata* have different mean consumption rates on 3rd 200 mosquito larvae with 1 liter of water volume (187 ± 13.5 by female, 75 ± 11.6 by male, with an average of 175 ± 15.6 (female) and 72 ± 11.8 (male). The data indicated that *P.reticulata* have different mean consumption rates on 4th 100 mosquito larvae with 1 liter of water volume (25 ± 4.3 preys by female and 12 ± 2.7 preys by male with an average of 24 ± 1.8 (female) and 12 ± 0.9 (male) preys). The data indicated that wild guppies have different mean consumption rates on 4th 200 mosquito larvae with 1 liter of water volume (70 ± 17.8 preys by female and 34 ± 9.4 preys by male with an average of 68 ± 10.2 (female) and 33 ± 5.3 (male) preys). Larval consumption increased when the densities of prey increase until satiation level is reached, that is, when the fish becomes overwhelmed. Prey densities also influences predation activities and feeding rate.

Keywords: Predators, consumption rate, preys, instars

Introduction

Insects, invertebrate animal of the class Insecta of the phylum Arthropoda, like other arthropods, an insect has a hard outer covering, or exoskeleton, a segmented body and jointed legs. Adult insects typically have wings and are the only flying invertebrates.

There are about 900,000 known insects' species, three times as many as all other animal species together, and thousands of new ones are described each year. They are commonly grouped in 27 to 32 orders, depending upon the classification used (Borror and Delong, 2015).

There are about 3,500 species of mosquitoes throughout the world, and roughly 176 of them can be found in the United States. Thirty four mosquito species of five different genera were recorded within the altitudinal range of 300 to 2000 m from Garhwal region. Effect of natural factors like temperature, humidity and rain fall also have impact on the mosquitoes. Climate has been established as an important determinant in the distribution of vectors and pathogens (Pemola and Jauhari, 2006).

Mosquito larvae differ greatly in appearance and morphology from adults. Larvae are adapted for an aquatic existence, and their feeding and breathing structures reflect this. In general, mosquito larvae are easier to identify to species than are adults. This is because of the characteristic patterns of setae (hairs) that can be studied in microscopic slide-mounted specimens (Eldridge, 2008).

According to Job (1940), larvivorous fishes feeding on immature stages of mosquito form an efficient bio-control agent. Larvivorous fish must be small, hardy, drought resistant and a

[1] Dr, Lecturer, Zoology Department, University of Magway
[2] Dr, Associate Professor, Zoology Department, University of Magway
[3] Dr, Lecturer, Zoology Department, University of Magway

prolific breeder in confined water with a short life span. It should be a surface feeder and carnivorous in habit with a preference for mosquito larvae.

Guppies, whose natural range is in Northeast South America, were introduced to many habitats and are now found all over the world. Male guppies, which are smaller than females, have ornamental caudal and dorsal fins, while females are duller in color. Guppies generally feed on a variety of food sources, including benthic algae and aquatic larvae (Allen, 2002).

In the 21st century, biological control using larvivorous fish, was become an important tool for mosquito borne diseases control, particularly in urban and periurban areas **(**Karlekar and Andrew, 2016).

The present investigation has been undertaken to find the consumption level of larvivorous fish on mosquito larvae. Hence, the present work was conducted with the following objectives:

- to elucidate the consumption rate of *Poecilia reticulata* on third and fourth instars of *culex* mosquito larvae
- to compare the consumption rate between male and female of *Poecilia reticulate* on third and fourth instars of *culex* mosquito larvae
- to assess the relationship of feeding rate with the number of prey densities.

Materials and Methods

Study Area

Magway Township has tropical climate and it is located in the central dry zone. It lies between 20° 07′ to 20° 15′ N and 94° 55′ to 94° 92′ E. Zoology Department, Magway University Campus is situated between 20° 08′ N and 94° 56′E. (Fig -1)

Source: Google Earth (2019)

Figure 1 A map of Magway University Campus

Study Period

Duration of study period was from October 2018 to October 2019

Plate1(A)The place where the mosquito fish were taken for experiment

Plate1(B) The place where the mosquito larvae were taken for experiment

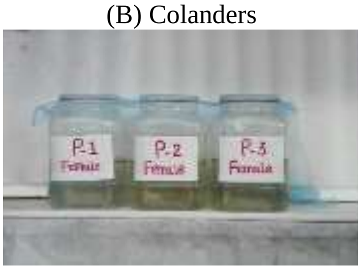
Equipments used for experiment

(A) Mosquito fish net

(B) Colanders

(C) Mosquito larvae counted spoon

(D) Dissecting microscope



(E) Experiment on the consumption rate of predator female on mosquito larvae

(F) Experiment on the consumption rate of predator male on mosquito larva

Plate 2 Equipment used during the experiment

Collection of Specimen

Poecilia reticulata and *Culex* mosquito larvae were collected from a natural ditch habitat, located at Moe Kaung street, Soe Kaw Min quarter. (Plate1. A, B)

The fishes were then taken to the laboratory. For investigation of consumption rate, *P.reticulata* (male and female) were kept separately in two liters plastic troughs (12x12x17cm) together with mosquito larvae. Three replications (three males and three females) were made for each of 3^{rd} and 4^{th} instars. The number of larvae consumed per fish during 24 hours period was recorded. After 24 hrs, uneaten larvae were removed and counted. Daily consumption rates of female and male of *P.reticulata* on 3^{rd} and 4^{th}instar of mosquito larvae were recorded after every 24 hrs for one week. Fresh 3^{rd} and 4^{th} instar of mosquito larvae were provided every 24 hrs after scoring.

The second experimental setup was to assess the relationship of feeding rate with the number of prey densities. This procedure was done in three replicates for each instar the experimental protocols used is: female fishes (1x1x100): single fish with 1 liter of water volume and 100 3rd instar of mosquito larvae. Male fishes (1x1x100): single fish with 1 liter of water volume and 100 3rdinstar of mosquito larvae. Female fishes (1x1x200): single fish with 1 liter of water volume and 200 3rdinstar of mosquito larvae. Male fishes (1x1x200): single fish with 1 liter of water volume and 200 3rdinstar of mosquito larvae.

Female fishes (1x1x100): single fish with 1 liter of water volume and 100 4thinstar of mosquito larvae. Male fishes (1x1x100): single fish with 1 liter of water volume and 100 4thinstar of mosquito larvae. Female fishes (1x1x200): single fish with 1 liter of water volume and 200 4thinstar of mosquito larvae. Male fishes (1x1x200): single fish with 1 liter of water volume and 200 4thinstar of mosquito larvae.

Data Analysis

Larva consumption rate was calculated by Microsoft Excel Software.

Identification of the Specimens

The identification of Poeciliid predators and mosquito larvae were carried out according to Talwar and Jhingran (1991), Dehghan and Sadraei, (2016) and Froese and Pauly (2017).

Results

Consumption Rate of Predator *Poecilia reticulata* on Prey *Culex* Mosquito larvae

The consumption rate of *Poecilia reticulata* (male and female) on 100 preys 3rd mosquito larva with 1 liter of water volume is shown in Table 1. It was observed that differential consumption rates of guppies on mosquito larvae were found.

The maximum numbers of 95 larvae were consumed by the fishes *Poecilia reticulata* (female) in the first replicate followed by 68 in the second replicate and 66 in the third replicate. The maximum numbers of 39 larvae were consumed by the fishes *Poecilia reticulata* (male) in the first and third replicates followed by 33 in the second replicate.

The minimum numbers of 40 larvae were consumed by the fishes *Poecilia reticulata* (female) and 17 larvae by the fishes (male).The data indicated that *Poecilia reticulata* have different mean consumption rates on 3rd mosquito larvae (64 ± 17.1 preys by female, 28± 6.7 preys by male, with an average of 56 ± 8.8 (female) and 26 ± 4.8 (male). It showed that prey consumption rate of female was higher than the male. (Fig. 2)

The prey consumption rate of *Poecilia reticulata* (male and female) on 200 prey 3rd mosquito larva with 1 liter of water volume is shown in Table 2. The maximum numbers of 200 larvae were consumed by the fishes *Poecilia reticulata* (female) in the first and second replicates followed by 194 in the third replicate. The maximum numbers of 98 larvae were consumed by the fishes *Poecilia reticulata* (male) in the third replicate followed by 91in the second replicate and 87 in the first replicate.

The minimum numbers of 134 larvae were consumed by the fishes *Poecilia reticulata* (female) and 51 larvae by the fishes (male). The data indicated that guppies have different mean consumption rates on 3rd mosquito larvae (187± 13.5 preys by female and 75 ± 11.6 preys by male with an average of 175 ± 15.6 (female) and 72 ± 11.8 (male) preys). It showed that prey consumption rate of female was higher than the male. (Fig.3).

The prey consumption rate of *Poecilia reticulata* (male and female) on 100 prey 4th mosquito larva with 1 liter of water volume is shown in Table 3. The maximum numbers of 32 larvae were consumed by the fishes *Poecilia reticulata* (female) in the second replicate followed by 28 in the third replicate and 27 in the first replicate. The maximum numbers of 16 larvae were consumed by the fishes *Poecilia reticulata* (male) in the second replicate followed by 14 in the first and third replicates.

The minimum numbers of 19 larvae were consumed with by the fishes *Poecilia reticulata* (female) and 9 larvae by the fishes (male).The data indicated that guppies have different mean consumption rates on 4th mosquito larvae (25 ± 4.3 preys by female and 12 ± preys by male with an average of 24 ± 1.8 (female) and 12 ± 0.9 (male) preys). It showed that prey consumption rate of female was higher than the male. (Fig. 4)

The prey consumption rate of *Poecilia reticulata* (male and female) on 200 prey 4th mosquito larva with 1 liter of water volume is shown in Table 4. The maximum numbers of 109 larvae were consumed by the fishes *Poecilia reticulata* (female) in the second replicate followed by 97 in the third replicate and 95 in the first replicate. The maximum numbers of 48 larvae were consumed by the fishes *Poecilia reticulata* (male) in the second replicate followed by 45 in the third replicate and 41 in the first replicate.

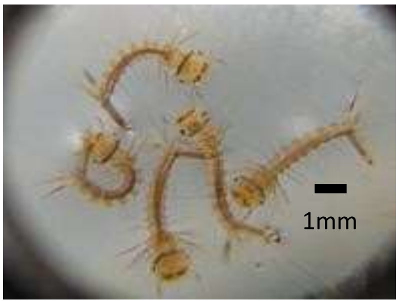
The minimum numbers of 43 larvae were consumed by the fishes *Poecilia reticulata* (female) and 15 larvae by the fishes (male).The data indicated that guppies have different mean consumption rates on 4th mosquito larvae (70 ± 17.8 preys by female and 34 ± 9.4 preys by male with an average of 68 ± 10.2 (female) and 33 ± 5.3 (male) preys). It showed that prey consumption rate of female was higher than the male. (Fig. 5)

In this study, 100 and 200 3rd instars of mosquito larvae with 1 liter of water volume were used in the predation experiment. The average numbers of 56±8.8 larvae were consumed with 100 3rd instars of mosquito larva by the fishes *Poecilia reticulata* (female) and 26±4.8 larvae by the fishes (male). The average numbers of 175±15.6 larvae were consumed 200 3rd instars of mosquito larva by the fishes *Poecilia reticulata* (female) and 72±11.8 larvae by the fishes (male).

In this study, 100 and 200 4th instars of mosquito larvae with 1 liter of water volume were used in the predation experiment. The average numbers of 50±2 larvae were consumed with 100 4th instars of mosquito larva by the fishes *Poecilia reticulata* (female) and 30±2 larvae by the fishes (male). The average numbers of 37±7.7 larvae were consumed with 200 4th instars of mosquito larva by the fishes *Poecilia reticulata* (female) and 16±1.8 larvae by the fishes (male). Larvae consumption increased, as there was an increase in prey densities, until satiation level was reached. (Table. 5, Fig.6).

(A) *Poecilia reticulata* (Male)

(B) *Poecilia reticulata* (Female)



(C) Third instar stage of mosquito larvae

(D) Fourth instar stage of mosquito larvae

Plate 3 Mosquito fish (male and female) and *Culex* mosquito larvae (3^{rd} and 4^{th} instars)

Table 1 Consumption rates of predator (*Poecilia recticulata*) on 100 preys (3rd instar of *Culex* mosquito larva) with 1 liter within 24 hrs in laboratory condition

Sr. No.	Date	No. of 3rd instar mosquito larva consumed / 24 hrs							
		P1♀	P2 ♀	P3♀	Mean ± SD	P1♂	P2♂	P3♂	Mean ± SD
1	2.1.2019	44	48	47	46±1.7	17	25	23	22±3.4
2	3.1.2019	43	40	46	43±2.4	19	29	18	22±5.0
3	4.1.2019	77	68	66	70±4.8	24	18	27	23±3.7
4	5.1.2019	66	47	55	56±7.8	27	27	26	27±0.5
5	6.1.2019	68	55	53	59±6.6	23	22	36	27±6.4
6	7.1.2019	57	58	50	55±3.6	30	20	29	26±4.5
7	8.1.2019	95	48	48	64±22	39	33	39	37±2.8
	Mean±SD	64±17	52±9	52±6	56±8.8	26±6.8	25±5	28±7	26±4.8

Replica P1, P2, P3

Table 2 Consumption rates of predator (*Poecilia recticulata*) on 200 preys (3^{rd} instar of *Culex* mosquito larva) with 1 liter within 24 hrs in laboratory condition

Sr. No .	Date	No. of 3rd instar mosquito larva consumed / 24 hrs							
		P1♀	P2♀	P3♀	Mean ± SD	P1♂	P2♂	P3♂	Mean ± SD
1	2.1.2019	167	166	134	156±15	51	59	62	57±4.6
2	3.1.2019	193	191	139	174±25	71	76	73	73±2.1
3	4.1.2019	200	199	194	198±3	65	79	83	76±7.7
4	5.1.2019	165	162	143	157±9.7	51	59	62	57±4.6
5	6.1.2019	193	191	134	173±27	71	91	74	79±8.8
6	7.1.2019	198	200	194	197±2.5	87	86	98	90±5.4
7	8.1.2019	192	190	137	173±25.5	75	78	72	75±2.4
	Mean±SD	187±14	186±14	154±26	175±15.6	67±12	75±11	75±12	72±11.8

Replica P1, P2, P3

Table 3 Consumption rates of predator (*Poecilia recticulata*) on 100 preys (4th instar of *Culex* mosquito larva) with 1 liter within 24 hrs in laboratory condition

Sr. No.	Date	No. of 4th instar mosquito larva consumed / 24 hrs							
		P1♀	P2♀	P3♀	Mean ±SD	P1♂	P2♂	P3♂	Mean ± SD
1	9.1.2019	27	20	28	25± 3.6	14	13	11	13±1.2
2	10.1.2019	23	23	25	24±0.9	14	9	12	12±2.0
3	11.1.2019	21	21	24	22±1.4	12	15	10	12±2.1
4	12.1.2019	19	28	19	22±4.2	13	10	12	12±1.2
5	13.1.2019	22	30	28	27±3.4	12	9	9	10±1.4
6	14.1.2019	24	24	26	25±0.9	11	16	10	12±2.6
7	15.1.2019	20	32	27	26±4.9	9	10	14	11±2.2
	Mean±SD	22±3	25±4	25±3	24±1.8	12±2	12±3	11±2	12±0.9

Replica P1, P2, P3

Table 4 Consumption rates of predator (*Poecilia recticulata*) on 200 preys (4th instar of *Culex* mosquito larva) with 1 liter within 24 hrs in laboratory condition

Sr. No.	Date	No. of 4th instar mosquito larva consumed / 24 hrs							
		P1♀	P2 ♀	P3 ♀	Mean ± SD	P1 ♂	P2♂	P3♂	Mean ± SD
1	9.1.2019	47	51	61	53± 5.9	41	18	41	33±10.8
2	10.1.2019	45	53	72	57±11.3	37	38	45	40±3.6
3	11.1.2019	79	77	97	84±9.0	25	32	35	31±4.1
4	12.1.2019	53	53	85	64±15.1	18	48	41	36±12.8
5	13.1.2019	71	61	79	70±7.4	41	35	36	37±2.6
6	14.1.2019	95	72	51	73±18	32	18	15	22±7.4
7	15.1.2019	79	109	43	77±26.9	39	31	28	33±4.6
	Mean±SD	67±18	68±19	70±18	68±10.2	33±8	31±10	34±9	33±5.3

Replica P1, P2, P3

Table 5 Feeding rate of male and female guppies in 24 hour on arvae of mosquito with the variations in mosquito densities

Sex of guppy	Mosquito larva stage	Fish (n)	Water volume	Mosquito densities	Mean ±SD
Female Guppy	3rd instar	1	1	100	56±8.8
male Guppy	3rd instar	1	1	100	26±4.8
Female Guppy	4th instar	1	1	100	24±1.8
male Guppy	4th instar	1	1	100	12±0.9
Female Guppy	3rd instar	1	1	200	175±15.6
male Guppy	3rd instar	1	1	200	72±11.8
Female Guppy	4th instar	1	1	200	68±10.2
male Guppy	4th instar	1	1	200	33±5.3

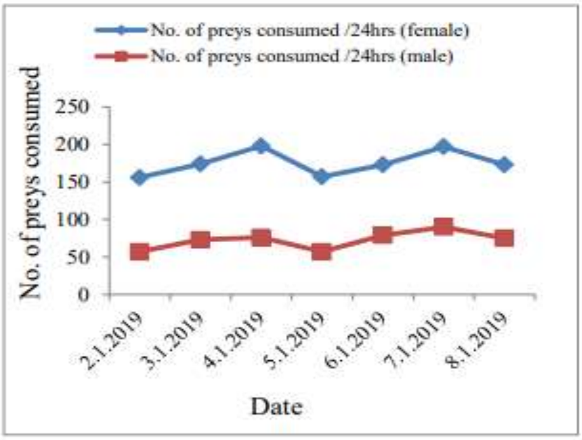


Figure 2 Consumption rate of predator on prey (3^{rd} 100) with 1 liter within 24 hrs during one week

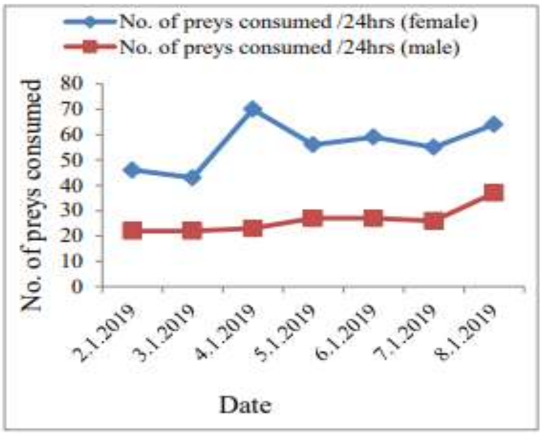


Figure 3 Consumption rate of predator on prey (3^{rd} 200) with 1 liter within 24 hrs during one week

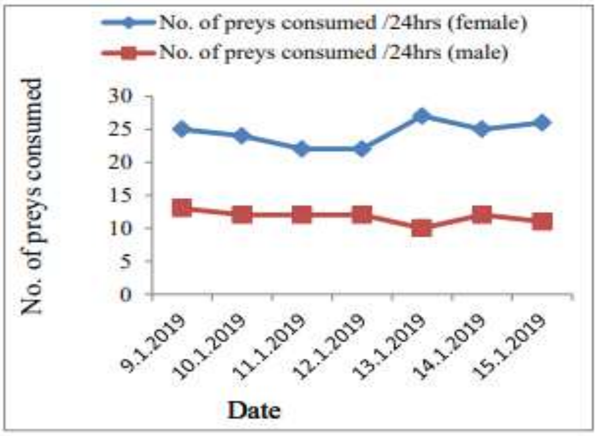


Figure 4 Consumption rate of predator on prey (4^{th} 100) with 1 liter within 24 hrs during one week

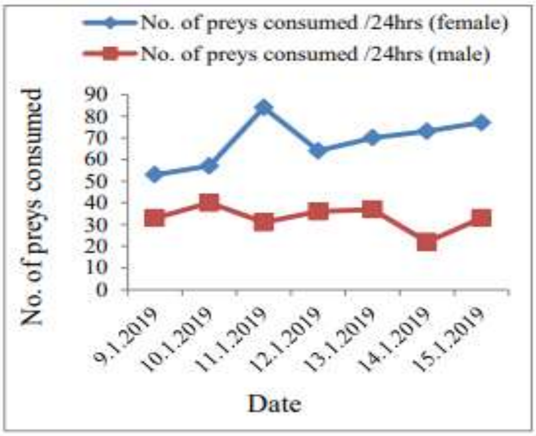


Figure 5 Fig 5. Consumption rate of predator on prey (4^{th} 200) with 1 liter within 24 hrs during one week

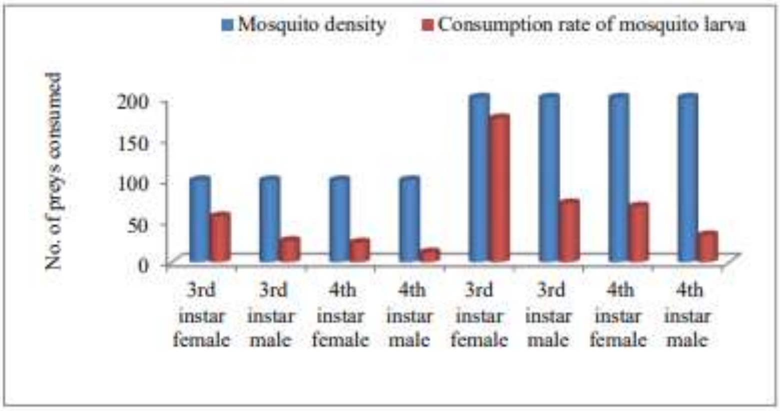


Figure 6 Consumption rate of predator on prey (3rd 100,200 and 4th100,200) within 24 hrs during one week

Discussion

This research investigated the under laboratory condition at 21.5±1.3˚C and 80.78±5.7% RH. In this study guppies were used as predators against *Culex* mosquito larvae in Magway University.

The consumption rate of *Poecilia reticulata* (male and female) on prey one hundred larvae of 3rd instar stage is shown in Table 1. It was observed that differential consumption rates of guppies on 3rd instar mosquito larvae were found. The data indicated that *P.reticulata* have different mean consumption rates on 3rd mosquito larvae (64 ± 17.1 by female, 28 ± 6.7 by male, with an average of 56 ± 8.8 (female) and 26 ± 4.8 (male). In this study, it was observed that female wild guppies ate 3rd mosquito larvae more than male guppies did.

The consumption rate of *P. reticulata* (male and female) on prey two hundred larvae of 3rd instar stage is shown in Table 2. The data indicated that *Poecilia reticulata* have different mean consumption rates on 3rd mosquito larvae (187 ± 13.5 by female, 75 ± 11.6 by male, with an average of 175 ± 15.6 (female) and 72 ± 11.8 (male). In this study, it was observed that female guppies ate 3rd mosquito larvae more than male guppies did.

This result supports the findings of Seng *et al* (2008): female guppies ate more than male guppies. This is due to the larger size of the female guppies. Therefore, female guppies can consume more mosquito larvae than male guppies can. Karlekar and Andrew (2016) found that the consumption rate on fifty larvae of 3rd instar stage of *Culex* with 800 ml of water volume on average of 27±2.5, which is comparatively different to the results of the present findings. Because of feeding rate increased with the increase in prey and predator densities.

The consumption rate of *Poecilia reticulata* on prey 4thinstar stage is shown in Table3. The data indicated that wild guppies have different mean consumption rates on one hundred larvae of 4thinstar stage (25 ± 4.3 preys by female and 12 ± 2.7 preys by male with an average of 24 ± 1.8 (female) and 12 ± 0.9 (male) preys).

The consumption rate of *Poecilia reticulata* on prey 4thinstar stage is shown in Table 4. The data indicated that guppies have different mean consumption rates on two hundred larvae of 4thinstar stage (70 ± 17.8 preys by female and 34 ± 9.4 preys by male with an average of 68 ± 10.2 (female) and 33 ± 5.3 (male) preys).

This result also supports the finding by Cavalcanti *et al* (2007) who report that the efficacy as predator depends on its weight and sex. Saleeza *et al*., (2014) found that the consumption rate on Two hundred larvae of 4th instar stage with on average of 50±5.2 (male) and 94±6.3 (female), which is comparatively different to the results of the present findings. Manna *et al* (2008) the number of prey consumed varies with the difference in body size. This means that prey consumption increases with body size.

In term of feeding rate in this study, female guppies had their feeding rate increased when the prey densities were increased. This result supports the findings of Anyaele and Obembe (2010). They reported that larval consumption increased when the densities of prey increase until satiation level is reached, that is, when the fish becomes overwhelmed. Guppy fish have been described as effective biological agents for the control of mosquito larvae (Rozendaal, 1997).

Mosquito borne diseases or mosquito borne illnesses are diseases caused by bacterial, viruses and parasites transmitted by mosquitoes. Nearly 700 million people get a mosquito borne illness each year resulting in greater than one million deaths. Mosquito cause more human suffering than any other organism (Douglas**,** 2004).

Biological control of mosquitoes was very popular during the early part of the 20th century, but this type of control has been replaced with the insecticidal control due to easy availability of chemicals such as organochlorines and organophosphates. However, because of problems with insecticide resistance and greater awareness of environmental contamination, there has been renewed interest in biological methods (Service, 2000).

Saleeza *et al* (2011) reported that the three common mosquito larvae are commonly found in residential areas in both urban and sub urban areas. A number of studies have indicated that guppies of the *Poecilia reticulata* species are good predators, as they can control mosquito larvae population. Saha *et al* (1986) studied on the use of *Poecilia reticulata* (guppy) as a powerful biocontrol agent in the field of mosquito eradication.

Conclusion

Jayapriya and Shoba, (2014) stated that Biological control has many advantages as compared to chemicals. Because it can be effective and safe to human and non-target populations. It has low cost of production and lower risk of resistance development. *Poecilia reticulata* is excellent agents for use as biological control of mosquito larvae. Thus, it can be seen that there is a great need for the identification of the Mosquitofish and mosquito species of economic importance.

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ABUNDANCE AND DIVERSITY STATUS OF SOME CEPHALOPOD ALONG CHAUNGTHA COASTAL AREA, AYEYARWADY REGION

Thida Soe[1], Thein Soe[2], Lei Lei Oo[3], Mu Mu Aung Soe[4]

Abstract

The present study was to assess the abundance and species diversity of cephalopods along Chaungtha coastal area. It was conducted from December 2018 to November 2019. Identification was followed after Jereb and Roper (2005) and Jereb *et al*. (2010,2014).The key of species richness index, Simpson's index of diversity and Shannon-Wiener's diversity index were used in this research. A total of 14 cephalopod species, belonging to six genera, four families under four orders were recorded within the study area. Maximum number of cephalopod individuals was recorded in cold season. The highest number of individual was also observed for the species *Uroteuthis duvauceli*. The second highest number of individual was examined in the hot season. The least number was in the wet season. Fourteen species were recorded in all season. According to Shannon index the highest value (1.828295) was in wet season while the lowest value (1.708776) was in cold season. According to Simpson's index the highest value (0.250587) was recorded in cold season and the lowest value (0.22207) was observed in wet season. The output was expected to promote the cephalopod fishery in future fishery sector of coastal region in Myanmar.

Keywords: Cephalopod species, abundance, diversity, Chaungtha coastal area

Introduction

Cephalopod is the most morphologically and behaviorally complex class in phylum Mollusca. They are a very diverse and abundant group inhabiting all marine environments of the world, from surface waters to more than 5000 m depth (Jereb and Roper 2005). Cephalopods are an important component of many marine ecosystems from the tropics to the poles (Jackson and O'dor, 2002). The coastal and marine environs comprise some of the high biodiversity area (Khan *etal*., 2005). Cephalopods are a diverse group of species. There are over 800 extant species of cephalopod in the world. Four groups of cephalopods are squid, cuttlefish, octopus and chambered nautilus (Roper *et al*.1984, Jereb and Roper, 2005). Three major orders; Octopoda, Sepioidea, Teuthoidea live in the continental shelf (Boyle and Daly, 2000). These species are abundant and ecologically important (Duysak,*etal*., 2008). In common with many marine animal groups, the highest diversity of the octopus occurs in the tropical Indo-West Pacific region, particularly the Indo-Malayan Archipelago (Jereb *et al*., 2014).

Cephalopods are a worldwide commercial interest with several fisheries providing significant quantities for human consumption, animal feed and fishing bait (Pierc and Guerra, 1994). Cephalopods are .most commonly eaten in Southeast Asia and Southern Europe (Mouritsen, 2018). Cephalopods started gaining importance in India and neighbor countries such as Myanmar, with the development of export markets and consequent are cepholopods fauna. Myanmar is rich in natural resources along coastal area including diverse cephalopods fauna (Wye, 2003). In coastal area, cephalopods dwell in sandy, rocky and muddy area. *Sepia, Loligo* and *Octopus* are edible mollusks and economically importance of Myanmar marine ecosystem (Khin Myat Myat Tun, 2000).

Ayeyarwady Region is abundant of coastal area and fishery; it produces million viss of fishes and prawns. Chaungtha beach is a part of Rakhine coast on the Bay of Bangal. Fishery sector

[1] Dr, Lecturer, Zoology Department, Pathein University
[2] Dr, Professor and head, Zoology Department, Pathein University
[3] Dr, Lecturer, Zoology Department, Pathein University
[4] Dr, Lecturer, Zoology Department, Pathein University

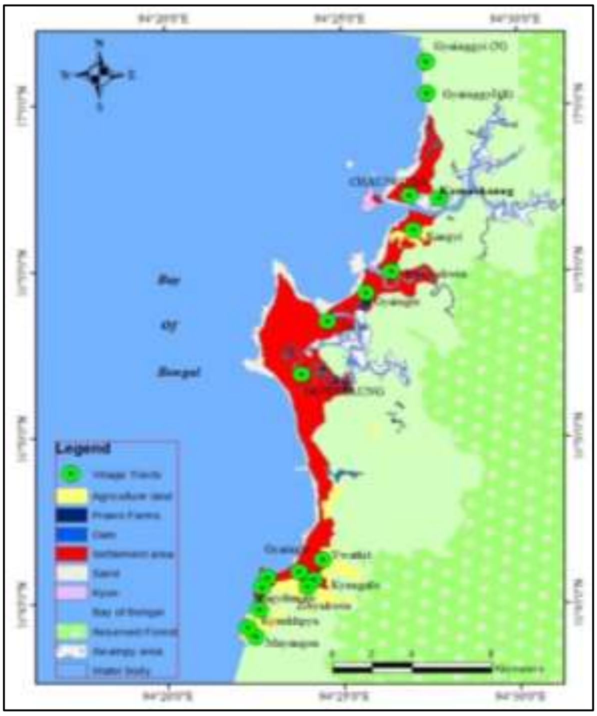
is the most important for local people because it provides the socio economic importance for local fisher families and fishery industry. The general aims of this research are:

- to identify the cephalopods species in Chaungtha coastal area
- to investigate the seasonal abundance of cephalopods species
- to assess the diversity of cephalopods species recorded from the study area

Materials and Methods

Study area and Study period

Chaungtha beach is located in the northwest of Pathein Township, Ayeyarwady Region. It is situated at latitude 16˚ 57' N and longitude 94˚ 30' E (Fig.1). The study period lasted from December, 2018 to November, 2019.



(**Source:** Department of Geography, Pathein University)

Figure 1 Map of Chaungtha environs

Data collections

Monthly data collection was carried out in study site. Cephalopod species and individuals were recorded and noted. Interview survey was made with local fishermen and depots, dealing with catch number, commercial status and fishing gear for cephalopod fishery. Spawning season of finfish and shell fish was investigated from fishery department of Ngwe Saung Township. The morphological and taxonomic characters, from fresh specimens were taken photographs. Then, they were preserved in 10% formaldehyde solution.

Species identification

Specimens were identified by distinctive characters and texture, colouration, proportion of carapace, shape of mantle, rows of sucker, tentacles shapes and photo key according to Jereb and Roper (2005), and Jereb *et al*., (2010,2014).

Estimates of species diversity

Three indices of species diversity, Dominant index, Shannon index, Simpson index, and its evenness were used to assess species diversity of cephalopods. Index value of each cephalopods species collected from study sites and seasons were compared (Krebs, 2001, and Stiling, 1999).

The formula of Dominant index is as:

$$\text{Dominant index} = \frac{n}{N} \text{ x100}$$

Where n is the number of individuals in each species and N is the total population number.
The formula of Richness index as:

$$\text{Richness index} = n + (n-1)/n$$

Where n is the number of individuals in each species.
The formula of the Simpson index of the species diversity is as:

$$D_s = \sum_{i=1}^{s} \frac{(n_i(n_i-1))}{(N(N-1))}$$

The formula of the Shannon index of species diversity is as:

$$H' = -\sum P_i \text{ Ln } P_i$$

$$\text{Evenness} = H' / \text{Ln}$$

Where, S is total number of species. Evenness is usually range between 0 and 1.0.

Results

A total of 14 species of cephalopods belonging to six genera of four families under four orders were recorded during the study period. (Table.1, Plate.1)

Species Composition

. A total of 14 species belong to six genera of four families under four orders of class cephalopods were recorded. The recorded cephalopods include under four orders of Sepiida, Sepiolida, Teuthida and Octopoda.

The order Sepiida included the highest number of species and percent (seven species, 50 percent of recorded cephalopods) among the collected cephalopods. The second highest number of species (three species, 22 percent of recorded cephalopods) was observed in Octopoda, and the least number of species (two species, 14 percent recorded cephalopods) in the order Teuthida and Sepiolida. (Table.1,Fig. 2)

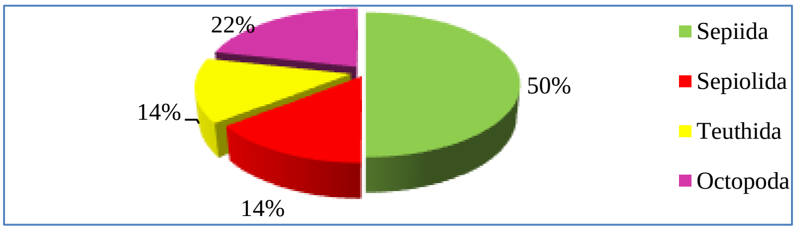


Figure 2 Species composition in different orders by percentage

Table 1 Species composition of cephalopods along Chaungtha coastal area

Order	Family	Scientific name	Connom name	Local Name
1. Sepiida	Sepiidae	*Sepia braggi*	Slender cuttlefish	Yay Kyat
		S . esculenta	Goldencuttlefish	Yay Kyat
		S . prabahari	Small strip cuttlefish	Yay Kyat
		S . aculata	Needle cuttlefish	Yay Kyat
		S. pharaonis	Pharaoh cuttlefish	Yay Kyat
		S . recurvirostra	Curve spine cuttlefish	Yay Kyat
		Sepiella inermis	Spineless cuttlefish	Yay Kyat
2.Sepiolida	Sepiolidae	*Euprymna hyllebergi*	Bobtail squid	Sin Na Ywet
		Euprymna moresi	Mimika bobtail squid	Sin Na Ywet
3.Teuthida	Loliginidae	*Uroteuthis duvauceli*	Indian squid	Kin Mon
		U.chinensis	Mitre squid	Kin Mon
4.Octopoda	Octopodidae	*Octopus dollfusi*	Marble octopous	Yay Myuk
		Amphioctopus aegina	Webfoot octopus	Yay Myuk
		A.neglectus	Webfoot octopus	Yay Myuk

Seasonal abundance of cephalopods

A total of 14 species of cephalopod were recorded at the study area in all season. Maximum number of cephalopod's individuals was observed in cold season. The highest catch number of individuals was also observed for the species *Uroteuthis duvauceli*. The second highest catch number of individuals was examined in hot season. The least number was in the wet season (Table.2, 3).

Hot season

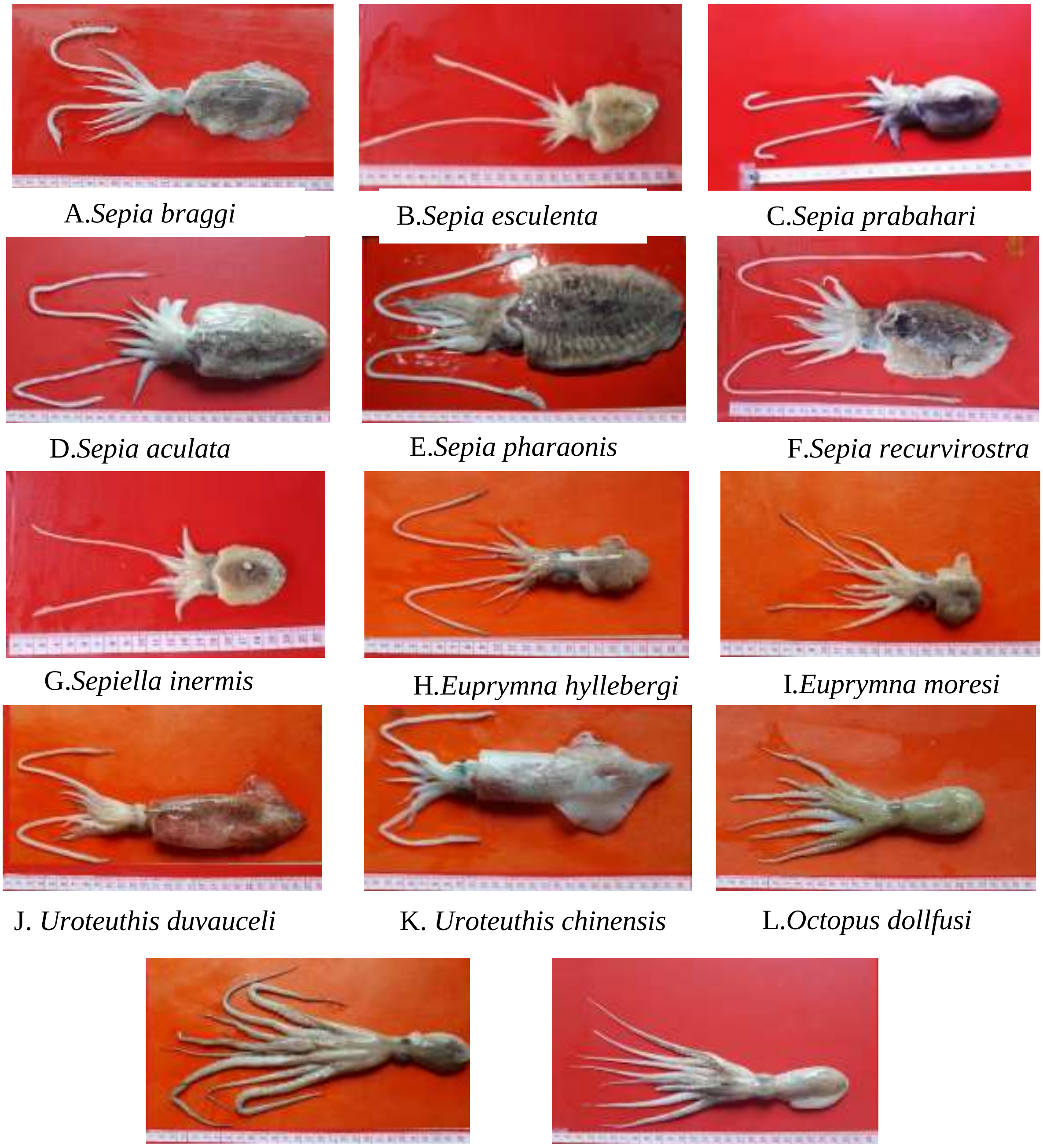
In hot season, 14 species and 582887individuals were observed from the study site. Among the recorded four orders in hot season, order Sepiida had the highest number of species (7 species) .The order Teuthida had the highest catch number of individuals (386580 individuals).The second highest number of species was recorded in order Octopoda (3 species) which was successively followed by order, Sepiolida and Teuthida (2 species). The order Sepiida had the second highest catch number of individuals (186717 individuals).Order Sepiolida had the least number of species and individuals (two species and 1049 individuals). In hot season *Uroteuthis duvauceli* was recorded to have the maximum number of individuals (193740 individuals) (Table.2 and Fig.3A).

Wet season

In wet season, 14 species and 263042 individuals were recorded in which the highest number of species (7 species) was observed in order Sepiida and the highest number of individuals (166500 individuals) was recorded in order Teuthida. The second highest number of individuals (91843 individuals) was observed in order Sepiida which was successively followed by order Octopoda (4390 individuals). The least number of species (two species) was observed in order Sepiolida and Teuthida, and order Sepiolida had the least number of species and individuals (two species and 309 individuals).The species *Uroteuthis duvauceli* was the maximum number of individuals in this season (87500 individuals) (Table.2 and Fig.3B).

Cold season

In cold season, 14 species and 1204830 individuals were collected. Among the recorded four orders, order Sepiida had the highest number of species (7species). The highest catch number of individuals (818324 individuals) was recorded in order Teuthida which was successively followed by order Sepiida including 370365 individuals and the order Octopoda was 14116 individuals. Two orders Teuthida and Sepiolida were observed to obtain two species. The least number of species and individuals (two species and 2025 individuals) was recorded for order Sepiolida Maximum number of individuals, 409612 individuals was observed for the species *Uroteuthis duvauceli* in this season (Table.2and Fig.3C).



A.*Sepia braggi* B.*Sepia esculenta* C.*Sepia prabahari*

D.*Sepia aculata* E.*Sepia pharaonis* F.*Sepia recurvirostra*

G.*Sepiella inermis* H.*Euprymna hyllebergi* I.*Euprymna moresi*

J. *Uroteuthis duvauceli* K. *Uroteuthis chinensis* L.*Octopus dollfusi*

M.*Amphioctopus aegina* N. .*Amphioctopus neglectus*

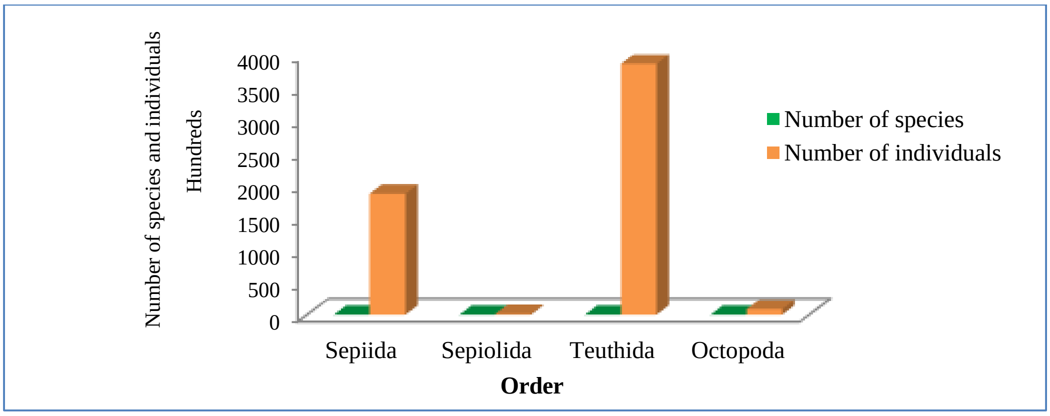
Plate 1 Recorded cephalopods belonging to order Sepiida, Sepiolida, Teuthida and Octopoda

Table 2 Seasonal abundance of cephalopods along Chaungtha coastal area

Order	No	Species	Hot	Wet	Cold	Total
1. Sepiida	1	*Sepia braggi*	27407	11450	44060	82917
	2	*S . esculenta*	25250	12870	45756	83876
	3	*S . prabahari*	27740	12100	45185	85025
	4	*S . aculata*	19821	11840	45073	76734
	5	*S. pharaonis*	6777	2583	10795	20155
	6	*S.recurvirostra*	59933	24450	137321	221704
	7	*Sepiella inermis*	19789	16550	42175	78514
Total			**186717**	**91843**	**370365**	**648925**
Sepiolida	8	*Euprymna hyllebergi*	522	145	1005	1672
	9	*Euprymna moresi*	527	164	1020	1711
Total			**1049**	**309**	**2025**	**3383**
3.Teuthida	10	*Uroteuthis duvauceli*	193740	87500	409612	690852
	11	*U.chinensis*	192840	79000	408712	680552
Total			**386580**	**166500**	**818324**	**1371404**
4. Octopoda	12	*Octopus dollfusi*	2865	1390	4456	8711
	13	*Amphioctopus aegina*	2836	1530	4775	9141
	14	*A.neglectus*	2840	1470	4885	9195
Total			**8541**	**4390**	**14116**	**27047**

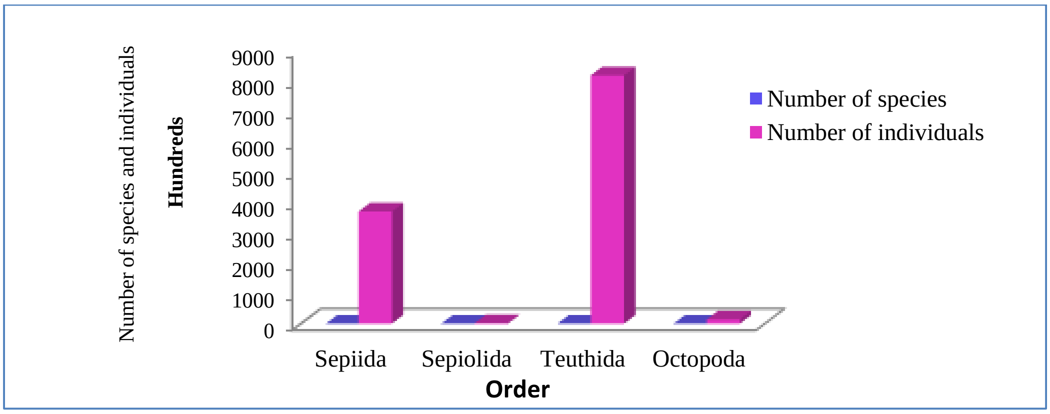
Table 3 Comparison seasonal abundance of cephalopods in three seasons

	Hot	**Wet**	**Cold**
Number of species	14	14	14
Number of individuals	582887	263042	1204830



(A) Hot Season

(B)Wet Season



(C) Cold Season

Figure 3 Seasonal abundance of cephalopods from study area

Species diversity

Species diversity of three seasons represented by different indies was found to vary as follows;

Hot Season

In hot season, *Uroteuthis duvauceli* Indian squid under the order Teuthida had the highest value of species diversity, richness index (193741.00), Shannon index (-0.366109), Simpson's index (0.110476). *Uroteuthis chinensis* of the order Teuthida was the second highest diverse species which had the highest index value of species richness (192841.00), Shannon index (-0.365948), Simpson'sindex (0.109452). The species *Euprymna hyllebergi* had the least value of species diversity, richness index (522.9980843), Shannon index (0.006285), Simpson's index (0.000001).

Wet season

In wet season, the highest value of species richness was (87500.99999), that of Shannon index (-0.366136), that of Simpson's was (0.110653) in the species *Uroteuthis duvauceli*, Indian squid of order. The second highest value of species diversity, richness index (79000.99999), Shannon index (-0.361259), Simpson's index (0.090199) was observed for the species *Uroteuthis chinensis* mitre squid of order Teuthida , The species *Euprymna hyllebergi* was the lowest value of species diversity richness was (145.9931034), Shannon index(-0.004136), Simpson's index (0.000000).

Cold season

In cold season**,** the highest value of species diversity, richness index (409613.00), Shannon index (-0.366793), Simpson's index (0.0115583) was recorded for the species *Uroteuthis duvauceli*, Indian squid of the order Teuthida. *Uroteuthis chinensis*, mitre squid had the second highest value of species diversity, richness index (408713.00), Shannon index (-0.366734), Simpson's index (0.115075).The species *Euprymna hyllebergi* was the lowest value of species diversity richness index (1005.999005), Shannon index (-0.005913), Simpson's index (0.000001).

Table 5 Diversity indies of cephalopods during hot season

Sr No	Species	n	Richness n+(n-1)/n	Dominance index n/N*100	Shannon index Pi Ln Pi	Simpson's index n(n-1) /N(N1)
1	*Sepia braggi*	27407	27407.99996	4.70194	-0.143747	0.002211
2	*S . esculenta*	25250	25250.99996	4.33189	-0.135985	0.001876
3	*S . prabahari*	27740	27740.99996	4.75907	-0.144919	0.002265
4	*S.aculata*	19821	19821.99995	3.40049	-0.114979	0.001156
5	*S.pharaonis*	6777	6777.999852	1.16266	-0.051790	0.000135
6	*S.recurvirostra*	59933	59933.99998	10.28210	-0.233894	0.010572
7	*Sepiella inermis*	19789	19789.99995	3.39500	-0.114848	0.001153
8	*Euprymna hyllebergi*	522	522.9980843	0.08955	-0.006285	0.000001
9	*Euprymna moresi*	527	527.9981025	0.09041	-0.006337	0.000001
10	*Uroteuthis duvauceli*	193740	193741.00	33.23800	-0.366109	0.110476
11	*U.chinensis*	192840	192841.00	33.08360	-0.365948	0.109452
12	*Octopus dollfusi*	2865	2865.999651	0.49152	-0.026126	0.000024
13	*Amphioctopus aegina*	2836	2836.999647	0.48654	-0.025911	0.000024
14	*A.neglectus*	2840	2840.999648	0.48723	-0.025941	0.000024
	Total	**582887**	**582900.9947**	**100.00000**	**-1.762821**	**0.239369**

Table 6 Diversity indies of cephalopods during wet season

Sr No	Species	n	Richness n+(n-1)/n	Dominance index n/N*100	Shannon index Pi Ln Pi	Simpson's index n(n-1)/N(N-1)
1	*Sepia braggi*	11450	11450.99991	4.35292	-0.136435	0.001895
2	*S. esculenta*	12870	12870.99992	4.89275	-0.147635	0.002394
3	*S. prabahari*	12100	12100.99992	4.60003	-0.141640	0.002116
4	*S.asculata*	11840	11840.99992	4.50118	-0.139574	0.002026
5	*S.pharaonis*	2583	2583.999613	0.98197	-0.045400	0.000096
6	*S.recurvirostra*	24450	24450.99996	9.29509	-0.220822	0.008640
7	*Sepiella inermis*	16550	16550.99994	6.29177	-0.174026	0.003958
8	*Euprymna hyllebergi*	145	145.9931034	0.05512	-0.004136	0.000000
9	*Euprymna moresi*	164	164.9939024	0.06235	-0.004601	0.000000
10	*Uroteuthis duvauceli*	87500	87500.99999	33.26465	-0.366136	0.110653
11	*U.chinensis*	79000	79000.99999	30.03323	-0.361259	0.090199
12	*Octopus dollfusi*	1390	1390.999281	0.52843	-0.027706	0.000028
13	*Amphioctopus aegina*	1530	1530.999346	0.58166	-0.029938	0.000034
14	*A.neglectus*	1470	1470.99932	0.55885	-0.028988	0.000031
	Total	**263042**	**263055.9841**	**100.00000**	**-1.828295**	**0.222070**

Table 7 Diversity indies of cephalopods during cold season

Sr No	Species	n	Richness n+(n-1)/n	Dominance index n/N*100	Shannon index Pi Ln Pi	Simpson's index n(n-1)/N(N-1)
1	*Sepia braggi*	44060	44060.99998	3.656947	-0.120992	0.001337
2	*S . esculenta*	45756	45756.99998	3.797714	-0.124215	0.001442
3	*S . prabahari*	45185	45185.99998	3.750322	-0.123135	0.001406
4	*S.aculata*	45073	45073.99998	3.741026	-0.122923	0.001399
5	*S.paraonis*	10795	10795.99991	0.895977	-0.042245	0.000080
6	*S.recurvirostra*	137321	137322.00	11.397542	-0.247529	0.012990
7	*Sepiella inermis*	42175	42175.99998	3.500494	-0.117346	0.001225
8	*Euprymna hyllebergi*	1005	1005.999005	0.083414	-0.005913	0.000001
9	*Euprymna moresi*	1020	1020.99902	0.084659	-0.005989	0.000001
10	*Uroteuthis duvauceli*	409612	409613.00	33.997493	-0.366793	0.115583
11	*U.chinensis*	408712	408713.00	33.922794	-0.366734	0.115075
12	*Octopus dollfusi*	4456	4456.999776	0.369845	-0.020711	0.000014
13	*Amphioctopus aegina*	4775	4775.999791	0.396321	-0.021919	0.000016
14	*A.neglectus*	4885	4885.999795	0.405451	-0.022332	0.000016
	Total	**1204830**	**1204843.997**	**100.00000**	**-1.708776**	**0.250587**

Table 8 Comparison of diversity indies of cephalopod species diversity in three seasons

	Seasons	Hot season	Wet season	Cold season
1	No of species	14	14	14
2	No of individuals	582887	263042	1204830
3	Species richness	582900.9947	263055.9841	1204843.997
4	Shannon index	1.762821	1.828295	1.708776
5	Shannon evenness	0.66797	0.69278	0.64749
6	Simpson index	0.239369	0.22207	0.250587

Comparison of species diversity in season

The value of two parameters of species diversity such as Shannon index and Simpson’s index were compared for season.

Both indices of Shannon index and Simpson’s index expressed the highest value of (1.828295) and (0.250587) were observed in the cephalopod species in wet and cold season. The least value of Shannon index (1.708776) was found in cold season while that of Simpson’s index (0.22207) was also recorded in wet season (Table.4).

Discussion

A total of 14 species belong to six genera of four families under four orders of class cephalopods were recorded. The recorded cephalopods include under four orders of Sepiida, Sepiolida, Teuthida and Octopoda. Order Sepiida was represented by seven species, order Octopoda was represented by three species and two orders Sepiolida and Teuthida were represented by two species.

In addition, 14 cephalopod species were found in all season. The highest catch number of individuals was observed in cold season (1204830 individuals). The second highest catch number of individuals was found in hot season (582887 individuals) and followed by wet season (263042 individuals). The least catch number was collected in wet season (263042 individuals).In all season, the highest catch number of individuals was recorded in the species *Uroteuthis duvauceli*.

The highest catch number of individuals was observed in cold season. It was agree with Thin Thin Maw (2009). She investigated the squid fishing and catch rate based on some fishery depots of Yangon environs. In her observed, the highest catch number was found in November December, January and February.

The least catch number was found in wet season. Zero catch number was observed in June, July and August. It may be assumed that the fishery department doesn't permit catching fish in these months of all coastal regions. Because June, July and August are spawning seasons of marine fish and prawn.

Fourteen cephalopod species were found in all season. This may be due to the fact that most cephalopods showed their continuous reproductive breeding period. Cephalopods have a rapid growth rate and short life spans. Spawning time and spawning vary among marine species; it's correlated with temperature, though cephalopods in shallow water spawn in cold months so that the offspring would hatch at warmer temperature. (Vidal, 2015)

Species diversity may be related with season. According to the different index value of species diversity, the Shannon index was weight to rare species which collected the least number while the Simpson's index was weight to common species. The value assess by Simpson's index for the least number (collected number is only one individual) of a particular species assessed value could not mentioned at all. Hence the former index should be suitable for the common species.

The species representing the highest value of cephalopod species diversity by Shannon index (-0.366109), Simpson's index (0.110476) was *Uroteuthis duvauceli*, which was followed by *Uroteuthis chinensis* Shannon index (-0.365948), Simpson's index (0.109452) in hot season. The species *Euprymna hyllebergi* had the lowest value of Shannon index (-0.006285), Simpson's index (0.000001).

The species evaluated as the highest value of cephalopod species diversity by Shannon index (-0.366136), Simpson's index (0.110653) was *Uroteuthis duvauceli*, which was followed by *Uroteuthis chinensis* Shannon index (-0.361259), Simpson's index (0.090199) in wet season. The species *Euprymna hyllebergi* had the lowest value of Shannon index (-0.004136), Simpson's index (0.000000).

The higest value of cephalopod species diversity by Shannon index (-0.366793), Simpson's index (0.0115583) was represented by *Uroteuthis duvauceli*, which was followed by *Uroteuthis chinensis* Shannon index (-0.366734), Simpson's index (-0.115075) in cold season. The species *Euprymna hyllebergi* had the lowest value of Shannon index (-0.005913), Simpson's index (0.000001).

It may be assumed that the highest value of species diversity may coincide with the breeding season of those species. The least value of species diversity may probably be due to the prebreeding season or may be the scarcity of those species in the natural habitat.

The present study was also agreed with Mae May Paw (2014). She observed the ecological aspects of cephalopod fauna from Chaungtha environs. She stated that the breeding of all cephalopod were in cool and dry season. Most ovigerous females were captured in cold season and dry season for all recorded species. It might be related to the period of after breeding season. Grist and des Clers (1999) stated that temperature was also an important parameter incorporated into population dynamic models for short-lived animals.

Value of Shannon diversity index for real community is between1.5 to 3.5 (Krebs, 2001). In the present research, Shannon index ranges from 1.708776 to 1.828295 in seasonally. In the present study, the diversity of some cephalopods along Chaungtha coastal area revealed as the real community for the cephalopods species.

Conclusion

According to result, 14 cephalopod species found in three seasons. The highest catch number of individuals was observed in cold season. Analyzing to diversity indices, the highest diversity value was assessed in cold season. These findings indicate that cephalopods revealed as the real community along Chaungtha coastal area. Findings of the survey can provide cephalopod fauna. However, local people and fishery department should protect cephalopod species extinction as well as maintaining the fishery sector of coastal region. Because fishery sector is important for local people of Chaungtha beach, coastal region and our country.

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ROOSTING AND HABITAT PREFERENCE OF INDIAN FLYING FOX, *PTEROPUS GIGANTEUS* (BRUNNICH, 1782) AT CENTRAL DRY ZONE MYANMAR

Nang Aye Aye Shein [1], Mie Mie Kyaw[2], Nwe Nwe Oo[3] and Tin Tin Lynn[4]

Abstract

In Myanmar the Indian flying fox, (*Pteropus giganteus*) is found in forested habitats and near the urban areas. We have learned roosting and habitat preference of the Indian flying fox at central dry zone Myanmar. Conserving a species depends on an understanding of its habitats requirements. Studied were made on (Site I) for a period of five months extending from October 2015 to February 2016 and (Site II) from October 2016 to February 2017. Direct roost count method was counted to estimate the population size of the colony. At the study Sites I total number of 146 trees (above 5 m) were observed. Among them, numbers of 13 trees were rested roost site by the flying Fox. Out of 13 trees, four trees of Tamarind (*Tamarindus india*) and one tree of Rain tree (*Samanea saman*) were rested the study period. The highest number of flying foxes were recorded in October 2015 (854 bats) and the lowest numbers were in February 2016 (716 bats). In the vicinity of the study site (Site II) had 25 trees (above 5 m). At the study Site II, there were six trees of *Bombox ceiba* (kapok). The highest numbers of bats were observed in February 2017(976 individuals) and the lowest numbers were in November 2016 (261 individuals).Thus, *P.giganteus* preferred to roost on tall and large tree, close to paddy fields and stream. Seasonal shifting pattern from one roost tree to another increase and decrease in bats were observed in study area. Habitat destruction and diverse threats to species in the sites were recorded in these areas.

Keywords: Roosting and Habitat Preference, Fruit Bats, Indian flying Fox, *Pteropus giganteus*, microclimate

Introduction

In Myanmar the Indian flying fox, (*Pteropus giganteus*) is found in forested habitats and near the urban areas. Bats fly mainly at night and spend the day in roosts which provide shelter from extremes of temperature, other climatic variables and predators (Furey and Racey, 2016). All bats comprise the Order Chiroptera (neuweiler, 2000), meaning 'hand-wing' in Greek (Tun Yin, world, Chiroptera is the second largest order of mammals. The Order Chiroptera can be divided into two suborders; Megachiroptera (the Old world fruit bats) and Microchiroptera (Insectivorous bats). The mircobats comprise 17 families but the Megachiroptera include only one family that is Pteropoidae (Corbet and Hill, 1992). Megachiroptera are very different with Microchiropera (Tun Yin, 1967). Old World fruit bats do not emit the echolocation (with the exception of *Rousettus*) but rely on olfaction and vision when foraging (Kunz and Fenton, 2003).

Roosting habitats of bats are often partitioned by roost type. Bats spend over half of their life time inside their roost environments. Bats roosts provide sites for mating, hibernation and rearing young, they promote social interactions and the digestion of food, and they offer protection from adverse weather and predators (Kunz, 1982)., Kunz also stated that the roosting habits of bats may be influenced by roost abundance and availability, risk of predation, the distribution and abundance of food resources, social organization and an energy economy imposed by body size and the physical environment. Disturbance and destruction of day roost sites is a fundamental factor for decline of bat population (Kunz, 1982).

[1] Associate Professor, Department of Zoology, Mohnyin Degree Collage
[2] Lecturer, Department of Zoology, University of Mandalay
[3] Associate Professor, Department of Zoology, Myityina University
[4] Lecturer, Department of Zoology, Magway University

Bats are found in all areas of the world especially throughout the temperate and tropical regions (Corbet and Hill, 1992) except in Arctic and Antarctic, in certain remote oceanic islands in eastern pacific (Tun Yin, 1967).At present there are more than 1300 species of bats distributed throughout the world (Voigt and Kingston, 2016). The distribution of bats is largely dependent on the spatial and temporal variation of their abundance of the food (Mickleburg et al., 1992).

Bats are ecologically and economically important animals. They are the only true flying mammals, and they occupy a wide arrange of ecological, niches. Bats shelter in tree cavities, crevices, caves and buildings and the rest exposed on trees (Fenton, 1983). Flying foxes are relatively large, their body weights ranging from 100g to 1000 g and wingspan up to 1.7 m (Neuweiler, 2000). Bats are threatened by human's activities and natural factors (Mickleburg et al.,1992). *P.giganteus* is assumed to be locally threatened by cutting down of roosting trees, hunting or other purposes (IUCN, 2008).

The present study was conducted to learn the roost site and habitat preference of *Pteropus giganteus* and to record the fluctuation of the bat population in the study area.

Materials and Methods

Study area

The roosting site of Indian Flying Fox, *Pteropus giganteus* was in Shwe Sedi Pagoda Precinct, Hpayar Pyan Village, Wetlet Township, Sagaing Region. (Site I). It is located at 22° 15' N and 95°43' 52' E. The elevation of this study site is approximately 90 m (Figure 1).

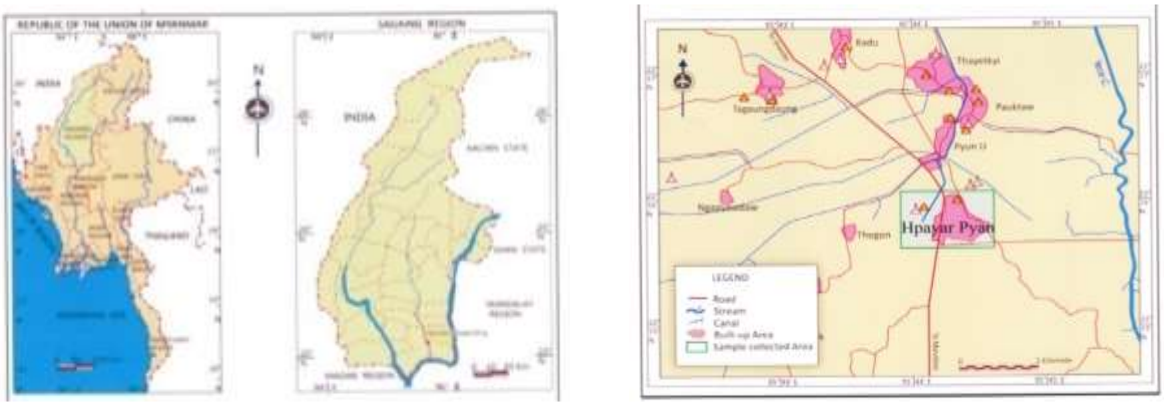
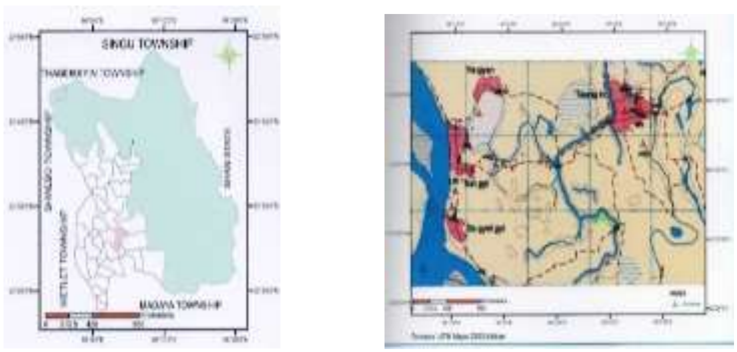


Figure 1 Location map of Hpayar Pyan Village, Wetlet Township, (Sagaing Region)

The roosting site of Indian Flying Fox, *Pteropus giganteus* was in Taunginn village, Mandalay Region (Site II).It is located at 22° 25' N and 96°02' E. The elevation of this study site is approximately 76 m (Figure 2).



(**Source:** Geography Department of MU)

Figure 2 Location map of Taunginn village in Sintgu Township (MandalayRegion)

Study period

The survey was carried out of for five months from October 2015 to February 2016 (Site I) and from October 2016 to February 2017 (Site II).

Data collection

Monthly field surveys were carried out twice a month; the second week and last week of each month. The trees which have over 5 m height were focused.

Identification of species

Identification of Indian Flying Fox, *Pteroopus giganteus* was followed after by Corbet and Hill (1992) and Bates and Harrison (1997). The nomenclature of the trees was based according to Kress et al. (2003).

The measurement of roost trees

The diameter of the trunk as well as the height of each roost trees were measured by the aid of clinometers (Suunto MC2, Pro-Compass) and measuring tape. The height of tree was calculated by using the following equation (Bower *et al.*, 1988) (Fig. 3).

$$H = (d \times \tan\theta) + h$$

where, H = the height of a tree

h = the height of eye above ground

d = distance, tan θ = the angle of elevation

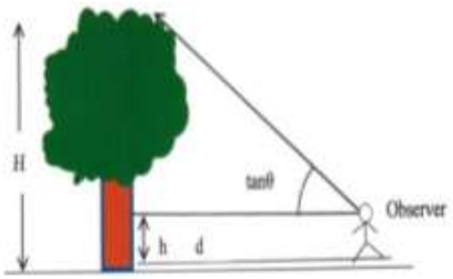


Figure 3 Measuring of the height of the tree

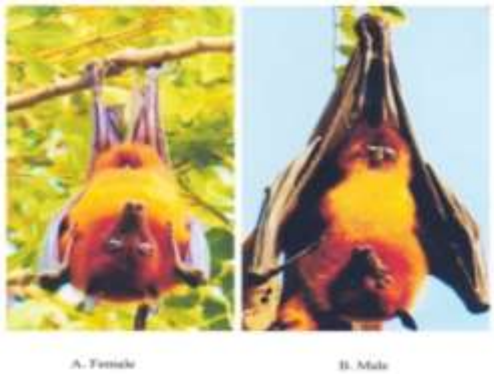


Figure 4 *Pteropus giganteus*

Results

Systematic position of Indian Flying Fox

Kingdom	-	Animalia
Phylum	-	Chordata
Class	-	Mammalia
Order	-	Chiroptera
Suborder	-	Megachiroptera
Family	-	Pteropodidae
Genus	-	*Pteropus*
Species	-	*P. giganteus* (Brunnich, 1782)

Morphological Structure -*P. giganteus* is a very large fruit bat. The snout is long and the ears are tall and pointed. The wings are massive. The first digit has a large claw. The crown of the head was a chestnut brown colour and the baculum is large and semicircular. (Fig 4)

Global distribution - This species is distributed extending from Pakistan, India, Nepal, China and Maldives through to Myanmar, Bangladesh, Bhutan, Sri Lanka, source; Corbet and Hill,1992 and Bates and Harrison, 1997.

The selection of roost site

In the study site, the total numbers of 146 trees (above 5 m height) were observed at Site I. These trees belong to eight species, eight genera and seven families. Among them 13 trees were observed as the roosting trees for Indian flying fox (Table 1). These were six trees of Tamarind (Magyi) *Tamarindus indica* and Palm (Htan) *Borassus flabellifer* and one tree of Rain tree (Kokko) *Samanea saman*.(Table 1).The total numbers of 25 trees (above 5 m height) were observed. These trees belong to seven species, seven genera and seven families at Site II (Table 5). Although there were six trees of *Bombax ceiba* (Kapok), the roosting trees for Indian flying fox used five threes as roosts (Table 6). All roosts were located beside the Nut-min Stream, near the Tasaintpay In. The heights and diameters of *Bombax ceiba* were roosted.

Site I flying foxes were collected the highest number in October 2015 (854 bats) and the lowest numbers were in February 2016 (716 bats) (Table 4). Site II the highest numbers of bats were observed in February 2017 (976 individuals) and the lowest number was in November 2016 (261 individuals) (Table 8). The temperature and humidity of the roost site was almost stable without much variation at site I the ambient temperature ranged from 20°C to 23°C and humidity ranged from 64% to 71% (Table 3). Site II at the ambient temperature ranged from 20.1˚C to 25.9˚C and the humidity ranged from 52.6% to 61%. (Table 7)

Table 1 Number of different trees species from October 2015 to February 2016 at (Site I - Hpayar Pyan Village)

Sr No	Family	Scientific Name	Common Name	No	Root Tree
1	Casealpiniac	*Tamirindus inidca*	Tramarind tree	59	6
2	Arecaceae	*Borassus flabellifer*	Palm tree	72	6
3	Anacardiacea	*Magnifera indica*	Mango tree	3	0
4	Fabaceae	*Sammanea saman*	Rain tree	2	1
5	Meliaceae	*Azadirachta indica*	Neem tree	3	0
6	Arecaceae	*Coco snucifera*	Coconut tree	4	0
7	Rosaceae	*Prunus domestica*	Plum	2	0
8	Minosocea	*Acacia leucophliea*	White barked acacia	1	0
		Total		**146**	**13**

Table 2 Height and diameter of roost trees (Site I - Hpayar Pyan Village)

Roost tree species	No. of roost tree	Height (m)	Diameter (m)
Tamarindus indica	6	24.6m	1.97m
Borassus flabelifer	6	23.33m	1.43m
Samanea saman	1	31m	3.38m

Table 3 Monthly populations of *P. giganteus* in relation to temperature and humidity from October 2015 to February 2016 (Site I)

Monthly/year	Population	Temperature	Humidity (%)
Oct-2015	854	23.2	66.3
Nov-2015	785	22	64
Dec-2015	779	20.2	71
Jan-2016	762	23	664
Feb-2016	716	22.7	65

Table 4 Monthly populations of *P.giganteus* on different roosted trees Site I

Sr no	Roost tree species	Roost tree Number	Oct	Nov	Dec	Jan	Feb
1	*Tamarindus indica*	T1	69	61	23	20	-
2	*Tamarindus indica*	T2	350	334	311	328	319
3	*Tamarindus indica*	T3	101	84	76	122	89
4	*Tamarindus indica*	T4	16	13	9	20	12
5	*Tamarindus indica*	T5	36	30	15	13	-
6	*Tamarindus indica*	T6	10	17	5	22	17
7	*Borassus flabelifer*	P1	10	4	-	-	-
8	*Borassus flabelifer*	P2	9	5	-	-	-
9	*Borassus flabelifer*	P3	2	2	-	-	-
10	*Borassus flabelifer*	P4	2	-	-	-	-
11	*Borassus flabelifer*	P5	4	-	-	-	-
12	*Borassus flabelifer*	P6	4	-	-	-	-
13	*Samanea saman*	R1	241	235	340	237	279
Total			**845**	**785**	**779**	**762**	**716**

Table 5 Number of different tree species at study site during October 2016 to February 2017 (Site II -Taunginn Village)

Sr no	Family	Scientific name	Common name	Local name	No.of tree
1.	Ameacardiacea	*Magifera indica* (L.)	Mango	Thayet	7
2.	Rhamnaceae	*Ziziphus jujuba*	Jujube	Zi	2
3.	Bombacaceae	*Bombax ceib* L.	Kapok	Letpan	6
4.	Rubiaceae	*Naucle aorientalis*	Bur Tree	Ma-u	5
5	Mimosaceae	*Albizia lebbek*	Lebbek Tree	Kokko	1
6.	Boraginaceae	*Cordia dichotoma* Forst..	Bird Lime	Thanat	3
7.	Fabaceae	*Butea frondosa* Roxb.	Palas Tree	Pauk	1
		Total			**25**

Table 6 Height and diameter of roosted trees (*Bombox ceiba*) Site II

Sr no.	Roost tree	Height (m)	Diameter	Roost
1	I	25.34	7.2	+
2	II	17.13	5	+
3	III	17.5	4.3	+
4	IV	11.12	1.5	-
5	V	15.91	3.5	+
6	VI	21.5	6	+

\+ Presence, - Absence

Table 7 Monthly populations of *P.giganteus* in relation to temperature and humidity from October 2016 to February 2017 Site II.

Month/year	Population	Temperature (°C)	Humidity (%)
Oct-2016	272	25	61
Nov-2016	261	24.5	56
Dec-2016	481	20.1	58
Jan-2017	475	21.5	52.6
Feb-2017	976	25.9	56

Table 8 Monthly populations of *P.giganteus* on different roost trees (*Bombox ceiba*) Site II

Srno	Roost tree	Oct	Nov	Dec	Jan	Feb
1	I	93	97	-	69	581
2	II	84	79	59	20	180
3	III	95	85	186	45	215
4	IV	-	-	-	-	-
5	V	-	-	78	-	-
6	VI	-	-	158	341	-
	Total	**272**	**261**	**481**	**475**	**976**



Plate I. Roosting of Indian flying foxes at the study sites

Discussion

The present study was carried out in Shwe Sedi Pagoda Precinct as Site I from October 2015 to February 2016. In this study, *P. giganteus* roosted on 13 trees, comprising three species of trees and the population decreased month by month throughout the study period. However, in the same study site, Moe Moe Aung (2005) recorded that bats used 20 trees as roosting site, including five tree species and the populations of bats increased within her study period (from 2003 to 2005).

During the present investigation, *P.giganteus* was found to root in larger trees. The heights of all roost tree ranged from 23.33m (*Borassus flabellifer*) to 31 m (*Samanea saman*), while the diameters ranged from 1.43 m (*Borassus flabellifer*) to 3.38 m (*Tamarindus indica)* respectively. The ambient temperature ranged from 20.2˚C to 23.2˚C and the humidity ranged from 64% to 71%. The maximum roost of bats was found in *Tamarindus indica* T_2 (319 individuals). The minimum roost of bats was found in (*Borassus flabellifer)* P_3 (2 individuals). Flying foxes were collected the highest number in October 2015 (854 bats) and the lowest numbers were in February

2016 (716 bats). In study site I, the maximum number of roosts was recorded on the tamarind trees in October and minimum number in February.

According to the present results, the populations were assumed to decrease in the present study period. These study months were cold season. The days are shorter in cold season. Since the sunset time is earlier, the emergence time is earlier in the cold season. Similarly, it was assumed that the food production was lower in the cold season thus the bats may need to travel longer distances to search food. Therefore, the population of *P.giganteus* clearly decreased throughout the entire study period from October 2015 to February 2016. Moe Moe Aung (2005) also recorded that the numbers of bats decreased from September through December in Shwe Sedi Pagoda. Moe Moe Aung (2005); Sein Sein Win (2006) and May Myo Nyunt (2007) also stated that bats emerged earlier from the roosts site during the cold season compared to the wet and the hot seasons. The present finding also agrees with the statement of previous local workers.

The present study was also carried out in Taunginn village, Mandalay Region as Site II from October 2016 to February 2017. The flying fox (*P.giganteus*) from the same study site was previously observed by Sein Sein Win (2006).

During the short period of five months study, In the vicinity of the study site has 25 trees (above 5 m) belonging to seven species, seven genera, and seven families. At the study site, there were six trees of *Bombox ceiba* (kapok). Among them, *P.giganteus* roosted on five trees of this species. The highest numbers of bats were observed in February 2017 (976 individuals) and the lowest numbers were in November 2016 (261 individuals). The maximum roost of bats was in RT I (581 individuals) with highest 25.34 m and diameter 7.2 m. The minimum roost of bats was in RT II (20 individuals) with height 17.13 m and diameter 5 m. No bat was observed in RT IV with height 11.12 m and diameter 1.5 m. The ambient temperature was ranged from 20.1˚C to 25.9˚C and the humidity was ranged from 52.6% to 61%. All root trees located along side of Nut-min Stream and surrounded by paddy field. Similarly, Sein Sein Win (2006) recorded that the population of bats was decreased in November, December and February, 2005. It was assumed that the decrease of the bat populations may be due to the lower food availability.

Kunz and Fenton, 2003 stated that bats have evolved specialized thumbs and feet for roosting. It was assumed that they are shrouded by their wings, giving them the appearance of dead leaves so they may reduce the detecting by predators. Similarly, IUCN, 2008 stated that *P.giganteus* roots on larger trees, close to cultivated fields and ponds. Elangovan and Kumar (2015) reported that *P.giganteus* live in large diurnal roost and usually located on the branches of larger trees. Gulraiz et al., 2015 recorded that seasonal shifting pattern from one roost tree to another increase and/or decrease in bats were observed at both sites.

This finding was agreed with Moe Moe Aung (2005), Sein Sein Win (2006) and May Myo Nyunt (2007), who stated that the roost trees occupied by the flying foxes in general are quite large and high.

From this result, all of the roost trees occupied by *P.giganteus* were tall and large in size, thus it may be recorded that *P.giganteus* could prefer to roost on large and tall trees. At the study site, from this result, we were assumed in the field human impacts and climate change are found. We were learned the comparison two study sites, Site I data collected throughout decreased at roost the study period. Site I area environment is closed near the pagoda, village and human impacts. Site II data collected throughout increased at roost the study period. Site II area environment is situated near the river, paddy field and not human impact. Habitat destruction and diverse threats to species in the sites were observed in these areas.

Conclusion

Seasonal shifting pattern from one roost tree to another increase and decrease in bats were observed in the study area. Conserving a species depends on an under-standing of its habitats requirements. So, the long term conservation of the species, need to protect their habitat in this area as well as the other parts of Myanmar.

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SOLITARY BEHAVIOUR OF INDIAN FLYING FOX *PTEROPUS GIGANTEUS* (BRUNNICH, 1782) FROM SALINGYI TOWNSHIP, SAGAING REGION

Soe Soe Htun[1]

Abstract

Salingyi is a township in Yinmabin District in the Sagaing Region of Myanmar. The behaviour of *Pteropus giganteus* was conducted for twelve months; from December 2017 to November 2018. The purposes of this study are to quantify directly the daytime behaviours categories and to know solitary behavioural of roosting bats with relating to ambient temperature. Data collection was conducted clearly visible on a single roost tree purposively sampled for studies of daytime behaviour. Focal sampling and scan sampling were utilized according to Martins and Bateson (2007). The number of individual bats counted in *Sizygium jambos* colony ranged between a minimum of 174 individuals and maximum of 566 individuals. The diurnal activity patterns consisted of the solitary activities of roosting, sleeping and grooming, stretching and funning. Indian flying foxes were found to sleep most in the early morning (20°C-25°C) and became active in the afternoon and evening (30°C-35°C). Fanning was more frequently recorded during the afternoon (30°C-35°C) than other time periods in a day. Grooming and wing stretching were increased with ambient temperature (25°C-30°C). The present study indicates that the ambient temperature has a profound effect on the behaviour of Indian flying foxes.

Keywords: *Pteropus giganteus*, solitary behaviour, ambient temperature, Sagaing Region

Introduction

Bats (order Chiroptera) are unique in being the only group of mammals that have developed sustained flight. Approximately 25% of all chiropteran species (nearly 238 species) are considered threatened by the International Union for Conservation of Nature (IUCN) (Kumar and Kanaujia, 2009). Order Chiroptera includes more than 1,300 extant species and are and unique among mammals in their evolution of powered flight (Kingston and Voigt, 2016). After rodents, the second most diverse group of small mammal is bat (Hutchins *et al*., 2003). In addition, the suborder Megachiroptera contains one family (Pteropodidae) that includes 186 species of mainly frugivorous bats (Simmons, 2005). At the present, the known bat species from Myanmar is 103 (Bates, *et al*, 2020). The Indian flying fox is found in Bangladesh, Bhutan, China, India, the Maldives, Myanmar, Pakistan and Sri Lanka and it is widely distributed across most of Nepal (Jnawali *et al.*, 2011).

Nearly all species of bat (except species of the genus *Pteropus*) occupy a wide variety of habitats like caves, crevices, temples, ruined buildings and foliage. Bats living in such habitats are well protected from predators and the abiotic factors like sunlight, ambient temperature, humidity, thunder and rain. Whereas, bats belonging to the genus *Pteropus* live in trees by exposing themselves to these abiotic factors (Bates and Harrison, 1997).

Their diurnal roosts are found in various types of large and tall trees, including *Ficus bengalensis*, *F. religiosa*, *Tamarindus indica*, *Mangifera indica*, *Dalbergia sissoo* and *Eucalyptus* sp (Vendan, 2003). During hot summer days, majority of the individuals gently flap the distal end of one of their wings, which is attributed to thermoregulation (Mathur *et al*., 2012).

The flying foxes are very conspicuous among tree roosting bats and thus many studies have been carried out on various aspects such as population ecology (Mathur *et al*., 2012; Manandharet *et al*., 2017), reproductive behaviour (Maruthupandian and Marimuthu, 2013), roosting ecology

[1] Dr, Associate Professor, Department of Zoology, Kyaing Tong University

(Hahn *et al*., 2014; Gulraiz *et al*., 2014), distribution (Kumar *et al*, 2017) and conservations issues (Senthilkumar and Marimuthu, 2012).

The behaviour of *P. giganteus* considered as an interesting phase because it bifurcates the diurnal and nocturnal activities. The nocturnal behaviour of *P. giganteus* begins with the emergence and ends with home flight. During the day, these animals sleep, hanging upside down by their feet with their wings wrapped around themselves. They also fan themselves to aid in thermoregulation, move around in the roosting tree, and communicate with each other (Nowak, 1999).

Bat generally prefers to roost during in diversified roosting habitats. Roosting site selection depends on their abundance, risk of predation, availability and distribution of food resources, body size and physical environment (Kunz, 1982). Similarly, Khan *et al.* (2020) also reported that *P. giganteus* utilizes different feeding and roosting sites including diverse roost. Local climate, seasonal food availability and social interactions among bats are the main factors responsible for evolving solitary or gregarious foliage roosting behavior in bats (Kunz, 1982).

The purposes of this study are to quantify directly the daytime behaviours categories of *P. giganteus* and to know solitary behavioural patterns of roosting bats with relating to ambient temperature

Materials and Methods

Study area

Salingyi is a township in Yinmabin District in the Sagaing Region of Myanmar. Bonsanwaddy monastery, Kyardet village, Salingyi Township was chosen as the study area. It is located at 21° 50' 29.58" N and 94° 56' 10.57" E and situated at South-West from Salingyi Township (Plate.1).

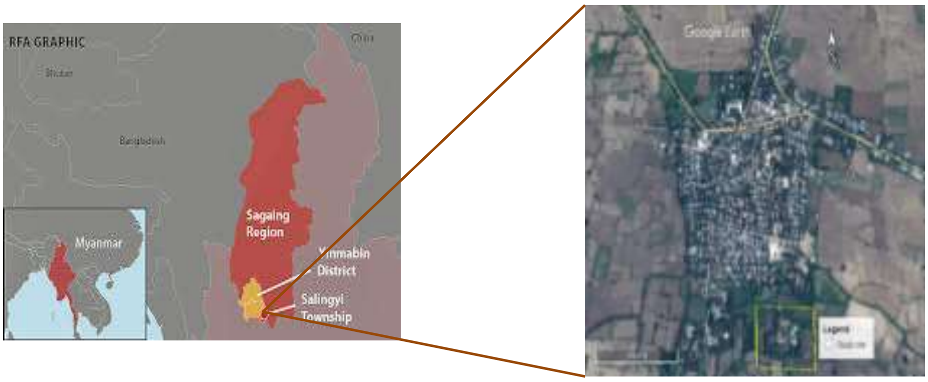

Plate 1 Location map of study area

Study period

The study was conducted for twelve months; from December 2017 to November 2018.

Identification of the specimens

The identification of the specimen was according to Bates and Harrison (1997).

Data collection

The population of Indian Flying Fox, *Pteropus giganteus* roosted on 11 trees, *Sizygium jambos* (one) and *Tamarindus indicus* (10). Among the 11 roosting trees, countings were only conducted in *Sizygium jambos* roosting tree. The behaviors of roosting flying foxes were recorded three times in a month. Observation days were spread across three times (morning, afternoon and evening). Observation started at (8:00 hr) and ended at (17:00 hr). Focal sampling and scan sampling were utilized according to Martins and Bateson (2007).

Data collection was conducted on a clearly visible single roost tree purposively sampled for studies of daytime behaviour. The ambient temperature at the study locations was recorded from morning to evening using a thermometer.

Results

The number of individual bats counted in *Sizygium jambos* colony ranged between a minimum of 174 individuals and maximum of 566 individuals. The numbers of bat increased to peak population in April 2018 but the decreasing of bat population was observed in January 2018 (Fig. 1).

Solitary behaviours of *P. giganteus*

Sleeping

Sleeping bats hung claws at the twig while their wing membranes or patagium were wrapped up and covering the head and ventral parts of the body but sometimes it was also seen even with an open arms or wings (Plate 2 A).

Grooming

Bats used their hindclaw, foreclaw, mouth, tongue and teeth for grooming. This behaviour was manifested by licking the fur and inner and outer membrane, licking genitals, licking mouth, scratching body, cleaning ears. Actually, the grooming behaviour was observed after the bat perching on the twigs during the field observations (Plate 2 B).

Stretching

Flying foxes did stretching by spreading out their wings one after the other or even simultaneously (Plate 2 C and D).

Fanning

Flying foxes were observed to do fanning in afternoon during fair weather conditions. An individual was observed to cling its hind feet on to the branch and flapped its wing membranes towards the bodies (Plate 2 E).

Daytime behaviours

Bats behavior is of different kind, however, here 13 type of behavior were observed and recorded. Among the 13 observed behaviours, sleeping was the most frequent behviour (18.28%) followed by stretching (18.09%), grooming (14.68%) and funning (14.29%). Other activities, such as locomotion, aggression, searching, courtship, flying, copulating, and urinating, defecating and maternal care were much less frequently observed (Fig. 2).

The diurnal activity patterns of *P. giganteus* consisted predominantly of the solitary activities of roosting, sleeping and grooming, stretching and funning. Most frequently, Indian flying foxes were seen sleeping throughout the day. In October, grooming was recorded at higher frequencies (20.25%) than in other months. Wing stretching was higher frequently recorded in April (22.83%) and lowest in August (9.47%). The frequency of wing fanning was found to be highest in April (22.83%) and decreased in October (2.53%) (Fig.3).

Daytime behaviour in response to time of day

Indian flying foxes were found to sleep most in the early morning and became active in the afternoon and evening. Sleeping behaviour was recorded least frequently during morning in April (4.90%) and higher frequency from December to March and June, July, September and October (8.82%) respectively (Fig.4). Grooming was more frequent in the morning in December, January, February, March, July, October and November (10%) each and less frequent during the afternoon and evening in August (3.84%) and November (2.17%) (Fig.5). Stretching was more frequent in the morning from February to July (9.37 %) each and least during afternoon (4.47%) and evening (3.03%) in August (Fig.6). Fanning was more frequently recorded during the afternoon than other time periods in a day. It was recorded maximum during morning September (16.36%) while maximum flapping activity was observed in the afternoon from February to May (10.97%). However, the least frequency was found in the morning in December (10.81%) and lack of activity was recorded in October (0%) (Fig.7).

Daytime behaviour in response to ambient temperature

Changes in behaviour of bats in response to ambient temperature showed that the behaviour relied on ambient temperature. Sleep occurred frequently in all the temperatures in the study area. The frequency percentage of sleep gradually decreased with rising in temperature up to (30°C-35°C) but it increased at (20°C-25°C). And also the frequency percentage of groom gradually decreased with rise in temperature up to (30°C-35°C) and increased at (25°C-30°C). Wing stretching increased with ambient temperature (25°C-30°C). Fanning was recorded more frequently in raised temperature (30°C-35°C) and least frequently in temperature (20°C-25°C) (Fig. 5). Other activities were mostly recorded in temperature (25°C-30°C) and (30°C-35°C) (Fig.8).

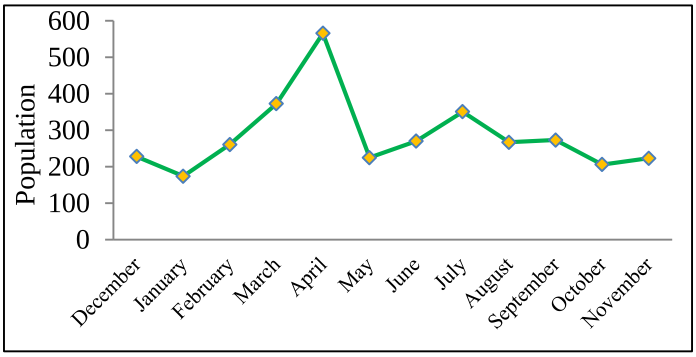

Figure 1 Population of bats in study area

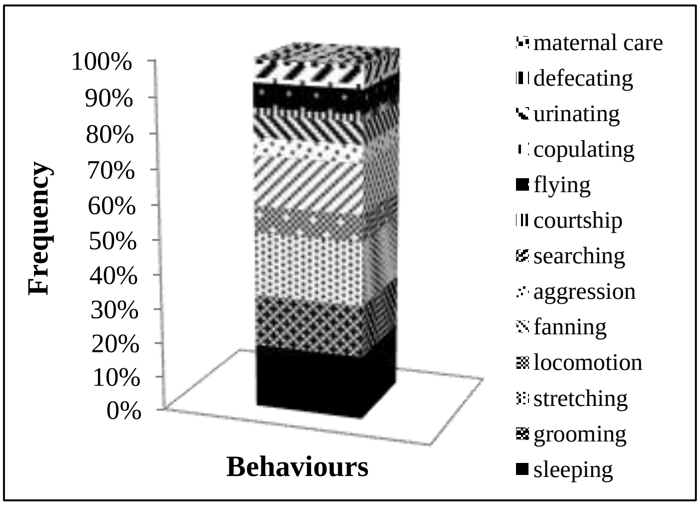

Figure 2 Daytime behaviour of Indian Flying Fox (*P. giganteus*)

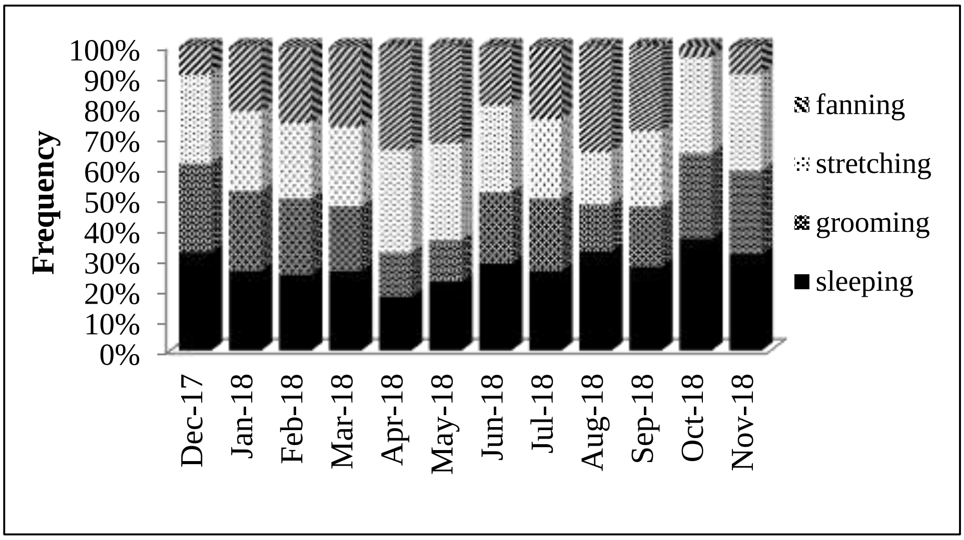

Figure 3 Frequency of solitary behaviours at Salingyi Township

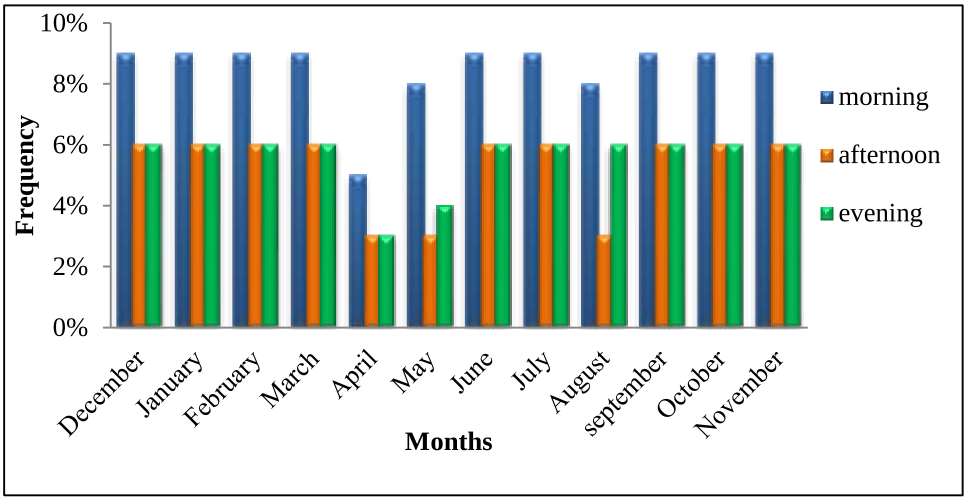

Figure 4 Monthly frequency of sleep in different hours of day

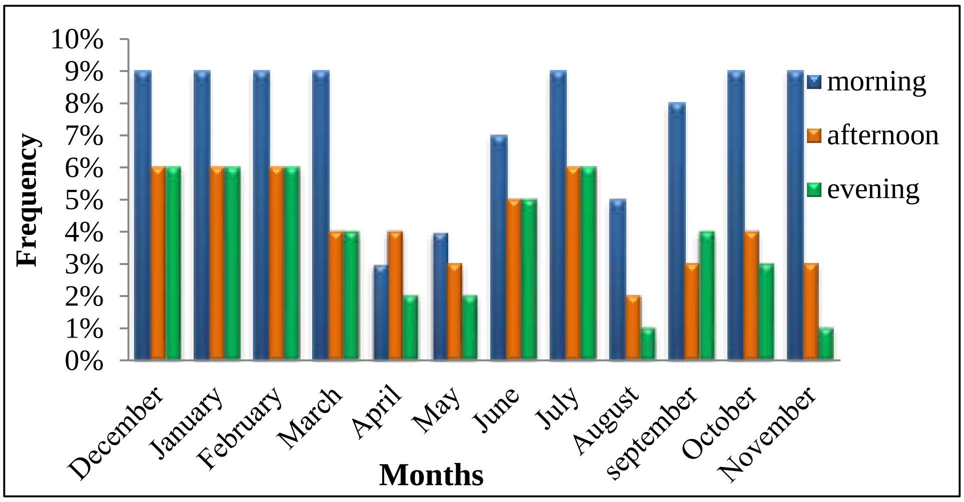

Figure 5 Monthly frequency of grooming in different hours of day

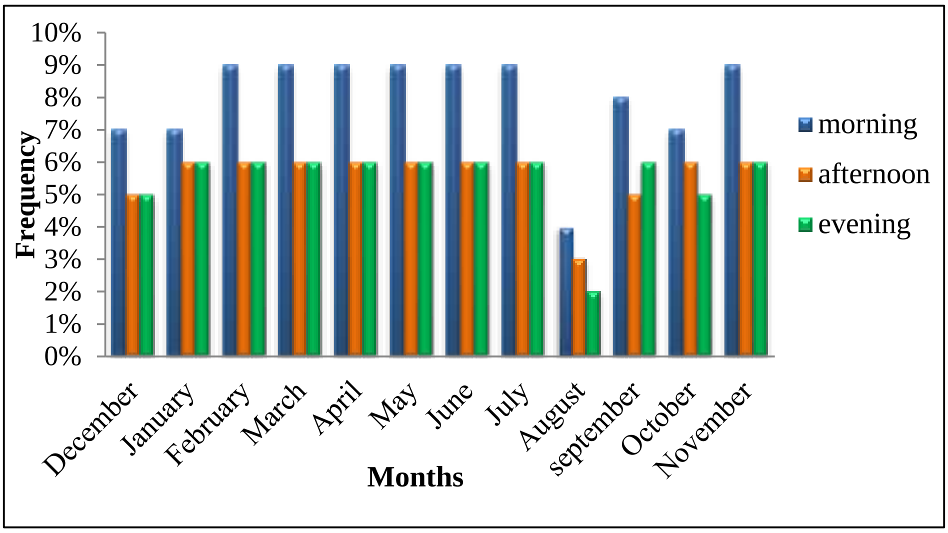

Figure 6 Monthly frequency of stretching in different hours of day

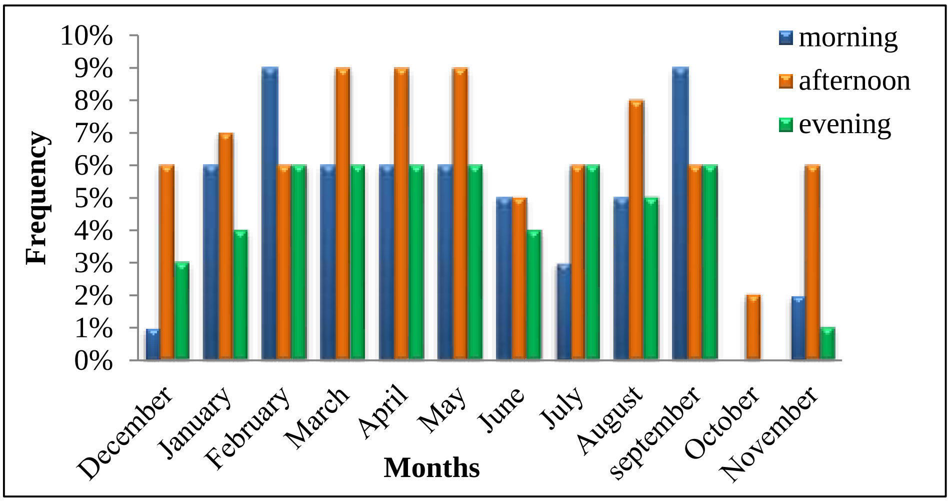

Figure 7 Frequency of funning in different hours of day

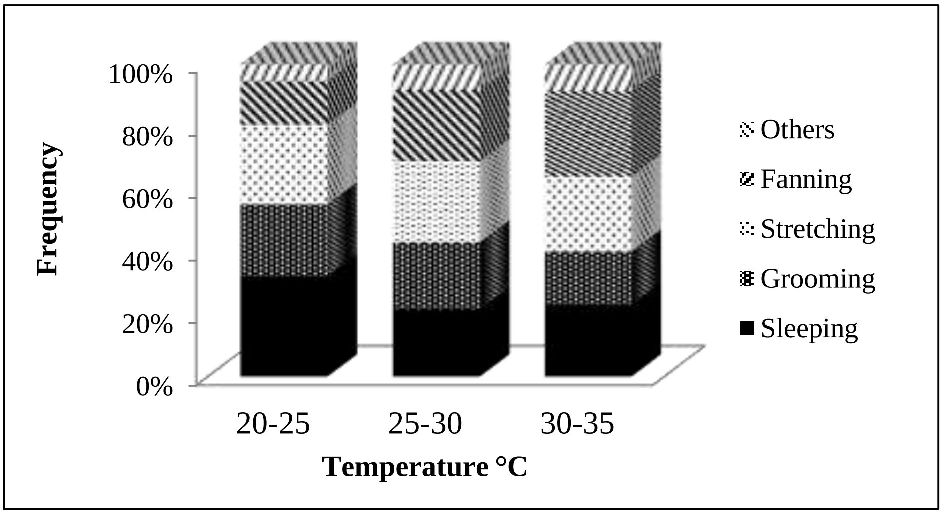

Figure 8 Frequency of behaviours at different temperatures

A. Sleeping

B. Grooming

D. Stretching (one wing)

C. Stretching (both wings)

E. Fanning

Plate 2 Solitary behaviour of *Pteropus giganteus*

Discussion

Indian flying fox exhibited various behaviours during daytime with fluctuation in the frequency. Sleep and stretch behaviours were recorded as frequently observing activities while courtship, copulation and defecation were less than two percent of behavioural composition but the maternal care was the least, 0.17% in the present study. Sleep, groom, mate/courtship and wing spread were the most frequently occurring behaviours of Grey-headed Flying Fox (Connell *et al*., 2006). In the present study, behaviours of *Pteropus giganteus* also revealed similar results.

The frequency of sleep changed significantly throughout the day, the sleep behaviour was recorded largely in the early morning than other periods of the day time and it seems to be the bats has just arrived back from foraging. However, the sleep behaviour continued in other parts of day since it saves energy to fly out in the evening for foraging (Manandhar, *et al*., 2017). During the study period, sleeping behavior was most occurred in the morning.

Bats are nocturnal animal and day times are expected to be the inactive period for them. The lowest percentage sleeping behavior was recorded with rises temperature (30°C-35°C) and higher frequency in (20°C-25°C) were recorded. Funakoshi *et al.*, (1991) reported that sleeping duration is closely linked with the ambient temperature.

The Indian flying fox were found involved in grooming during the early morning in study area. Morning grooming activity was often triggered by the first rays of sun reaching bats in the tops of trees and comprised of extensive and thorough cleansing of all body surfaces (Markus and Blackshaw, 2002). Nelson (1965) suggested that it is important to keep the wing soft and flexible by spreading the lipid droplets around wing membrane, in addition it is a behavioral strategy for reducing the ectoparasite density. During the entire field observation, individual self- groom was also documented several times a day.

In the present study, the highest occurrence of wing stretching was observed in the morning. Wing stretch activity had begun with sunrise and continued to the afternoon when it was hot. Brooke (2000) described bats roosting on prominent and exposed branches of trees that opened one or both wings as sunlight first fell on them, faced towards the sun and slowly rotated back and forth. Previous workers also reported that the roosting trees provide a great protection from environmental perils and also protection from predators (Richmond *et al*., 1992, Altringham, 2011 and Khan *et al*., 2020). Therefore, the wing stretching behaviour seems to be associated with thermoregulation or energetic savings during roosting.

During the study period, wing fanning was mostly observed during afternoon as compared to other parts of day. Wing flap of Indian flying fox was frequently observed during the maximum temperature (25°C-35°C). However, this behavior was relatively decreased with temperature (20°C-25°C) in the day time. It may be assumed that when the air temperature rises, intensity of heat of the sun becomes higher, so in order to keep them cool, they flap their wings. Neuweiler (2001) also stated that fanning and also stretching are probably strategies to lower the body temperature by creating convection currents of the air that can help cool down the body of the bats.

Although *P. giganteus* was a nocturnal flying mammal but daytime roosting has to rest or sleep because the bats spend most of their lives in their roosts (Altringham, 2011). They performed some other activities at daytime roosting which included grooming, fanning, aggression, stretching, flying, mating, occasional daytime defecation, but still then resting or sleeping was the main activity at daytimes. The present study indicates that the high temperature has a profound effect on the Indian flying foxes. The temperature has been recorded to range from maximum of 35°C to minimum of 20°C. Neuweiler (2001) described in the active state, fruit bats could maintain their body temperature between 31°C and 39°C.

Conclusion

Pteropus species live mainly on trees exposing themselves to the daylight. All other species live in closed areas such as caves, crevices, unused buildings etc. Hence individuals of *Pteropus* must adapt themselves to hot and cold temperatures. The current study provides baseline information for future investigations on the behaviours of *P. giganteus.* Among the different daytime behaviour of this species, sleeping, grooming and wing stretching were more active in morning than fanning. This is followed by a lull in activity in afternoon and evening. During the study period the average temperature in roosting tree was a maximum 35°C and minimum 20°C. Observed that the environmental factor like temperature is important factor to determine roosting behaviour in *P. giganteus.*

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BEEKEEPING METHOD AND LIFE CYCLE OF EUROPEAN HONEYBEE, *Apis mellifera* Linnaeus, 1758 IN MAGWAY REGION

Thida Wai[1], Lai Lai Phyu [2], Cherry Soe [3], Nyo Nyo Lwin [4]

Abstract

Beekeeping method and life cycle of European honeybee, *Apis mellifera* Linnaeus, 1758 were conducted in the campus of Pakokku University and Pauk Kone Village, Magway Region during September, 2017 to May, 2018. Migratory beekeeping method was more suitable in the study area instead of stationary methods due to unavailable food sources in main station throughout the year. It was observed that three different castes of honeybee were included in a colony (queen, worker and drone). Honeybees have five stages in their life cycle: egg, larva, prepupa, pupa and adult. The developmental period of the drone from egg to adult stage was the longest (24 days) and followed by worker (21 days) and queen (16 days). Among the casts, queen is the largest and heaviest than drone and worker. Understanding of life stages of *Apis mellifera* may help the proper management of honeybee colonies. The sizes of honeybee were assumed to be depended on the sufficient of food sources and environmental factors. The data of the present study would provide the information to further researches concerned with beekeeping and ontogenetic development of the honeybee and other related researches. Beekeeping could create the good opportunities of job for livelihood of rural people.

Keywords: European honeybee, *Apis mellifera*, Beekeeping methods, life cycle.

Introduction

Honey bees are the main insects which help in pollination of different species of plants (Sawyer, 1981). Honey bees play an important role for cross-pollination, or the transfer of pollen from one plant to the stigma of another plant leading to the process of fertilization (Suwannapong *et al*., 2012).

In Asia, a total of 11 honey bee species were recorded (Michener, 2000). Some species are being domesticated and employed as pollinator and production of bee product such as honey, beeswax, bee pollen etc, were used in various ways (McGregor, 1973). A colony of honeybee consists of a queen, several thousand workers and in a certain season of the year- a few hundred drones. Among the members of the colony there is a division of labor and specialization in the performance of biological functions (Wintson, 1987).

Beekeeping is one of the oldest known forms of food production. Some of the earliest known evidences of beekeeping are from a rock painting, dating to around 13,000 BC (Kleinjans *et al*., 2012). Beekeeping can also create social benefits when small-scale farmers join together to form an association, either formal or informal (Hilmi *et al.*, 2011).

Beekeeping had been practiced with two methods such as stationary and migratory method for providing food for human and serving as pollinators to improve the crop quality and quantity. In stationary method, the beehives were maintained in only one place which gives enough food for the bees. When the food sources were rare in that place, the bees can be kept by giving them supplementary feeding without migrating to other places for food sources. In migratory method, the beehives were always moved to various places where plants were enough to provide sufficient food sources for bees throughout the year.

[1] Lecturer, Department of Zoology, Magway University
[2] Lecturer, Department of Zoology, Magway University
[3] Assistant Lecturer, Department of Zoology, Magway University
[4] Professor and head, Biology Department , Yangon University of Education

The present work was conducted with following objectives: to investigate the suitable beekeeping method practicing in the study sites of Magway Region, to record the life cycle of European honeybee, *Apis mellifera* keeping in the study sites.

Materials and Methods

Study Area and Study Period

The study was carried out in Pakokku University Campus, 21° 21' 06.11" N and 95° 04' 18.44" E, and Pauk Kone Village, 20° 26'27.24" N and 94°49' 41.59" E in Magway Region during September, 2017 to April, 2018.

Plate 1 Map showing the study sites (Source: Google Earth 2018)

Apparatus Used in Beekeeping

Apparatus used in beekeeping were wooden box (61 cm × 43 cm × 34 cm), wooden frame (6 frames for each box), smoker, hive tool, bee brush, Bee veil, gloves and bee suit.

Source of Honeybees

Three beehives of honeybees, *Apis mellifera*, were purchased from Department of Apiculture, Magway Region.

Food Source Plants in Study Sites

In Pakokku University Campus, Magway Region, there are several plum trees and various flowering plants providing nectar and pollen as favorable food source for honeybees. In Pauk Kone Village, Magway Region, honeybees preferred food source such as sunflower, gram and maize were abundant during the study period.

Data Collection

Beehives were checked weekly at the apiary of study sites between 8:00 AM to 11:00 AM. Each 20 samples for all life stages such as eggs, larva, prepupa (cell capped), pupa and adult stages were collected and recorded their length and weight.

Identification

The honeybees collected were identified according to Bingham (1897).

Beekeeping Methods

Beekeeping was practiced according to Cramp (2008).

Environmental Parameters

Monthly mean temperature, relative humidity and rainfall were recorded from Hydrology and Metrology Department, Magway Township, Magway Region.

Statistical analysis

Collected data were statistically analyzed by using Microsoft Excel (2010) and presented as mean and standard deviation, and expressed with graph.

Results

Characteristic of Recorded Honeybee, *Apis mellifera*

Body length of worker is 12.2 ± 0.54mm (n=20). The male drone is 15.3 ± 0.67mm (n=20), and the queen is 19.5 ± 1.35mm (n=20) long. Body is golden brown and black, with pale yellow rings on the abdomen. The head, antenna, and legs are almost black; fine hairs cover the thorax and only lightly cover the abdomen. Wings are translucent. Pollen baskets are made of specialized hairs and are located on the outer surface of the tibiae of the hind legs. All observed characters of recorded bee are similar to the characters of *Apis mellifera* mentioned by Bingham (1897).

Classification of *Apis mellifera*

Phylum - Arthropoda

Class - Insecta

Order - Hymenoptera

Family - Apidae

Genus - *Apis*

Species - *A. mellifera* Linnaeus, 1758

Beekeeping Method Used in the Present Study

A total of three beehives were designated as hive A, B and C. In each hive there are stacks of six vertical wooden square frames with two parallel steel strings. In each cell frame, there were need to be a full of honeybees. So that, each beehive colony was prepared placing a queen, about 9000 workers and a few hundreds of drones. And then, these beehives were placed near the plum trees in the Pakokku University Campus. Beekeeping in the Pakokku University Campus was started in September 2017. At that time, sufficient food source nectar from plum trees were sufficient for honeybees. Regular monitoring was weekly conducted in the beehives to know the situation of honeybee such as death rate, presence of predators, parasitic infection and other diseases.

In February 2018, the flowering season of plum tree was ended, food sources from plum trees were insufficient for honeybees, besides, other available food sources were also became rare in the Pakokku University Campus. Therefore, honeybees were fed the supplement food as sugar solution placing near the beehives.

In March 2018, populations of honeybee in hives were decreased due to scarcity of food sources for honeybees in Pakokku University Campus. Therefore, these beehives were moved to Pauk Kone Village, Magway Region where food sources from sunflower, maize and various bean plants were available to forage by honeybees. After moving to food sources abundant place, the populations of honeybee in studied beehives were increased again.

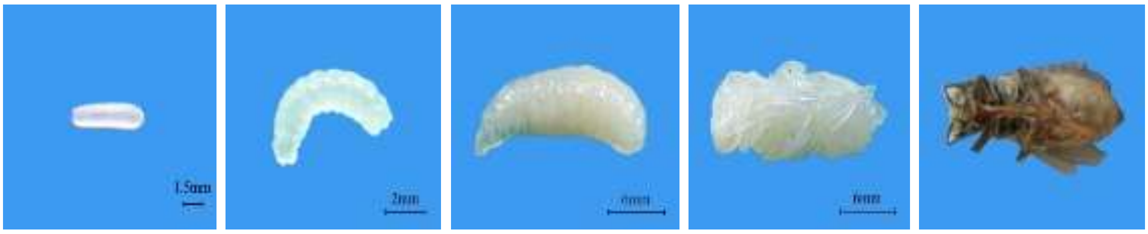
According to the present experiment, migratory beekeeping method was the more suitable than stationary method in the study area, since availability of food sources for honeybee is the main factor in beekeeping during the study period.

Life cycle of studied bee, *Apis mellifera*

In the studied beehives, altogether five stages such as egg, larva, prepupa, pupa and adult were observed in all casts (Plate 2). In the colony, egg and larva are called open brood and prepupa and pupa are referred to as sealed brood because of the capped cells. Among the casts, different durations of developmental stages were recorded (Figure 1).

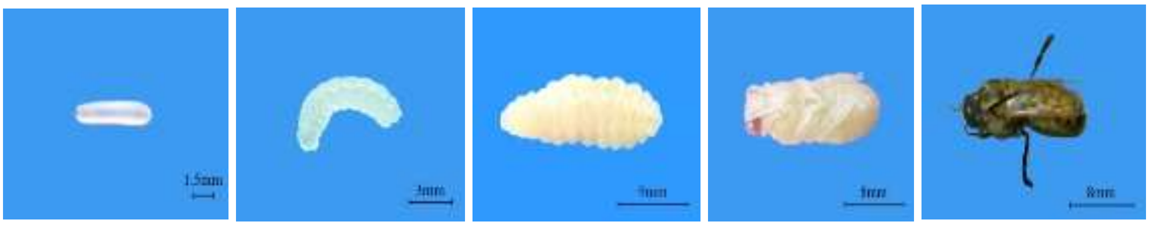
Egg Larva Prepupa Pupa Adult

(A) Developmental stages of queen



Egg Larva Prepupa Pupa Adult

(B) Developmental stages of worker



Egg Larva Prepupa Pupa Adult

(C) Developmental stages of drone

Plate 2 Developmental stages of honeybee, *Apis mellifera*

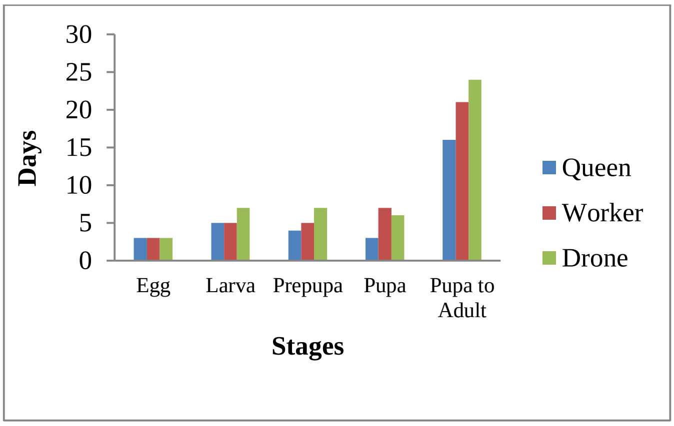


Figure 1 Duration of developmental stages of three casts of *Apis mellifera*

Egg

The queen laid eggs in wax cells in the brood area of the comb (Plate 1). The position of the egg after immediately laid by queen was upright about 90° at the bottom of the cells. In the next day, the egg was slightly tilting about 45° and in the 3^{rd} day, the egg was horizontal position in the wax cell. After 3 days, egg hatched and larvae emerged. The egg of honeybee was very tiny and difficult to see, but their presence was indicated by a laying queen in the colony, or with the help of light or under sunlight. The duration of egg to emerging larva can be determined by looking its position. The egg lasted 1-3 days to hatch (Table 1). The length of egg was measured about 1.5 mm and the weight of egg was less than 0.001 g (Table 2, 3).

Larva

The duration of the larval stage varies among the casts, the duration of larval development was recorded in queen (5 days), workers (5 days) and drones (7 days), respectively (Table 1). Morphological features of larvae were whitish wormlike grub with no leg, eyes, antennae, wings, or sting (Plate 2). It was "C" shaped and easily seen at the bottom of their wax cell. In larva stage, drone and queen showed the same length (15 mm) and worker had the shortest length (9.6 mm) (Table 2). The weight of the drone showed the highest (0.28g), followed by worker (0.15g) and queen (0.11g) (Table 3).

Prepupa

The duration of this stage varied among the caste, drone showed the longest development time span was recorded in drone (7 days) and followed by worker (5 days) and queen (4 days), respectively (Table 1). The last larval stage was referred to as the prepupal stage, since the final larval molt included a metamorphosis into the pupae (Plate 2). The last few days of larval life were spent constructing a cocoon within the cell. To spin the cocoon, the larvae uncurled and stretched out fully in the cells with their heads toward the capped end. In prepupa stage, drone had the longest length (17 mm), followed by queen (16.9 mm) and worker (10.9 mm) (Table 2). The weight of the drone showed the highest value (0.36g), followed by queen (0.28g) and worker (0.18g) (Table 3).

Pupa

This stage lasted 7 days for worker, 6 days for drone and 3 days for queen (Table 1). The pupal stage is the last period before the final molt to the adult. The head, eyes, antennae, mouthparts, legs, thorax and abdomen showed adult characteristics, but only the wings were still small and undeveloped. As the pupa developed, coloration of eyes gradually changed from light pink to dark pink, purple and finally dark color. The color of body also changed gradually from white to dark color (Plate2). In pupa stage, the longest length was recorded in drone (16.5 mm), followed by queen (15.7 mm) and worker (12.2 mm) (Table 2). The weights of pupae were found to be varied among the casts as queen (0.28g), drone (0.26g) and worker (0.22g) (Table 3).

Adult

After complete metamorphosis, adult emerged from its cell by chewing the capped cell with its mouthparts. The duration of emergence from the cell varied among the castes: drone showed longest duration (24days), followed by worker (21days), and queen (16 days) (Table 1). A newly emerged honeybee was pale in color. In adult stage, queen had the longest length as 17.6 mm, followed by that of 16.8 mm in drone and 12.2 mm in worker (Table 2). The weights of the bees among the different casts were recorded as drone (0.29g), queen (0.26g) and worker (0.22g), respectively (Table 3).

Table 1 Duration of the developmental stages of three casts of *Apis mellifera*

Cast	Egg hatched (days)	Larva stage (days)	Prepupa/ capped stage (days)	Pupa stage (days)	Developmental period (Days until emergence)
Queen	3	5	4	3	16
Worker	3	5	5	7	21
Drone	3	7	7	6	24

Table 2 Maximum length of developmental stages of honeybee

Cast	Open Stage		Seal Stage		Adult (mm)
	Egg (mm)	Larva (mm)	Prepupa (mm)	Pupa (mm)	
Queen	1.5±0.00	15.0±0.35	16.9±0.55	15.7±0.27	17.6±1.52
Worker	1.5±0.00	9.6±0.46	10.9±0.53	12.2±0.34	12.2±0.54
Drone	1.5±0.00	15.0±0.5	17.0±0.35	16.5±0.35	16.8±0.27

Table 3 Maximum weight of developmental stages of honeybee

Cast	Open Stage		Seal Stage		Adult (g)
	Egg (g)	Larva (g)	Prepupa (g)	Pupa (g)	
Queen	0.001±0.00	0.11±0.01	0.28±0.01	0.28±0.01	0.26±0.01
Worker	0.001±0.00	0.15±0.01	0.18±0.01	0.22±0.02	0.22±0.01
Drone	0.001±0.00	0.28±0.01	0.36±0.01	0.26±0.01	0.29±0.01

Discussion

Beekeeping of honeybee species *Apis mellifera* involved two methods, the stationary and migratory methods applied based on the availability of food sources (Cramp, 2008). Since the study area is situated in the central dry zone of Myanmar, the annual rainfall is low and growing of plants and flowering times are varying season to season. Therefore, beehives are not impossible to maintain constantly in only one place, Pakokku University Campus, because food sources for the bees were not enough throughout the year. As a result, beehives were move to Pauk Kone Village where seasonal crops were abundance as the food sources of honeybee. Cramp (2008) stated that when the food sources were rare in beekeeping place, the bees can be kept by giving them supplementary feeding without migrating to other places for food sources, otherwise moving them to food abundance places. The disadvantages of supplementary feeding are more expenditure and extra work for beekeeper, unhealthy of honeybee in long term feeding. Therefore, migratory beekeeping method was more suitable in the study area instead of stationary method. This migratory method is also commonly used by commercial beekeepers in the study area. Thus, migratory method was more promising than stationary method for beekeeping practice. Moreover, migratory beekeeping practices can increase dual incomes from the production of bee products, especially honey, and also in enhancing the crop yield by pollination of honey bees. Hence, there is a need to implement relevant beekeeping method for successful honey yield and crop yield as well.

According to Sharma, *et al*. (2013), migratory beekeeping are able to harvest 50-60 kg of honey/colony/year which is about five times more than obtained with stationary beekeeping. In addition, bee colonies could increase by at least 20% and save the maintenance of cost during dearth period by migratory beekeeping. This was confirmed with the present findings that migratory beekeeping was more profitable than stationary beekeeping for commercial beekeepers.

During the study, the duration of life cycle from egg to emerging bee varied to the caste, the queen emerge in 15-16 days, workers in 21 days and drones in 24 days. Stone, (2005) stated that honeybee exhibits a complete development or metamorphosis during their life including life stages as egg, larva, pupa and adult and development from egg to adult in general takes two to three weeks.

Among the casts in the experimental beehives of the present study, the queen (fertile female) had the higher length than drone (male) and worker (sterile female). This finding was in coincidence with the finding of Kilani (1999) that the queen was higher measurements than drone and worker due to having long and heavy abdomen of the queen.

The developmental stages of honeybees in the present study indicated that the duration of each developmental stage varied among the different casts in the same hive. The larval stage lasted 5 days for queen, 5 days for worker and 7 days for drone respectively. Tribe and Fletcher (1977) mentioned that the duration of the larval period was about 4.5 days for queen, 5.5 days for worker and 6.5 days for drone. This larval period was slightly different with the present data. It was assumed that the casts, genetic and environmental factors are depending to the duration of metamorphosis as stated by the previous authors (Tribe and Fletcher, 1977; Winston, 1992).

Fasasi *et al.* (2011) stated that measurement of adult drone is 19.7±0.2 mm, that of queen is 18.3±0.5 mm and that measurement of worker is 17.0±1.3 mm. In the present study, the length of drone was 16.8±0.27 mm, followed by that of queen was 17.6±1.52 mm and worker was 12.2±0.54 mm respectively. The size of all casts of honeybee in the present study was smaller than the bee recorded of Fasasi *et al*. (2011). It was assumed that the sizes of honeybee depend on the sufficient of food sources and environmental factors.

In this study, mean temperature, relative humidity and rainfall recorded were as low as 26.28°C, 51.08% and 0.2 mm and as high as 31.45°C, 80.55% and 9.65 mm respectively. No significant changes of the developmental period and measurement of life stages of *A. mellifera* were observed during the study period. Therefore, the result of this work showed that weather parameters had no effects on the life cycle of *A. mellifera*.

Therefore, the data recorded in the present work contribute the information to further researches concerned with beekeeping and ontogenetic development of the honeybee and other related researches.

Conclusion

It was concluded that *Apis mellifera* had been preferred for beekeeping which can provide dual income from the production of bee products and also enhancing the crop yield by pollination. Moreover, understanding of life stages of *Apis mellifera* may help the proper management of honeybee colonies to get more bee products and good quality of the crop. Therefore, beekeeping could create the good opportunities of job for livelihood of rural people.

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ANTIMICROBIAL ACTIVITIES OF LACTIC ACID BACTERIA FROM GUT OF *CATLA CATLA* (HAMILTON, 1822)

Win War War Kyaw[1], Mu Mu Aung Soe[2] and Kyaw Myo Naing[2]

Abstract

This research aims at assessing and determining the antimicrobial activity of Lactic acid bacteria (LAB) from the gut of *Catla catla* (Catla). Samples of fish were collected from fishery pond near Hinthada Township, Ayeyarwaddy Region. Isolation of LAB was undertaken by serial dilution method on deMan Rogosa Sharpe (MRS) medium, supplemented with 0.5% $CaCO_3$. They were identified based on colony morphology, cell morphology, gram staining, acid fast staining, MR, VP, indole, catalase and motility tests. Total of five strains were isolated. Most isolated strains were gram positive, catalase negative and non- motile, and all were non-acid fast. Colony forming unit (CFU/mL) of isolated bacteria were counted by spread plate technique. CFU were ranging from $24x10^8$ to $30x10^{10}$ CFU/mL. Isolated bacteria were also tested for antimicrobial activities using paper diffusion assay against *Saccharomyces cerevisiae, Argobacterium tumefaciens, Pseudomonas fluorescens* and *Escherichia coli*. The diameters of the inhibition zones were ranged from 8.4 to 14.7 mm. Out of five isolates, two strains (Cc1 and Cc2) showed antimicrobial activity against four test organism and inhibition zones were significantly higher ($p< 0.05$) than that of other isolates. These strains demonstrated high antimicrobial properties and could be used as starters for probiotic in fishery pond feeding.

Keywords: Lactic acid bacteria, Antimicrobial activities, *Catla catla*

Introduction

Lactic acid bacteria produce many kinds of metabolites which might affect the other microbes in the gut. Lactic acid bacteria (LAB) are the most common types of microbes used as probiotics which are safely applied in medical and veterinary function (Divakara *et al.*, 2010). Many lactic acid bacteria (LAB) are proved to function as probiotics, which are benefit to host health, when ingested in sufficient quantities.

The beneficial role played by this microorganism in humans and animals, including effect on the immune system has been extensively reported (Perdigon *et al.*, 1992). The Lactic acid bacteria are present in the intestine of most animals. The colonization of the gut by probiotic bacteria prevents growth of harmful bacteria by competition exclusion and by the production of organic acid and antimicrobial compounds (Buntin *et al.*, 2008).

Lactic acid bacteria were not dominant population in fish, it has been well documented in several investigations that lactic acid bacteria are a part of the native microbiota of aquatic animals from temperature regions (Ringø, 2004). Lactic acid bacteria (LAB) are a group of gram positive, cocci or rods, catalase negative and fastidious organisms. Lactic acid bacteria have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (O'sullivan *et al.*, 2002).

Yasuds and Taga (1980) suggested that probiotic bacteria would be found to be useful not only as food but also biological controllers of fish disease and activators of nutrient regeneration. The optimization of the use of probiotic *Lactobacilli* for the gastrointestinal disorders requires the knowledge of their antibiotic resistance to reinforce the concomitant antibiotic therapy (Salminen *et al.*, 1998).

[1] Assistant Lecturer, Department of Zoology, Hinthada University
[2] Lecturers, Department of Zoology, Pathein University

Probiotic bacteria would be found to be useful not only as food but also serves as biological controllers of fish disease and activators of nutrient regeneration. Nowadays the focus is not merely on bacteria collected from fermented dairy products but also bacteria collected from the intestine (Sanders, 1999). Fish intestinal tract is considered to be valuable waste and a good source for LAB isolation. LABs are a group of Gram-positive, non-motile, cocci or rods, catalase negative. Various authors have shown that lactic acid bacteria are also part of the normal intestinal flora of fish (Ringø *et al*, 1998).

There are increased understanding of probiotics use of LAB would lead to the development of natural antibiotic and reduce the dependency on chemical or drug uses in aquaculture (Subasinghe, 1997). In addition to the numerous investigations demonstrating the presence of LAB in the digest tract of several different fish species several studies have reported on the isolation of LAB from cold-smoked and fermented fish. Based on that this research was conducted to obtain Lactic acid bacteria, and then to select and characterize its ability.

In the future, lactic acid bacteria can be used as a probiotics to support the feeding of fishes. Keeping this in view, the present study was undertaken with the following objectives: to isolate the lactic acid bacteria from gut of *Catla catla* (Nga gaung pwa), to study the colony morphology of isolated LAB and investigate the cell characters and staining reactions of isolated bacteria species and to investigate the antimicrobial activities of isolated lactic acid bacteria from fish gut.

Materials and Methods

Study Period

This research was conducted at the laboratory of Zoology Department, Pathein University during July to November, 2019.

Collection of samples

Fish sample of *Catla catla* was collected from the fishery pond near Hinthada Township, Ayeyarwady Region (Site-I, 17°41′ 46″ N and 95°25′ 37″ E) and (Site -II, 17°41′ 46″ N and 95°25′ 38″ E) (Fig. 1). Fish samples were put into sterilized polythene bag and carried to the laboratory of Zoology Department, Pathein University by using ice-box. Samples were stored in the refrigerator at 4°C for further study.

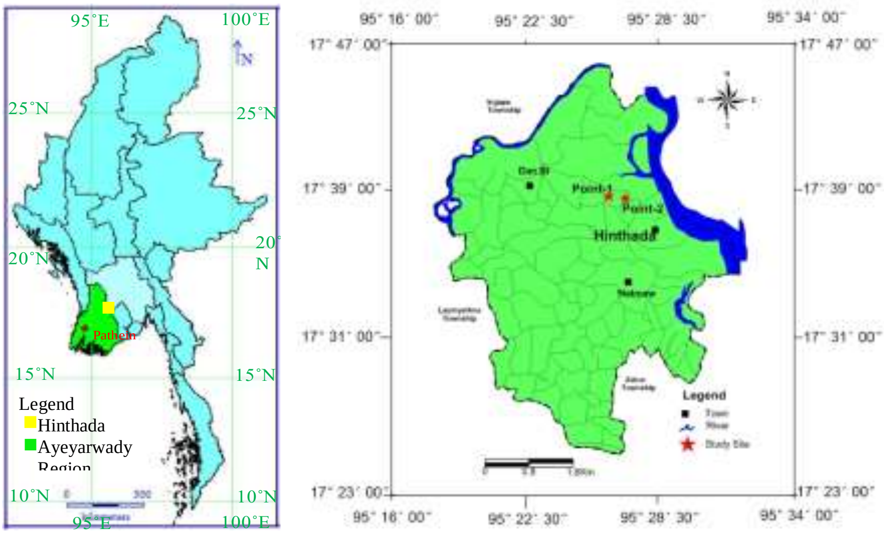


Figure 1 Map of sample collected area (Source: DIVA-

Culture of bacteria

The length and weight of the fish were measured before dissection. The skin was washed with 70% ethanol before opening the ventral surface with sterile scissors. The fish were dissected to remove the gut, one gram of intestine was taken from each fish sample.

Isolation of pure culture

Then the gut surface was sterilized with ethanol and washed with distilled water for three time and cut into small pieces with sterilized scissors. These pieces were mixed 9 mL of sterile saline diluent (0.85% NaCl). This mixture was shaking agitatedly. Then serially diluted to 10 fold dilutions and plated on deMan Rogosa Sharpe (MRS) media (Himedia, India) containing 0.5% $CaCO_3$. Then, 20 µL of bacterial suspensions from each dilution was inoculated onto MRS agar and incubated at 31℃ for three days. Clear zone forming colonies on MRS agar were selected as Lactic acid bacteria. Streak plate method was used to purify these selected bacteria.

For pure culture from plate to test tube, about 100mL of culture medium were separately distributed into test tubes. The test tube were plugged with cotton wool and sterilized by autoclaving at 1.05 kg per cm^2 (15 lb per in^2) for 15 minutes at 121℃. The sterilized media were cooled down. Each of separate colonies on petridishes was taken out to streak on the slant medium to obtain pure culture (Atlas, 1997).

Study of morphology

Colony and cells characteristics were studied that is size, shape, colour, opacity, elevation, margin, morphology, gram staining, acid fast staining nature.

Gram staining

A drop of normal saline was placed on clean grease free slide. A small loop of isolated bacteria was smeared on the slide and allows it to dry. The smear was fixed by passing the dried slide three or four times rapidly over a flame. Covered the slide with crystal violet stain and allow it to act for one minute. Then, the slide was rinsed with distilled water for a few seconds. The slide was covered with fresh iodine solution and allowed it to act for about one minute. Add the alcohol drop by drop and stop adding alcohol when no more colour flows out from the smear. As a counter stain, the smear was covered with safranin for about 20-30 seconds and washed with distilled water. Then the slide was dried. The stained slide was examined under the oil immersion objective of the microscope.

Acid fast staining

Bacterial was smeared on clean and grease free slide. Allow smear to air dry and then heat fix. A generous amount of carbol fuchsin stain was applied over the smear. Heat the stain until vapour. Allow the heated stain to remain on the slide for 5 minutes. After five minutes, the slide was cooled and gently washed with distilled water. Acid alcohol was used to decolorize the stain and then methylene blue was used to flood the smear for 2 minutes and air dry. Then, observed under oil immersion lens.

Catalase test

Two to three drops of 3% H_2O_2 were placed on a clean dry glass slide. A bacterial colony was picked using a clean sterile straight wire loop and mixed into the 3% H_2O_2. Production of bubbles in 5-10 second is a positive test. No appearance of bubbles or appearance after 30 seconds is considered negative.

Motility

Motility of the isolated bacteria can be detected in semi-solid agar medium. Ten milliliter of semi-solid agar was dispensed in test tubes. The tubes containing the medium were inoculated by stabbing with straight wire to about half the depth of the medium. After incubation, motile bacteria will spread into the medium and non- motile will confine to the stab.

Biochemical characterization

Methyl red test, Voges-Proskauer test and Indole test were performed.

Methyl red test

Sterilized MR broth was inoculated by a loopful of pure bacterial isolate and incubated at 32℃ for 24 hour. 5mL broth cultured was aseptically transferred into test tube and three drops of methyl red was added. Red colour formation within 15minutes is a positive result. No red colour formation after 15 minutes is a negative result.

Voges-Proskauer test

Sterilized VP broth was inoculated by a loopful of pure bacterial isolate and incubated at 32℃ for 24 hour. The broth cultured 5mL was dispensed into test tube to perform Voges-Proskauer test. Three drops of Barritt's reagent A was added to broth cultured and shaken slightly. And then equal amount of Barritt's reagent B was added into broth cultured. The test tube was kept still for 15 minutes. Appearance of red colour on the reagent layer signified positive. No change colour is negative.

Indole test

Test tubes containing 5mL of sterilized tryptone broth were inoculated with isolated bacteria. After 24h incubation, three drops of Kovac's reagent was added. A positive result is shown by presence of red colour in the surface alcohol layer of the broth. A negative result appears yellow.

Enumeration of lactic acid bacteria by standard plate count method

All isolated bacteria species associated from fish gut of *Catla catla* were enumerated. The isolated of lactic acid bacteria from slant cultures were placed and grow in peptone water about 24h and then streaked on de Man Rogosa Sharpe (MRS) agar medium plates. After growing, few bacteria are picked out with sterile loop and inoculated into the test tubes containing of peptone water. These tubes were incubated at 32℃ for 24h. Ten fold dilution were then prepared with sterile distilled water and 20μL of each dilution was spread on the surface of plate count agar with three replicates. The agar plates were incubated at 32℃ for 24h. After incubation the number of colonies was counted and the broth culture of viable cell per milliliter was calculated as suggested by Reynolds and Perez-Ramos (2009).

$$\text{Colony forming unit per milliter or gram} = \frac{\text{Number of colonies}}{\text{Dilution factor x amount plated}}$$

Antimicrobial assay

Antagonistic assay was done on assay medium. *Saccharomyces cerevisiae* NITE52847, *Agrobacterium tumefaciens* IFO5431, *Pseudomonas fluorescens* IFO94307 and *Escherichia coli* AHU5436 were sub cultured in nutrient agar medium. These test organisms were supported by

NITE Japan in 2004 and maintained at PBDC, Pathein University. Each test organism culture was suspended in peptone water. 0.2 mL suspension of test organisms was cultured on assay medium.

Isolated LAB strains were inoculated into 10 mL of seed medium at 32℃ for 3 days. One mL of seed medium culture was inoculated into fermentation medium at 32℃ for 3 days. And then sterilized paper discs were dipped into each LAB broth cultured (10^8 CFU/mL) of fermentation medium. And then paper discs were dried in the Biosafety cabinet for three hours and these discs were placed on assay medium with test organism. Control paper discs were also dipped into fermentation medium and placed on assay medium. Tests and control plates were incubated at 32℃ for 3 days and then measured the inhibition zone by using digital callipers. Five replicate were carried out to test the antimicrobial activity of isolated bacteria.

Identification of Bacteria and Plants

Bacterial species identification was followed after Breed *et al.,* (1957) Buchanan, Gibbons (1974) and Holt *et al*. (1994). Identification of the fish species was made according to Jayaram (2013).

Statistical analysis

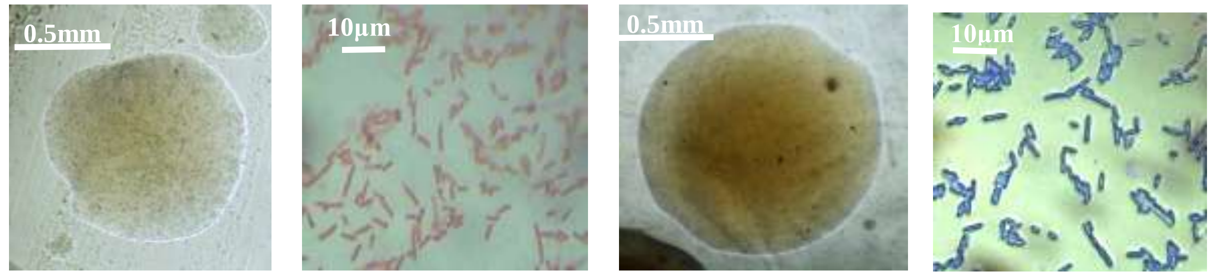
The antimicrobial activities were subjected to analysis of variance and means of sample were compared by least significance difference (LSD) using IBM-SPSS software (version 25).

Results

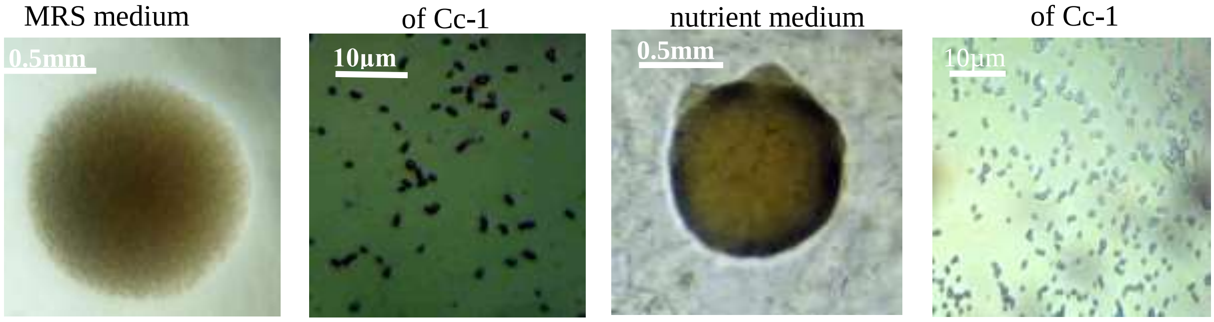
Totally five strains of lactic acid bacteria were isolated from the gut of *Catla catla*. Isolated strains from fish gut of *Catla catla* were designated as Cc-1 to 5. Most isolated strains were gram positive, catalase negative, non- motile, rod and cocci bacteria and all were non-acid fast. Methyl test, Voges-Proskauer test and Indole test were performed and all LAB strains showed negative in these tests (Table 1 and 2). Colony forming units of Lactic acid bacteria were ranging from 24.38 x 10^8 to 30.31 x 10^{10} CFU/mL. Lactic acid bacteria from gut of *Catla catla* Cc-1 (30.31 x 10^{10} CFU/mL), Cc-2 (32.81 x 10^9 CFU/mL) and Cc-3 (24.38 x 10^8 CFU/mL) were the good growth rate strains. The antimicrobial activity of LAB was tested against four test organisms by using paper disc diffusion method. The result showed that LAB inhibited *Saccharomyces cerevisiae, Agrobacterium tumefaciens*, *Pseudomonas fluoresence*, and *Escherichia coli*. The diameters of the inhibition zones were varied and ranged between 8.4 to 14.7 mm.

Lactic acid bacteria from gut of *Catla catla*

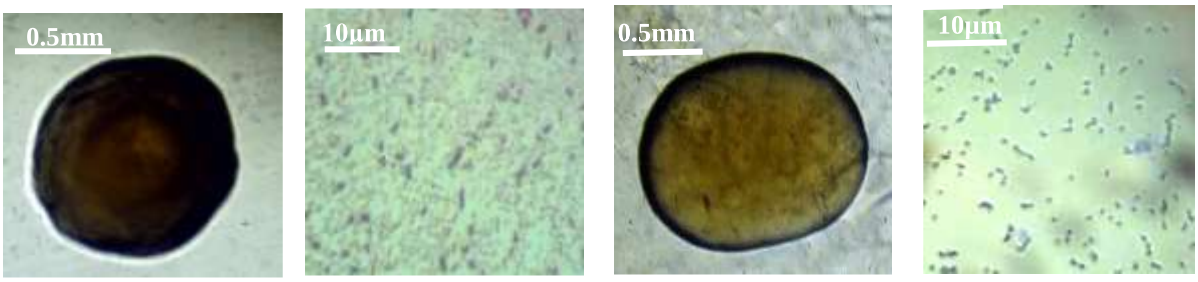
The colony morphology of Cc-1 was circular, Creamy-White, entire and convex. The single colony on MRS medium was 1.0-1.6 mm in diameter and on nutrient was 1.0-1.6 mm in diameter. Cells were rod shape with 2.25-3.6 µm in width, gram positive, non-acid fast, singly and pair, non-motile and catalase negative. The colony morphology of Cc-2 was circular, yellow, entire and convex. The single colony on MRS medium was 1.3-1.9 mm in diameter and on nutrient was 1.0-1.4 mm in diameter. Cells were rod shape with 1.8-2.7 µm in width, gram positive, non-acid fast, singly and pair, non-motile, and catalase negative. The colony morphology of Cc-3 was circular, yellow, entire and convex. The single colony on MRS medium was 1.0-1.6 mm in diameter and on nutrient was 1.0-1.4 mm in diameter. Cells were short rod shape with 1.8-2.7 µm in width, gram positive, non-acid fast, singly and pair, non-motile, and catalase negative. The colony morphology of Cc-4 was circular, yellow, entire and convex. The single colony on MRS medium was 1.0-1.5 mm in diameter and on nutrient was 1.0-1.5 mm in diameter. Cells were rod shape with 2.25 -3.6 µm in width, gram negative, non-acid fast, singly and pair, slightly motile and catalase positive. The colony morphology of Cc-5 was circular, yellow, entire and flat. The single colony on MRS medium was 1.0-1.4 mm in diameter and on nutrient was 1.0-1.5 mm in diameter. Cells were cocci shape with 1.8-2.7 µm in diameter, gram positive, non-acid fast, singly and chain, non-motile, and catalase negative (Fig. 2).



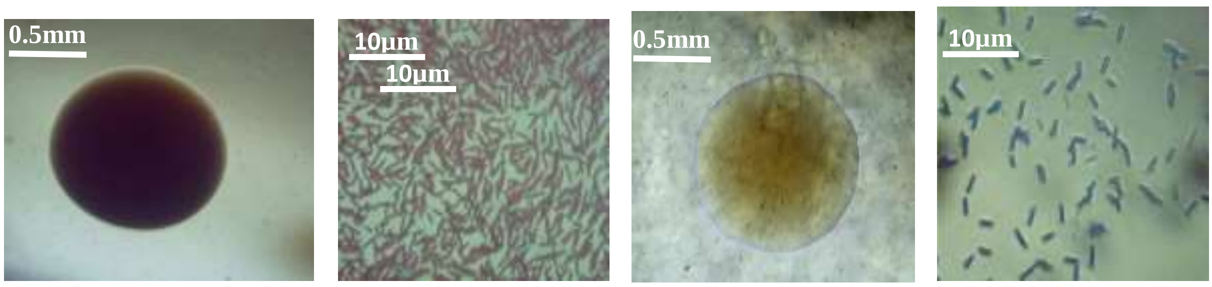
A. Colony of Cc-1 on MRS medium | B. Gram staining of Cc-1 | C. Colony of Cc-1 nutrient medium | D. Acid fact staining of Cc-1



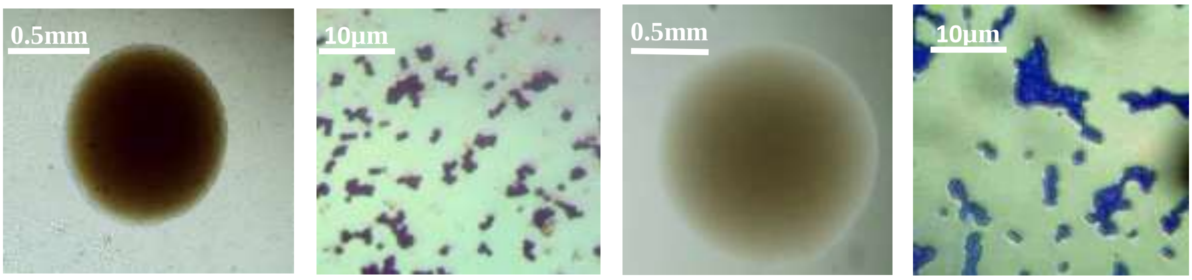
A. Colony of Cc-2 on MRS medium | B. Gram staining of Cc-2 | C. Colony of Cc-2 on nutrient medium | D. Acid fact staining of Cc-2



A. Colony of Cc-3 on MRS medium | B. Gram staining of Cc-3 | C. Colony of Cc-3 on nutrient medium | D. Acid fact staining of Cc-3



A. Colony of Cc-4 on MRS medium | B. Gram staining of Cc-4 | C. Colony of Cc-4 on nutrient medium | D. Acid fact staining of Cc-4

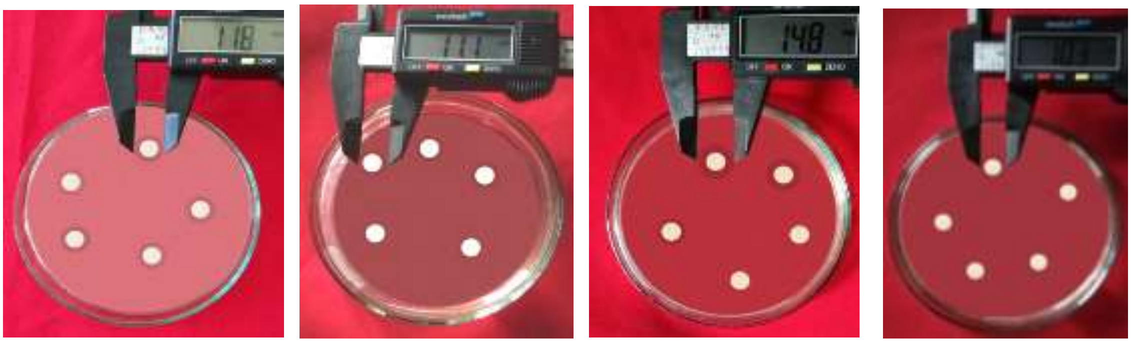


A. Colony of Cc-5 on MRS medium | B. Gram staining of Cc-5 | C. Colony of Cc-5 on nutrient medium | D. Acid fact staining of Cc-5

Figure 2 Colony and cell morphology of isolated bacteria

Antimicrobial activities of isolated lactic acid bacteria

The antimicrobial activity of LAB was tested against four test organisms by using paper disc diffusion method. The result showed that LAB inhibited *Saccharomyces cerevisiae, Agrobacterium tumefaciens, Pseudomonas fluoresence*, and *Escherichia coli*. For *S. cerevisiae* (11.44 mm, 12.38 mm, 10.8 mm, 0 mm and 10.94 mm respectively), for *A. tumefaciens* (12.06 mm, 13.72 mm, 0 mm, 10.16 and 0 mm respectively), for *P. fluoresence* (13.6 mm, 13.78 mm, 11.22 mm, 8.4 mm and 8.4 mm respectively), and for *E. coli* (10.3 mm, 14.7 mm, 10.84 mm, 0 mm and 8.4 mm respectively) (Fig. 3). Inhibition zone of isolated strains of LAB against test organisms were significantly different ($p < 0.05$) from each other. Cc-2 showed the highest zone of inhibition against four test organisms and significantly inhibited ($p<0.05$) test organisms than other LAB strains. Cc-1 showed second largest zone and significantly inhibited ($p<0.05$) the test organisms than Cc-3, Cc-4 and Cc-5. Cc-3 can inhibit only three test organisms while Cc-4 and Cc-5 can against only one test organism (Table 3, 4 and Fig. 4).



(i) *S. cerevisiae* | (ii) *A. tumefaciens* | (iii) *P. fluorescens* | (iv) *E. coli*

A. Antimicrobial activity of *Cc1* against four test organisms

(i) *S. cerevisiae* | (ii) *A. tumefaciens* | (iii) *P. fluorescens* | (iv) *E. coli*

B. Antimicrobial activity of *Cc2* against four test organisms

Figure 2 Antimicrobial activity of isolated bacteria against four test organisms

Table 1 Colony morphology of isolated LAB strains

LAB strains	Size (mm)	Shape	Colour	Margin	Elevation
Cc-1	1.0-1.6	Circular	Creamy-White	Entire	Convex
Cc-2	1.3-1.9	Circular	Yellow	Entire	Convex
Cc-3	1.0-1.6	Circular	Yellow	Entire	Convex
Cc-4	1.0-1.5	Circular	Yellow	Entire	Convex
Cc-5	1.0-1.4	Circular	Yellow	Entire	Flat

Table 2 Cells morphology, staining reactions and motility test of isolated LAB strains

LAB Strains	Cell size (µm)	Shape	Arrangement	Gram reaction	Acid fast reaction	Motility	Catalase test
Cc-1	2.25-3.6	Rod	Singly and pair	Positive	Non-acid fast	Non motile	Negative
Cc-2	1.8-2.7	Rod	Singly and pair	Positive	Non-acid fast	Non motile	Negative
Cc-3	1.8-2.7	Short rod	Singly and pair	Positive	Non-acid fast	Non motile	Negative
Cc-4	2.25-3.6	Rod	Singly and pair	Negative	Non-acid fast	Slightly motile	Positive
Cc-5	1.8-2.7	Cocci	Singly and chain	Positive	Non-acid fast	Non motile	Negative

Table 3 Analysis of antimicrobial activities of isolated LAB strains

		Sum of Squares	df	Mean Square	F	Sig.
S. cerevisiae	Between Groups	526.594	4	131.649	898.012	.000
	Within Groups	2.932	20	.147		
	Total	529.526	24			
A. tumefaciens	Between Groups	892.854	4	223.214	681.360	.000
	Within Groups	6.552	20	.328		
	Total	899.406	24			
P. fluorescens	Between Groups	140.124	4	35.031	63.600	.000
	Within Groups	11.016	20	.551		
	Total	151.140	24			
E. coli	Between Groups	593.314	4	148.329	879.766	.000
	Within Groups	3.372	20	.169		
	Total	596.686	24			

Table 4 Comparison of antimicrobial activities of isolated LAB strains (n=5)

LAB Strains	Inhibition Zone against Test Organisms (mean ± SD mm)			
	S. cerevisiae	***A. tumefaciens***	***P. fluorescens***	***E. coli***
Cc1	11.44 ± 0.38^{b}	12.06 ± 0.63^{b}	13.6 ± 0.90^{b}	10.3 ± 0.19^{a}
Cc2	12.38 ± 0.36^{c}	13.72 ± 1.10^{c}	13.78 ± 1.13^{b}	14.7 ± 0.53^{b}
Cc3	10.8 ± 0.52^{a}	-	11.22 ± 0.27^{a}	10.84 ± 0.48^{a}
Cc4	-	10.16 ± 0.15	+	-
Cc5	10.94 ± 0.44^{a}	-	+	+

+ = inhibition zone <10, - = no inhibition zone, Paper disc = 7mm

Means with different superscript within same column were significantly different at $p < 0.05$

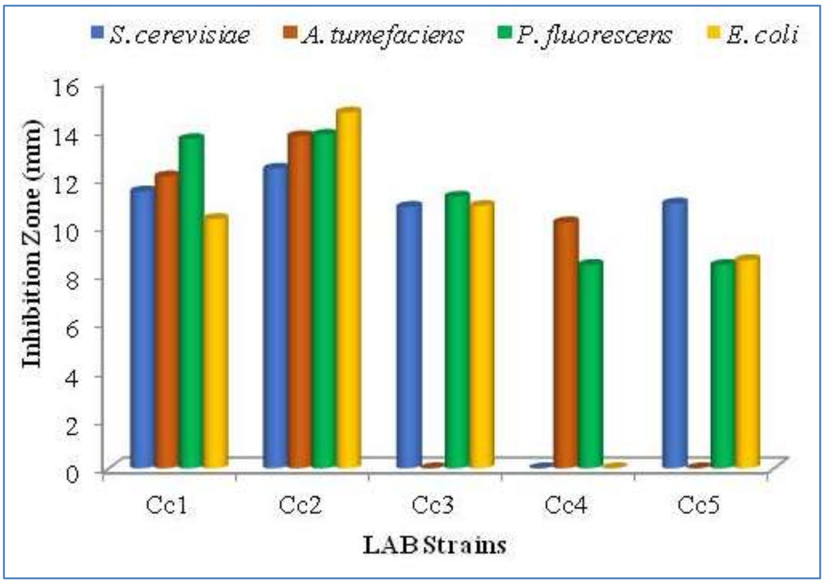


Figure 3 Antimicrobial activity of LAB strains against test organisms

Discussion

In this research, LAB strains were isolated by the formation of clear zone on the MRS medium. The clear zone appearance is due to the dissolution of $CaCO_3$ on MRS medium by acid agent (Panthavee *et al*., 2007). The clear zone around colonies indicated that the bacteria were able to produce acid substances. *Bacillus sp*. as non-lactic acid bacteria was competent in producing acids (Prihanto *et al*., 2020). These selected isolates have similar characteristic which are gram positive bacteria, rod shaped, catalase negative, non-motile. These results were also in line with the finding of Lawalata *et al*. (2011) who reported that the LAB isolates are gram positive, rod shape, catalase negative and non-motile. LAB colonies were circular, flat, low convex with entire margin. All member of lactic acid bacteria were gram positive rods or cocci found in chain, singly and in pairs (Bukola and Onilude, 2008). The nature of lactic acid bacteria was positive in gram character, non-motile, and negative calalyze (Axelsson, 2004).

They can live singly in pairs or irregular cluster and sometimes in chains of varying length. Isolated bacteria in this work are similar with the above statements for lactic acid bacteria. Five strains were isolated of which micromorphologically one was coccus, four were bacilli. Jack *et al*. (1995) reported that the isolated LAB species conformed by the methyl red and catalyzed negative test. In this work isolation of lactic acid bacteria are methyl red and catalyzed negative test. Reynolds and Perez-Ramos (2009) used standard plate count method by spreading 100µL of a given dilution on the entire surface of nutrient agar plates. Only the dilutions producing 30 to 300 colony forming units were enumerated. In this study counting methods were the same above work. From the five isolates Cc-1 (30.31 $x10^{10}$ CFU/mL), Cc-2(32.81 x 10^{9} CFU/mL) and Cc-3 (24.38 x 10^{8} CFU/mL) were the best grown bacteria.

Davidson and Paris (1989) screened the antimicrobial activity of LAB from fish aganist *Escherichia coli* ATCC 35218, *Stapylococcus aureus* ATCC 25923, *Pseudomonas fluorescens* FNCC 0070 was performed. Five isolates of Lactic acid bacteria in this work were tested for the antimicrobial activity of LAB aganist *Saccharomyces cerevisiae, Agrobacterium tumefaciens, Pseudomonas fluorescens* and *Escherichia coli.*

Kim and Austin (2008) reported that antibiotic sensitivity test was carried out in aquaculture by disc diffusion technique. The plates were incubated at 32°C for 24 hour to observe and measure the inhibition zone. Hamid *et al*. (2012) reported that the antagonistic activity of the isolated LAB against *Salmonella typhimrium* and *Escherichia coli* were determined by using agar disc diffusion.

In this research antibiotic sensitivity test was carried out by using disc diffusion method and bacteria were incubated at 32℃ for 24 hours. The diameters of the inhibition zones of isolated bacteria were varied and ranged between 8.4 to 14.7 mm. This result was very close to the results of Lawalata *et al.* (2011) who studied the antimicrobial activity of LAB from fish and they stated that the diameters of the inhibition zones were ranged between 3.0 mm to 15.0 mm.

From the five isolates, two bacterial isolates Cc-1 and Cc-2 were the best LAB against on four test organisms; *S. cerevisiae, A. tumefaciens, P. fluorescens* and *E. coli*, and inhibition zones were significantly higher ($p< 0.05$) than other isolated LAB strains.

The report of these findings is similar to the work reported by Rodríguez *et al*. (2012) who reported that LAB were effective against many pathogenic bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. The antagonistic activity of LAB metabolites against the spoilage bacteria also agrees with the findings of Vasiee *et al*. (2018).

The inhibitory effect caused by LAB strains can be considered that the LAB produced organic acids, especially lactic and acetic acids, exerting a strong inhibitory effect on Gram-negative and positive bacteria (Muruzović *et al*., 2018).

LAB strains are effective against a variety of bacterial pathogens and some food borne microorganisms; they can serve as alternative antimicrobial agents and food preservatives. This potential can be harnessed by the food and animal feeding industries on a large scale as bio-preservatives and probiotic instead of the chemical preservatives and antibiotic that we use which have some side effects (Adeyemo *et al*., 2018).

Conclusion

In conclusion, among five, two strains were also active more against *S. cerevisiae, A. tumefaciens, P. fluorescens* and *E. coli* than other strains. Cc-1 and Cc-2 showed maximum inhibition zone against all tested microorganisms and inhibition zones were significantly higher ($p< 0.05$) than that of other isolated LAB strains. Vine *et al.* (2004) reported that many studies on probiotics in aquaculture have used *in vitro* models of specific bacteria as antagonists of pathogens. Using the culture as probiotics could be expected to reduce antibiotics usage for animal feeding. Therefore, isolated bacteria have antimicrobial activity and they could be used as starter culture for antibiotic feed of animals.

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ASSEMBLAGES OF WATER BIRD COMMUNITIES IN SOME WETLANDS OF SHWE TANTIT VILLAGE ENVIRONS, PAKOKKU TOWNSHIP, MAGWAY REGION

Yadanar Myo[1], Thant Zin[2]

Abstract

Since water bird communities are often considered biological indicators of wetland ecosystems, assemblages of water birds in wetlands of Shwe Tantit village, Pakokku Township were investigated during August 2019 to July 2020. A total of 37 water bird species belonging to 28 genera, 11 families and seven orders were recorded. Of these, 18 species were migrants and 19 species were residents. During the study period, one near threatened species *Threskiornis melanocephalus* (Black-headed Ibis) was recorded. In the study area, Glossy Ibis and Eastern Cattle Egret revealed to be highest in abundance and percent composition in bird communities in the study area. The results of present study indicated the importance of wetlands in the study area for the conservation of water birds due to assemblages of different bird communities.

Keywords: water bird communities, wetlands, Shwe Tantit, Pakokku

Introduction

Waterbirds depend on wetlands for avariety of activities which include feeding, breeding, nesting and moulting. The highest number of water birds is often found in wetlands which have the greatest diversity of plant species and vegetation types or where there is permanent water. Wetlands are biologically very productive and provide feeding grounds for a diverse range of resident and migratory waterbirds (Balla, 1994).

Wetlands are critical for many waterbird species mainly due to their high primary productivity. Their productivity leads to a high availability of resources which in turn favours the coexistence of many species. (Brandolin and Blendinger, 2015)

Wetlands are the most productive and biologically diverse in the world but very fragile ecosystem. Wetlands and water birds are inseparable elements and thus form a rich array of water bird sommunities. (Grimmett and Inskipp, 2005)

Water birds are an important component of most of the wetland ecosystems as they occupy several trophic levels in the food web of wetland nutrient cycles. Activities of water birds are considered as indicator of quality of the wetland ecosystem and form the terminal links in many aquatic food chains and as a result they reflect changes originating in several different ecosystem components. (Hussein, 2018)

Pakokku is located in the dry zone of central Myanmar. The study area of Shwe Tantit village is situated about 12 km away from the northeast of Pakokku and located near the Ayeyawady River. The water sources in the wetlands of ShweTantit village environs are available throughout the year due to irrigation system. Many paddy fields are also abundant in the study area for performing different activities of water birds. This research investigated the assemblages of water birds in Shwe Tantit village environs to provide the base line information of species composition and abundance of water birds including both residents and migrants in the wetlands of Shwe Tantit village environs and to determine the assemblages of water bird communities in the study area.

[1] Dr, Lecturer, Zoology Department, Myingyan University
[2] Dr, Professor and Head, Zoology Department, University of Mandalay

Materials and Methods

Study area

Shwe Tantit village lies between 21° 20' 36" to 21° 21' 39" N and 95° 8' 1" to 95° 9' 10" E. The total area is about 2.79 square kilometers. The habitat of this study area is open habitat type and microhabitats occurred mostly as woody trees, paddy fields, bushy area, medium and tall trees, cultivated area, garden, wetland with submerged plant, flood plain, Kyi village Dam and some portions of Ayeyawady River (Fig. 1).

(**Source:** Google earth)

Figure 1 A map of Shwe Tantit village environs

Study period

The present study was conducted from August 2019 to July 2020.

Study design

In terms of monitoring birds, the transect method is used. Transect surveys are used to record a variety of birds. It is a simple method that provides a uniform way of counting birds over time or across locations. Five transects were drawn for bird surveys in the study area. Transect line 1 measured in the length of 1.55 km, line 2 in 1.08 km, line 3 in 1.16 km, line 4 in 1.74 km and line 5 in 0.80 km. Therefore, a total of 6.33 km length of transect route was used. Species and individual counts were made along the transect routes by on foot. Birds were viewed by a pair of binocular while traversing the transect routes. Birds watching were taken from 6:30 AM to 10:30 AM. In each occasion of bird watching, bird species and their individuals were recorded. Field marks such as streaks, bars, wings, eyes, tail and birds' behavior were noted down. The photos of birds were taken immediately after viewing the birds.

Identification and classification of species

The identification of birds was made by referring to taxonomic descriptions given by Smythies (2001), Robson (2015) and Robson (2016). Classification of birds was followed after Birdlife International (2015).

Status

Status of the birds such as migrant or resident was worked out based on the presence or absence of bird species in each month in the study area according to King and Dickinson (1995).

M = migrant (bird migrate from cold northern to warm southern temperature regions at a definite time of each year to avoid hazard winter)

R = resident (birds that spend throughout the year in one place but some species show local movement).

Results

Throughout the study period from August 2019 to July 2020, a total of 37 water bird species belonging to 28 genera, 11 families and seven orders were recorded in the study area. The water bird species recorded were categorized into 18 migrants and 19 residents. Based on IUCN (2019), *Threskiornis melanocephalus* (Black-headed Ibis) was considered as Near Threatened species and the rest of the species recorded were considered as least concern (Table 1, Plate 1).

During the study period, the order Pelecaniformes was represented with the largest number of species (13 species, 35.14%), followed by the order Charadriiformes (nine species, 24.32%), Gruiformes (eight species, 21.62%), Anseriformes (three species, 8.11%), Suliformes (two species, 5.41%) and Podicipediformes and Ciconiiformes (one species, 2.70% each). (Fig. 2)

The highest number of two families and 13 species were recorded under order Pelecaniformes, followed by order Charadriiformes containing four families and nine species, and Anseriformes, Podicipediformes, Ciconiiformes, Suliformes and Gruiformes containing one family each (Fig. 2).

A total number of 37 species and 2654 individuals were recorded in the study period. The highest number of 33 bird species was recorded in each month of January and February, followed by December (29 species) and November (25 species). With regard to the individuals, the highest total number was observed in February (177 individuals), followed by January (550 individuals), March (439 individuals) and December (413 individuals). Moreover, according to the monthly data, *Plegadis falcinellus* (Glossy Ibis) showed the highest number of individuals (215) in December representing the occurrence of the highest number of single species (Table 2).

The population percentage of water bird communities in the study area was the highest in *Plegadis falcinellus* (Glossy Ibis) (34.63 %), followed by *Bubulcus coromandus* (Eastern Cattle Egret) (26.26 %), *Egretta garzetta* (Little Egret) (5.50 %) and *Anastomus oscitans* (Asian Openbill) (5.16 %) (Table 2).

In the study area, three species of water birds such as *Plegadis falcinellus* (Glossy Ibis), *Bubulcus coromandus* (Eastern Cattle Egret) and *Egretta garzetta* (Little Egret) were found in every month of the study period. (Table 2)

Table 1 List of water bird species recorded in Shwe Tantit village environs, Pakokku Township during August 2019 to July 2020

Order	Family	Sr. No.	Species	Common Name	Local Name	Residential Status	IUCN (2019) Status
Anseriformes	Anatidae	1.	*Dendrocygna javanica* (Horfield, 1817)	Lesser Whistling-duck	Sitsale-gaung-me	R	LC
		2.	*Tadorna ferruginea* (Pallas, 1764)	Ruddy Shelduck	Hin-thar	M	LC
		3.	*Anas zonorhyncha* Swinhoe, 1866	Chinese Spot-billed Duck	Wun-be	M	LC
Podicipediformes	Podicipedidae	4.	*Tachybaptus ruficollis* (Pallas, 1764)	Little Grebe	Than-sae-moke	R	LC
Ciconiiformes	Ciconiidae	5.	*Anastomus oscitans* (Baddaert, 1783)	Asian Openbill	Khayu-toke	M	LC
Pelecaniformes	Threskiornithidae	6.	*Threskiornis melanocephalus* (Mourer chauvire & Moutou, 1987)	Black-headed Ibis	Khayu-soke-aphyu	M	NT
		7.	*Plegadis falcinellus* (Linnaeus, 1766)	Glossy Ibis	Khayu-soke	M	LC
	Ardeidae	8.	*Ixobrychus sinensis* (Gmelin,1789)	Yellow Bittern	Chone-toe-byaing	R	LC
		9.	*Ixobrychus cinnamomeus* (Gmelin,1789)	Cinnamon Bittern	Sat-byaing	R	LC
		10.	*Nycticorax nycticorax* (Linnaeus, 1758)	Black-crowned Night-heron	Sat-byaing	R	LC
		11.	*Ardeola grayii* (Sykes, 1832)	Indian Pond-heron	Byaing-auk	R	LC
		12.	*Ardeola bacchus* (Bonaparte, 1855)	Chinese Pond-heron	Byaing-auk	R	LC
		13.	*Bubulcus coromandus* (Linnaeus, 1758)	Eastern Cattle Egret	Kywe-kyaung-byaing	R	LC
		14.	*Ardea cinerea*Linnaeus, 1758	Grey Heron	Nga-hit-mwe	R	LC
		15.	*Ardea purpurea*Linnaeus, 1766	Purple Heron	Nga-hit	M	LC
		16.	*Ardea alba* (Linnaeus, 1758)	Great Egret	Byaing-ngan	R	LC
		17.	*Mesophoyx intermedia* (Wagler,1929)	Intermediate Egret	Tha-ya-wadi-byaing	R	LC
		18.	*Egretta garzetta* (Linnaeus, 1766)	Little Egret	Byaing	R	LC
Suliformes	Phalacrocoracidae	19.	*Phalacrocorax niger* (Vieillot, 1817)	Little Cormorant	Tin-kyi	R	LC
		20.	*Phalacrocorax carbo* (Linnaeus, 1758)	Great Cormorant	Tin-kyi	R	LC
Gruiformes	Rallidae	21.	*Gallirallus striatus* (Linnaeus 1766)	Slaty-breasted Rail	Ye-ngone	M	LC
		22.	*Amaurornis phoenicurus* (Pennant, 1769)	White-breasted Waterhen	Ye-kyet-yin-phyu	R	LC
		23.	*Porzana pusilla* (Pallas, 1776)	Baillon's Crake	Ye-ngone	M	LC
		24.	*Porzana fusca* (Linnaeus 1766)	Ruddy-breasted Crake	Ye-ngone	M	LC
		25.	*Gallicrex cinerea* (Gmelin,1789)	Watercock	Baung-don	R	LC
		26.	*Porphyrio poliocephalus* (Linnaeus, 1758)	Grey-headed Swamphen	Mal-nyo	M	LC
		27.	*Gallinula chloropus* (Linnaeus, 1758)	Common Moorhen	Ye-kyet	R	LC
		28.	*Fulica atra* Linnaeus, 1758	Common Coot	Ye-kyet-don	M	LC
Charadriiformes	Recurvirostridae	29.	*Himantopus himantopus* (Linnaeus, 1758)	Black-winged Stilt	Daung-lan-chae-thauk	R	LC
	Charadriidae	30.	*Vanellusc inereus* (Blyth, 1842)	Grey-headed Lapwing	Tit-ti-du	M	LC
		31.	*Vanellus indicus* (Boddaert, 1783)	Red-wattled Lapwing	Sit-ta-laing	R	LC
		32.	*Charadrius hiaticula* Linnaeus, 1758	Common ringed Plover	Ta-ling-kaung	M	LC
		33.	*Charadrius dubius* Scopoli, 1786	Little ringed Plover	Ta-ling-kaung	R	LC
	Rostratulidae	34.	*Rostratula benghalensis* (Linnaeus, 1758)	Greater Painted-spine	Sanaik	M	LC
	Scolopacidae	35.	*Gallinago gallinago* (Linnaeus, 1758)	Common Snipe	Sanaik	M	LC
		36.	*Tringa ochropus* Linnaeus, 1758	Green Sandpiper	Ye-nyaunt-kaung	M	LC
		37.	*Tringa stagnatilis* (Bechstein, 1803)	Marsh Sandpiper	Ye-nyaunt	M	LC

R = Resident M = Migrant LC = Least Concern NT = Near threatened

Table 2 Monthly abundance of water birds in Shwe Tantit village environs during August 2019 to July 2020

Sr. No.	Scientific Name	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Jul.	Total	Population percentage
1.	*Dendrocygna javanica*	0	6	3	1	5	1	8	6	0	0	0	0	30	1.13
2.	*Tadorna ferruginea*	0	0	0	0	12	2	1	0	0	0	0	0	5	0.57
3.	*Anas zonorhyncha*	3	6	1	0	2	3	0	0	0	0	0	5	20	0.75
4.	*Tachybaptus ruficollis*	0	1	2	2	4	2	4	4	2	0	2	1	24	0.90
5.	*Anastomus oscitans*	0	2	4	10	24	29	35	14	4	8	5	2	137	5.16
6.	*Threskiornis melanocephalus*	2	0	0	5	7	10	5	7	0	0	0	3	39	1.47
7.	*Plegadis falcinellus*	30	35	30	42	215	150	200	150	35	7	5	20	919	34.63
8.	*Ixobrychus sinensis*	0	0	0	3	3	7	0	0	0	0	0	0	13	0.49
9.	*Ixobrychus cinnamomeus*	0	2	0	0	0	0	0	2	0	0	0	0	4	0.15
10.	*Nycticorax nycticorax*	0	0	0	0	0	0	2	0	0	0	0	0	2	0.08
11.	*Ardeola grayii*	6	5	10	11	9	8	8	4	0	0	0	7	68	2.56
12.	*Ardeola bacchus*	6	4	6	8	9	11	12	3	4	2	2	4	71	2.68
13.	*Bubulcus coromandus*	11	15	25	13	47	204	150	175	15	18	14	10	697	26.26
14.	*Ardea cinerea*	0	0	0	0	1	2	1	0	0	0	0	0	4	0.15
15.	*Ardea purpurea*	0	0	0	0	0	0	1	0	0	0	0	0	1	0.04
16.	*Ardea alba*	0	0	0	1	0	2	2	1	0	0	0	0	6	0.23
17.	*Mesophoyx intermedia*	0	5	0	4	6	5	4	4	0	0	0	0	28	1.06
18	*Egretta garzetta*	9	10	8	8	15	20	22	20	10	8	6	10	146	5.50
19.	*Phalacrocorax niger*	2	2	2	4	4	6	2	6	0	0	0	0	28	1.06
20.	*Phalacrocorax carbo*	0	0	0	0	0	0	2	0	0	0	0	0	2	0.08
21.	*Gallirallus striatus*	0	0	0	0	2	2	2	0	0	0	0	0	6	0.23
22.	*Amaurornis phoenicurus*	0	0	0	1	2	4	4	2	0	2	0	0	15	0.57
23.	*Porzana pusilla*	0	0	0	0	2	2	2	0	0	0	0	0	6	0.23
24.	*Porzana fusca*	0	0	0	0	1	2	3	0	0	0	0	0	6	0.23
25.	*Gallicrex cinerea*	0	0	0	2	3	2	2	1	0	0	0	0	10	0.38
26.	*Porphyrio poliocephalus*	0	0	0	0	0	2	0	0	0	0	0	0	2	0.08
27.	*Gallinula chloropus*	0	0	4	2	3	4	4	1	0	0	0	0	18	0.68
28.	*Fulica atra*	0	0	0	0	0	2	4	2	0	0	0	0	8	0.30
29.	*Himantopus himantopus*	7	3	8	6	4	26	40	20	3	0	0	2	129	4.48
30.	*Vanellus cinereus*	2	2	4	6	4	4	2	1	0	0	0	2	27	1.02
31.	*Vanellus indicus*	1	1	2	1	0	1	1	0	0	0	2	0	9	0.34
32.	*Charadrius hiaticula*	0	4	6	6	8	10	15	8	0	0	0	0	57	2.15
33.	*Charadrius dubius*	0	3	8	5	2	6	3	1	0	0	0	0	28	1.06
34.	*Rostratula benghalensis*	0	0	1	3	5	3	5	0	0	0	0	0	17	0.64
35.	*Gallinago gallinago*	0	0	1	1	1	2	3	0	0	0	0	0	8	0.30
36.	*Tringa ochropus*	1	1	2	3	5	6	10	5	0	0	0	2	35	1.32
37.	*Tringa stagnatilis*	0	0	2	3	8	10	4	2	0	0	0	0	29	1.09
	Total number of individuals	80	107	129	151	413	550	563	439	73	45	36	68	2654	
	Total number of species	12	18	20	25	29	33	33	23	7	6	7	12		

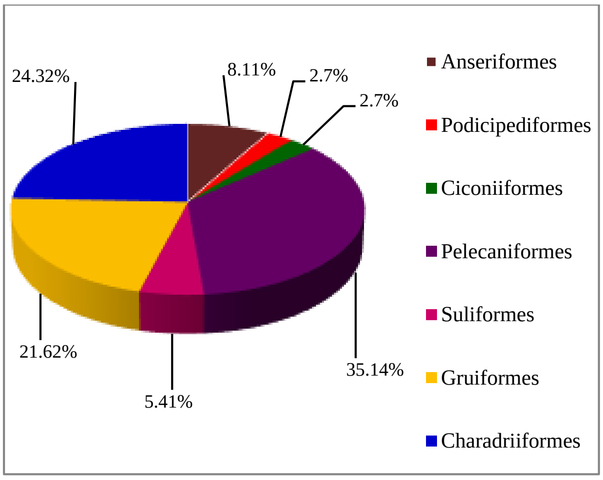

Figure 2 Percentage composition of water bird species in different orders in Shwe Tantit village environs

Discussion

Throughout the study period from August 2019 to July 2020, a total of 37 water bird species confined to 28 genera belonging to 11 families of seven orders were identified and recorded in some wetlands of Shwe Tantit village environs, Pakokku Township. Among them, 18 species were migrants and 19 species were residents. In this study, the highest number of 33 water bird species was found in each month of January and February. This may be due to complete arrival of winter migrants in addition to local residents.

Based on IUCN (2019), *Threskiornis melanocephalus* (Black-headed Ibis) is considered as near threatened and the remaining 36 species as least concern.

During the study period, three species of water birds were found in every month of the study period and these species may be considered very common and they are dominated species in the study area. It is evident that the area well supports the complete life stages of these water birds for the continuation of species existence.

Concerned with studies on the occurrence of bird species in different areas in Upper Myanmar undertaken by local researchers, Hla Toe (2012) stated nine species of migratory birds and 24 species of resident birds at Sunye In in Sintkaing Township, Mandalay Region; Nwet Nwet Win (2012) observed 13 species of migratory birds and 23 species of resident birds at Pauk In in Pakokku Township, Magway Region and Htay Khaing (2017) recorded 12 species of migratory birds and 19 species of resident at Inmagyi wetland in Myinmu Township, Sagaing Region. When comparison was made on the about mentioned studies from different areas, some species are common for all areas and some species are recorded in particular area. Variations in the number of abundance among different works may relate to the factors such as topography, habitat condition and availability of food sources, environmental conditions and habitat sensitivity of some bird species. In this work 18 species of migrants and 19 species of residents were recorded in ShweTantit Village environs of Pakokku Township.

In the study area, *Plegadis falcinellus* (Glossy Ibis) represented the highest population percentage, followed by *Bubulcus coromandus* (Eastern Cattle Egret), *Egretta garzetta* (Little Egret) and *Anastomus oscitans* (Asian Openbill). Therefore, these species are considered as predominant species in the study area. They utilized wetland areas, adjacent plantations and also human settlements for foraging, loafing and breeding purposes.

Bird species distribution and abundance are influenced by habitat structure. Habitat may be used for resources, survival, reproduction, foraging, cover, nesting, escape, denning or other life history traits. Thus, the conservation of natural habitats across the country is the most essential factor in maintaining bird populations.

The wetland habitats of Shwe Tantit village environs were inhabited with different life stages of bird species including juveniles, adult, male, female, breeding and non-breeding.

Based on the results, Shwe Tantit environs is considered good habitat for water bird species because this site has food resources, shady trees, dam and artificial ponds. Moreover, this area is located nearby lake and Ayeyawady River.

Conclusion

Occurrence of many water bird species of residents as well as migrants in the wetlands of Shwe Tantit village environs is evident for the depending of these species on these wetlands for their survival via performances of various activities of living processess and also reveals the importance of the area for the conservation of water bird species. Due to population increase and growing demand on fresh water resources there may be a heavy pressure on the wetlands of Shwe

Tantit and the concerned stakeholders should think about it and manage the long-term conservation of these wetlands and water birds.

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(A) *Dendrocygna javanica* (B) *Tadorna ferruginea* (C) *Anas zonorhyncha*



(D) *Tachybaptus ruficollis* (E) *Anastomus oscitans* (F) *Threskiornis melanocephalus*



(G) *Plegadis falcinellus* (H) *Ixobrychus sinensis* (I) *Ixobrychus cinnamomeus*



(J) *Nycticorax nycticorax* (K) *Ardeola grayii* (L) *Ardela bacchus*



(M) *Bubulcus coromandus* (N) *Ardea cinerea* (O) *Ardea purpurea*

Plate 1 Bird species recorded in the study area

(P) *Ardea alba*

(Q) *Mesophoyx intermedia*

(R) *Egretta garzetta*

(S) *Phalacrocorax niger*

(T) *Phalacrocorax carbo*

(U) *Gallirallus striatus*

(V) *Amaurornis phoenicurus*

(W) *Porzana pusilla*

(X) *Porzana fusca*

(Y) *Gallicrex cinerea*

(Z) *Porphyrio poliocephalus*

(AA) *Gallinula chloropus*

(BB) *Fulica atra*

(CC) *Himantopus himantopus*

(DD) *Vanellus cinereus*

Plate 1 Continued

(EE) *Vanellus indicus*

(FF) *Charadrius hiaticula*

(GG) *Caharadrius dubius*

(HH) *Rostratula benghalensis*

(II) *Gallinago gallinago*

(JJ) *Tringa ochropus*

(KK) *Tringa stagnatilis*

(LL) Assemblages of water bird communities mixing *Ardeola bacchus* with other water birds

(MM) Assemblages of *Tadorna ferruginea* and *Plegadis falcinellus*

Plate 1 Continued

SEASONAL STATUS OF REPTILIAN FAUNA IN KYAIKHTIYOE WILDLIFE SANCTUARY, MON STATE

Win Zaw[1], Thet Naing[2]

Abstract

Kyaikhtiyo Wildlife Sanctuary in Mon State is selected as study area, lies between (17º 24′ N - 17º 34′ N) and (97º 1′ E - 97º 10′ E), and is about 137 square kilometers (38600 acre) in area. The Mountain range of Wetwun Taung and its eastern environ including Wetwun Chaung and Kadat Chaung was chosen as Site A, Yathae Taung and its western environ as Site B, Wait Sar Taung and along its south-eastern face to near the highest peak at Kyaikhtiyoe Pogada as Site C, and the visitors-walking route from near Kinmon Sakan to Ye Myaung Gyi as Site D respectively. The specimens were collected by direct observation and visual encountered survey (VES). Seventeen reptile species of 11 genera belonging to 7 families of two orders are recorded in the study area. The most seasonal abundance of reptiles was concluded as hot season. the family Gekkonidae (60 individuals) was recorded to be highest number; second Agamidae (38 individuals), third Scincidae (26 individuals), and fourth Viperidae (3 individuals) were followed orderly, Platysternidae (1), Trionychidae (1), and Colubridae (1) as lowest number.

Keywords: wildlife Sanctuary, direct observation, visual encountered survey, seasonal abundance

Introduction

The reptiles are characterized as ‘crawling’ species, breathing with lungs and showing poikilothermy, variable body temperature. The Class Reptilia contain about 6600 species in four orders. Reptiles are divided into four orders. Testudines (turtles and tortoises); Crocodilians (crocodiles, alligators, and gharials); Rhynchocephalia (tuataras) and Squamata (scaled reptiles). Squamata are further divided into three suborders; Sauria or Lacertilia (lizards); Amphisbaenia (worm-lizards); and Serpents (snakes) (O’shea and Halliday, 2002).

Myanmar is the largest in mainland Southeast Asia and contains a magnificent variety of ecosystems. Myanmar supports some of the most intact natural habitats and species communities remaining in the Indo-Myanmar Hotspot, including many globally threatened species that are found in few or no other places in the world (Tordoff et. *al.,* 2005). The country has three seasons: wet (from mid-May to mid-October), cold (from early November to late February) and dry (from March to mid-May), and 172 Snake species, 87 Lizard species, and 32 chelonian species were inhabited in Myanmar (Forestry, 2015).

In spite of decreasing forest areas all over the world, Myanmar is able to maintain coverage of nearly half of its total land area with forests. In the Indo-Myanmar (Indo-Burma) Hotspot, comprehensive global threat assessments are only available for mammals, birds, amphibians and some groups of reptiles. Baseline data on species diversity in Myanmar is incomplete for most, if not all, major taxonomic groups, and the available data of the current status of the country’s diversity is mainly the globally threatened species that are currently listed in the *IUCN Red List of Threatened Species*. Ranking of 18 Asian countries by species richness from highest to lowest results, Myanmar possesses the second position with 27 species. Ranking by percentage of endemism from highest to lowest results, Myanmar possesses the third position with 22.2% (Forestry, 2011).

[1] Dr, Lecturer, Department of Zoology, University of Yangon
[2] Dr, Lecturer, Department of Zoology, Taungoo University

Twenty-four globally threatened reptile species have been recorded in Myanmar in 2010, most of them are turtles. The main threat to wild populations is over-exploitation, driven in most cases by the high value of turtles in the wildlife trade. According to Myanmar Biodiversity Conservation Investment Vision, there are twenty-six marine and non-marine turtle species (6-CR, 10-EN, 8-VU, 2-NT), fourteen snake species (1-EN, 1-VU, 1-NT, 11-DD), and seven lizard and gecko species (All are DD) recorded by WCS (2013) in Myanmar.

Ernst and Barbour (1989) recognized that turtle population has been declining at an alarming rate throughout the world in recent years. Adults of many turtles are harvested for food. Tordoff *et al.* (2005) examined the priority species for conservation investment in Myanmar, including 16 chelonian species which all are endangered. A recent conservation assessment made by IUCN (2010) recognized half of the Asian tortoise and freshwater turtle species to be endangered or critically endangered, primarily due to overexploitation for food and medicinal purposes. To prioritize where in Asian the most urgent attention toward conserving wild turtle populations used recently updated information to rank Asian countries according to richness, endemism, and threat level of their turtle farms. The results of biological analysis ranked China, Vietnam, Myanmar and Indonesia are the top four priority countries in Asia for turtle conservation (Stuart and Thorbjarnarson (2003). Bonin *et al.* (2006) explained that the most important message to bring to the public was the need to appreciate these delightful animals, yet to have them to remain in the wild rather than in private gardens. Protecting turtles thus requires that their habitats are protected, that commercialization be opposed, and that attitudes of certain people be encouraged to change with the times. Because turtles usually have low reproductive rates and late maturity, the exploitation of turtles and tortoises is generally considered to be unsustainable (Zhou and Jiang 2008). Win Maung and Win Ko Ko (2002) recorded 32 marine and non-marine chelonian species belonging to 28 genera from six families in Myanmar.

No fewer than 39 species of dangerously venomous snakes are currently known to inhabit Myanmar and the adjacent coastal waters. Of these, 15 are sea snakes and except for two, *Laticauda colubrina* and *Laticauda laticauda*, none voluntarily come onto land (occasionally, obligate marine species may be carried onto shore during severe weather by wave action or enter river deltas in brackish water). Of the remaining 24 species, all are terrestrial in the sense that none voluntarily enters coastal waters. And, of the terrestrial forms, several, such as *Trimeresurus albolabris*, favor arboreal habitats and are usually found resting on tree limbs. All of the terrestrial species can swim, and some are occasionally found swimming in the rivers and streams as well as in flooded rice paddies. Two families of dangerously venomous snakes are represented in the Myanmar herpetofauna: Elapidae (cobras, kraits, and coral snakes [subfamily Elapinae], and sea snakes and Australian elapids [subfamily Hydrophiinae]), and Viperidae (true vipers [subfamily Viperinae], pitvipers [subfamily Crotalinae], and *Azemiops* [subfamily Azemiopinae]) (Leviton et al. 2008).

Materials and Methods

Kyaikhtiyo Wildlife Sanctuary is located in Kyaikhto Township, Thaton district, Mon State. The selected study area lies between (17º 24′ N - 17º 34′ N) and (97º 1′ E - 97º 10′ E), and is about 137 square kilometers (38600 acre) in area. The Mountain range of Wetwun Taung and its eastern environ including Wetwun Chaung and Kadat Chaung was chosen as Site A, Yathae Taung and its western environ as Site B, Wait Sar Taung and along its south-eastern face to near the highest peak at Kyaikhtiyoe Pogada as Site C, and the visitors-walking route from near Kinmon Sakan to Yae Myaung Gyi as Site D respectively (Figure 1).

This study lasted from May 2019 to January 2020 from 7:00 am to 5:00 pm. The field survey was divided into three parts; May 2019 for hot season, September 2019 for wet season, and January 2020 for cold season. The study period of each season lasted about seven to ten days.

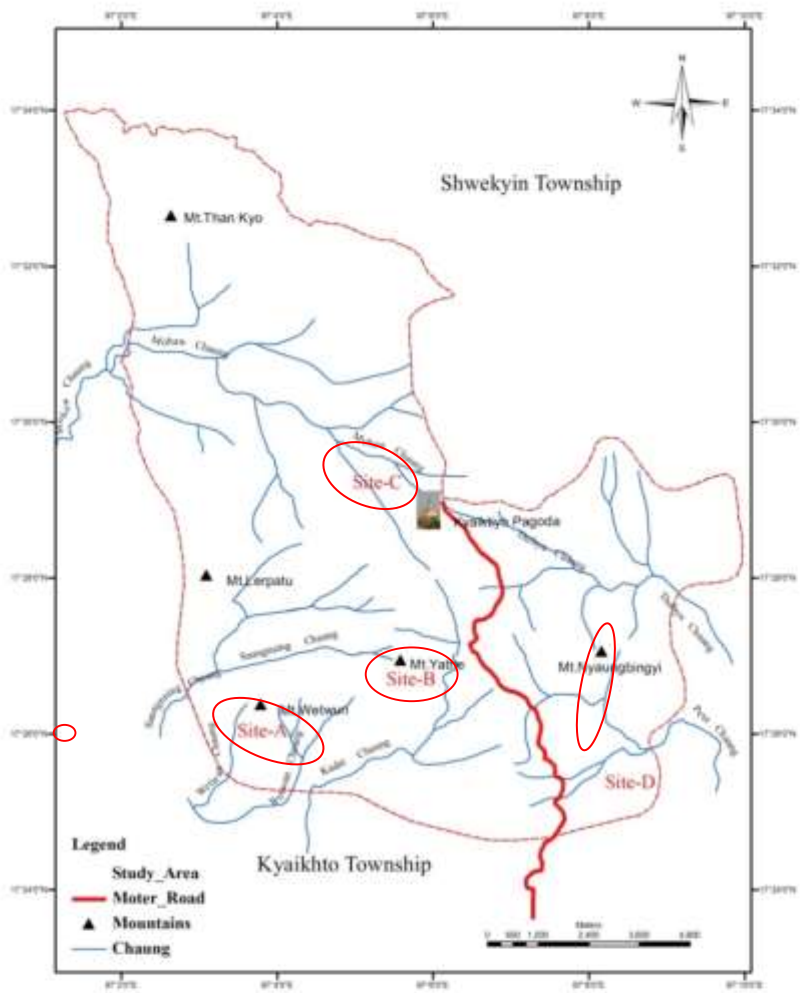
The specimens were collected from the study sites with the help of forest staffs by direct observation such as noosing and hand capturing, and visual encountered survey (VES) followed after Crump and Scott (1994). The captured specimens were treated with chloroform to record some morphometric data, and then replaced those to the detected habitats.

Hand capturing and noosing was used for geckos, skinks, and tree lizards found terrestrial and sheltered habitats to identify and record the studied specimens.

When the lizard faunas were arboreal and not easy to capture, visual encountered study (VES) was used with the help of binocular and digital camera along a survey path to identify the studied species. If the visual encountered specimens were not easy to be identified, they were not counted.

Identification of the specimens was followed after Boulenger (1890), Smith (1935), and Das (2010) by checking key characters of scalation and coloration. Identification of the turtle species was followed after Ernst and Barbour (1989), Bonin and Dupré (2006).

Body weight (BW) was taken by a digital balance to the nearest gram. Measurements such as snout-vent length (SVL), tail length (TL), head length (HL), and head width (HW) were measured by either a digital caliper or measuring tape to the nearest millimeters.



(**Source:** Kyaikhtiyoe Wildlife Sanctuary Office)

Figure1 Map of Kyaikhtiyoe Wildlife Sanctuary and its environs

Results

Species Composition of Study Area

Totally, seventeen reptile species of 11 genera belonging to 7 families of two orders were recorded in the study area. The classification of recorded species was shown in the table 1.

Seasonal abundance of reptiles in the study sites

The field survey was conducted into three seasons for the selected study sites to investigate the seasonal abundance of reptiles inhabited in Kyaikhtiyoe Wildlife Sanctuary. During this study period, 2 individuals of 2 turtle species, 4 individuals of 3 snake species, and 124 individuals of 12 lizard species were seasonally collected from various study sites (Table 2). According to the collected data, *Hemidactylus frenatus* was the most abundance in number, two turtle species (*P.megacephalum, L.scutata*) and two snake species (*P.popeiorum*, *M.bella*) were least in status (Fig. 2). Besides, individuals of the study species were peak number in hot season (74) and the least (23) in cool season (Fig. 3). Of these individuals, 2 individuals of turtles and 105 individuals of lizards recorded by direct observation, and 4 individuals of snakes and 19 individuals of lizards by VES (Photo-recorded).

Table1 The classification of the recorded species in the study area

Sr.	Order	Suborder	Family	Genus	Species	Common name
1	Testudines	Cryptodira	Platysternidae	*Platysternon*	*P.megacephalum*	Big headed turtle
2			Trionychidae	*Lissemys*	*L.scutata*	Myanmar flapshell turtle
3	Squamata	Serpentes	Viperidae	*Cryptelytrops*	*C.erythrurus*	Spot-tailed pit viper
4				*Popeia*	*P.popeiorum*	Popes's pit viper
5			Colubridae	*Maculophis*	*M.bella*	Dice-like trinket snake
6		Sauria	Agamidae	*Acanthosaura*	*A.crucigera*	Masked spiny lizard
7				*Calotes*	*C.htunwini*	Htunwin's forest lizard
8					*C.mystaceus*	Blue forest lizard
9					*C.versicolor*	Garden lizard
10			Gekkonidae	*Gekko*	*G.gecko*	Tokay gecko
11				*Hemidactylus*	*H.brookii*	Brooke's house gecko
12					*H.bowringii*	Bowring's house gecko
13					*H.frenatus*	Asian house gecko
14					*H.garnoti*	Garnot's house gecko
15			Scincidae	*Sphenomorphus*	*S.maculatus*	Spotted forest skink
16				*Eutrophis*	*E.multifasciata*	Common sun skink
17					*E.macularia*	Little ground skink

Morphometric data of the collected species

Two individuals of turtles and 105 individuals of lizards could be captured and recorded for some morphometric data. Some morphometric data such as body weight (BW), snout-vent length (SVL), tail length (TL), head length (HL), and head width (HW) are recorded for saurian specimens (Table 3); and body weight (BW), curved carapace length (CCL) and curved carapace width (CCW) for turtle specimens are measured nearest grams, millimeters and centimeters (Table 4).

Table 2 Seasonal occurrence of reptilian fauna in the study sites

Sr.	Specimen	Site A			Site B			Site C			Site D			Total
		H	W	C	H	W	C	H	W	C	H	W	C	
1	*P.megacephalum*	1	-	-	-	-	-	-	-	-	-	-	-	1
2	*L.scutata*	-	-	-	-	1	-	-	-	-	-	-	-	1
3	*C.erythrurus*	1	-	-	-	-	-	-	1	-	-	-	-	2
4	*P.popeiorum*	-	1	-	-	-	-	-	-	-	-	-	-	1
5	*M.bella*	-	-	-	1	-	-	-	-	-	-	-	-	1
6	*A.crucigera*	-	-	-	-	1	-	-	-	-	1	-	-	2
7	*C.htunwini*	1	-	1	1	-	-	1	1	-	3	1	1	10
8	*C.mystaceus*	2	1	1	1	-	-	3	1	-	2	-	1	12
9	*C.versicolor*	2	2	-	1	1	1	2	-	-	2	2	1	14
10	*G.gecko*	-	-	-	2	1	-	1	1	1	-	-	-	6
11	*H.brookii*	-	-	-	1	-	-	2	1	-	3	1	2	10
12	*H.bowringii*	1	-	-	2	1	1	1	-	-	2	2	1	11
13	*H.frenatus*	-	-	-	4	2	-	3	-	-	6	4	4	23
14	*H.garnoti*	1		-	3	1	1	1	-	-	2	-	1	10
15	*S.maculatus*	2	2	1	3	-	2	2	1	1	1	-	1	16
16	*E.multifasciata*	1	1	-	1	-	-	1	-	-	-	-	-	4
17	*E.macularia*	-	1	1	2	-	-	-	1	-	1	-	-	6
Total		**12**	**8**	**4**	**22**	**8**	**5**	**17**	**7**	**2**	**23**	**10**	**12**	**130**

H = Hot season, W = Wet season, C = Cool season

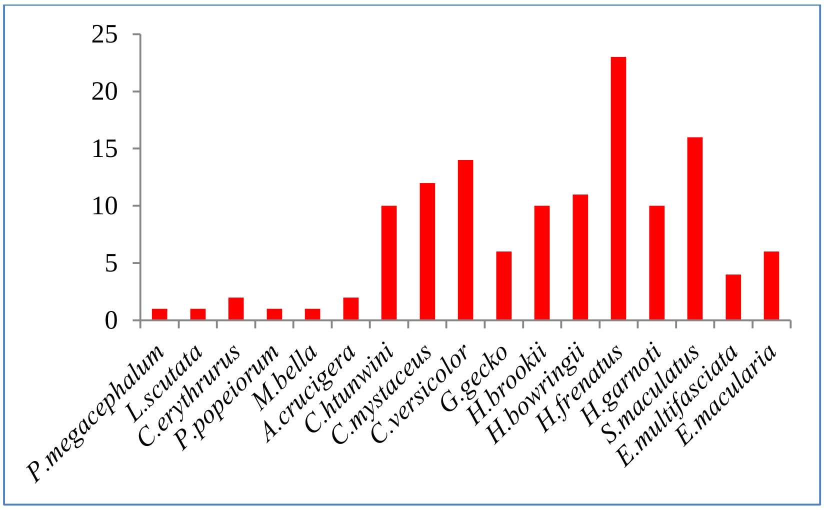


Figure 2 Abundance of reptile species in the study

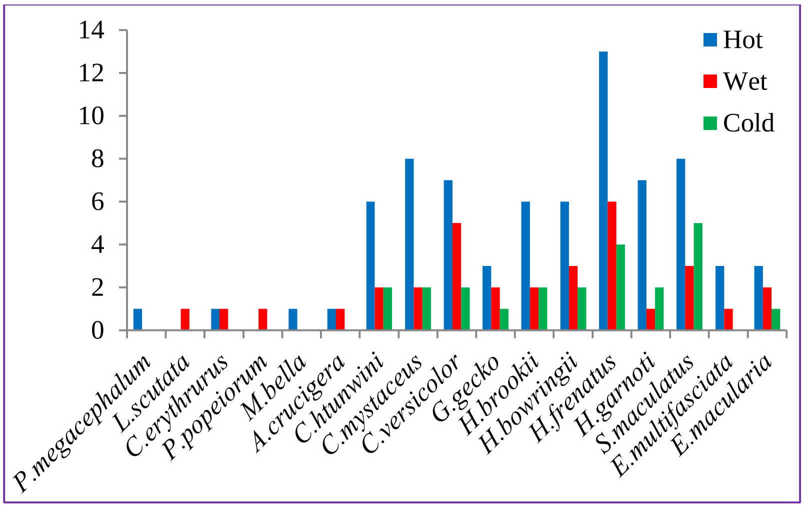


Figure 3 Seasonal abundance of reptiles in different study sites

Table 3 The morphometric data of the captured lizard specimens

Sr.	Species	n	BW (g) Min-Max	SVL (mm) Min-Max	TL (mm) Min-Max	HL (mm) Min-Max	HW (mm) Min-Max
1	*A.crucigera*	2	16.0 - 17.5	84.9 – 87.6	129.8 – 133.2	19.1 – 20.4	13.8 – 14.2
2	*C.htunwini*	9	2.5 – 8.0	48.2 – 68.7	134.0 – 207.2	14.9 – 18.6	9.4 – 11.2
3	*C.mystaceus*	9	9.5 – 10.5	78.5 – 89.2	172.4 – 194.9	16.8 – 22.1	13.6 – 15.1
4	*C.versicolor*	11	8.5 – 9.5	73.0 – 84.6	152.2 – 219.8	15.7 – 19.4	9.7 – 12.8
5	*G.gecko*	5	9.5 – 130	78.3 – 193.2	67.5 – 218.3	23.9 – 50.4	17.8 – 41.3
6	*H.brookii*	7	2 – 5.5	40.9 – 58.4	44.5 – 55.6	10.1 – 14.5	7.5 – 12.2
7	*H.bowringii*	19	2.5 – 4.5	44.9 – 55.7	46.0 – 56.3	11.1 – 12.7	8.0 – 10.8
8	*H.frenatus*	16	2 – 5.0	43.6 – 56.2	45.5 – 56.1	10.6 – 13.4	7.8 – 11.8
9	*H.garnoti*	8	2 – 5.5	44.2 – 56.6	45.8 – 56.8	10.7 – 14.9	7.8 – 11.3
10	*S.maculatus*	11	1. 5 – 6.0	27.7 – 58.4	45.5 – 90.5	6.6 – 12.7	4.0 – 6.1
11	*E.multifasciata*	3	5.5 - 6.5	74.2 - 83.7	119.3 - 137.8	11.7 - 12.8	5.9 - 6.7
12	*E.macularia*	5	0.5 – 4.5	34.3 – 52.5	49.8 – 88.6	7.0 – 9.5	4.8 – 5.8

n = numbers of captured specimens, BW = body weight, SVL = snout vent length, TL = tail length, HL = head length, HW = head width, Min = minimum, Max = maximum

Table 4 The morphometric data of the captured turtle specimens

Sr.	Species	n	BW (g)	CCL (cm)	CCW (cm)	IUCN status
1	*Platysternon megacephalum*	1	823	17.2	12.8	Endangered
2	*Lissemys scutata*	1	988	19.4	18.5	Data deficient

n = numbers of captured specimens, BW = body weight, CCL = Curved carapace length, CCW = Curved carapace width

Discussion

During this study, a total of 17 reptile species of 11 genera belonging to 7 families of two orders were observed as the species composition in Kyaikhtiyoe Wildlife Sanctuary, Mon State.

Mar Mar Thein (1981) was studied 27 species of 16 genera belonging to 7 families of lizards in Shwebo, Zee Gone, Alskapa, Let-Kha-Bin, Kyawt-Min (Sagaing Division); Taunggyi, Heho, Inpaw Khone- Inlay (Shan State); Matupi, Falum, Kale (Chin State); Mandalay, Maymyo (Mandalay Division); Moulmein, Set-se (Mon State); Mongmagan (Tenasserim Division).

Aye Aye Myat (2007) recorded a total of 17 lizard species belonging to 8 genera of 3 families in the study areas of Yangon, Bago, Mandalay, Ayeyarwaddy, Tanintharyi Divisions and Mon State.

Khin Than Kywe (2010) expressed that total 43 reptile species of 33 genera belonging to the 13 families of two orders were recorded in the Kyaikhtiyoe Wildlife Sanctuary from February 2005 to January 2009.

Kyaw Swar Aung (2012) observed 14 lizard species belonging to 8 genera of 2 families from Bago Region.

Thanda (2012) recorded a total of 15 lizard species of 3 families belonging to the order Squamata were recorded from in Hlegu and Hmawbi Environs.

Htay Htay Maw (2015) stated 18 species belonging to 5 families of lizards in Taungtha Reserved Forest area in Mandalay Region.

20 reptile species of 16 genera belonging to 7 families of two orders were collected in Sittway environs by San Htet San (2017).

Thu Zar Win (2018) recorded a total of seven species of six genera belonging to four lizard families in Hlawga Wildlife Park, Yangon Region.

Species composition of family Gekkonidae 74 percent, Agamidae and Scincidae 13 per cent were conducted by Thanda (2012) respectively. Aye Aye Myat (2007) discussed that species composition of family Agamidae was recorded to be the highest percentage (47 percent) in her study, and the remaining species composition were of the family Gekkonidae (35 percent), Scincidae (18 per cent) respectively. The highest relative abundance of lizard families conducted by San Htet San (2017) was Gekkonidae and Colubridae (18.52 percent), and followed by Agamidae (7.41 percent), Scincidae (3.7 percent) respectively. Zug (2013) said that no lizard is more familiar with us than the geckos in the tropics.

Htay Htay Maw (2015) concluded that agamids were regarded as dominant because they were recorded in the highest number (65) individuals in her study.

According to the species composition of the present study, the family Gekkonidae (60 individuals) was recorded to be highest number; the second Agamidae (38 individuals), third Scincidae (26 individuals), and fourth Viperidae (3 individuals) were followed orderly, Platysternidae (1), Trionychidae (1), and Colubridae (1) as lowest number.

Concerning with the seasonal abundance of reptiles, Khin Than Kywe (2010) stated that 180 individuals of reptile species recorded in hot season, 161 individuals in wet season, and 69 individuals in cool season respectively.

In this study, one individual of one respective turtle species was individually recorded in hot (*P.megacephalum*) and wet (*L.scutata*) seasons, four individuals of three snake species also in hot (*C.erythrurus*, *M.bella*) and wet (*C.erythrurus*, *P.popeiorum*) seasons. 71 individuals of lizards were occurred in hot, 30 individuals in wet, and 23 individuals in cool seasons respectively.

As shown in result, the most abundance of reptiles was recorded in hot season as 23 individuals of 10 species in study Site D and 22 individuals of 12 species in Site B, the least abundance in cool season as 2 individuals in Site C.

Aye Aye Myat (2007) recorded that the snout-vent length of *Gekko gecko* was measured about 150 mm as the longest body. Das (2010) expressed that the mean snout-vent length of *Gekko gecko* was measured about 185mm. Kyaw Swar Aung (2012) discussed that on the aspect of the mean morphometric measurement, the total length of *Gekko gecko* (34 cm, 30 – 38 cm) was observed as the longest body. G*ekko gecko* (TL = 13-16 cm) and *Sphenomorphus maculatus* (TL = 8-13 cm) were ranged as the species with long tail.

As the present study was expressed in result, the total length (SVL = 193.2 mm and TL = 218.3 mm) of *Gekko gecko* was recorded in 41.15 cm.

Conclusion

In the present study, the recorded reptile species of *Lissemys scutata* and *Calotes htunwini* were noted as the endemic species in Myanmar, and *Platysternon megacephalum* was endangered species in the conservation status of IUCN Red List.

According to the result, the species occurrence of reptiles in the present study was concluded as little in species number in contrast with that of San Htet San (2017) and Khin Than Kywe (2010). Besides, the collected number of lizard species in this study was fewer than that of above authors, except Thu Zar Win (2018).

The most seasonal abundance of reptiles was concluded as hot season.

According to the morphometric data, it was concluded that the maximum measurement of *Gekko gecko* in this study was the longest body than that species of Aye Aye Myat (2007), Das (2010), and Kyaw Swar Aung (2012) stated. In the present study, the maximum tail length (90.5 mm) of *Sphenomorphus maculatus* was shorter than that of Kyaw Swar Aung (2012) recorded

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OCCURRENCE, RICHNESS AND ABUNDANCE OF SOME AVIFAUNA IN HMANPYA HILL ENVIRONS, WAINGMAW TOWNSHIP, KACHIN STATE

Ohn Mar Tun[1], Htar Htar Aye[2] and Swè Nu Nu Tawng[3]

Abstract

The study on the occurrence, richness and abundance of avifauna in Waingmaw Township, Kachin State was conducted from September 2018 to January 2019. Hmanpya Hill in Maina Reserve Forest was allocated as the study area. A total of 496 individuals accounted from 30 species of birds distributed under 26 genera, representing 17 families and seven orders were recorded. Among these, the largest number of species and individuals were recorded in December and the lowest in September. Relative abundance indicated that five species were found as very common (vC), 11 species as Common (C) and 14 species as uncommon (uC). Species richness was found to be the highest (R = 3.19) in December and the lowest (R = 1.66) in October. According to the IUCN Red-list, 29 species were in Least Concern category while, *Psittacula alexandri* Red-breasted Parakeet with regarded as Near Threatened. Since, the present study area is located within the Maina Reserve Forest and harboured suitable habitats to support sufficiently large number of bird species, there is a need to maintain the friendly nature of the environment in order to safeguard the sustainability of the bird species that thrive in the area.

Keywords: Occurrence, Richness, Abundance, Avifauna, Reserve Forest

Introduction

Birds are good indicators of spatial biodiversity and sustainability, because they are high in the food chain, thus integrating changes at other levels and they occupy a broad range of ecosystems (Heath and Roument, 2001).

Birds are found in almost all types of habitats on the earth. The great diversity of habitats is reflected in a great diversity of birds. The number of bird species in a region is called species richness or species diversity. The simplest measure of species diversity is to count the number of species (Krebs, 2001).

Climate has a profound, but often indirect effect on bird distributions. Temperature and rainfall greatly influence the composition of plant communities, which, in turn, determines the availability of food, nest sites and protective cover for birds to use (Sibley, 2001).

Weather conditions have considerable influence on many aspects of bird migration. Every year, migratory birds usually fly about 26,000 kilometres from northern hemisphere, to escape from winter (Myanmar times, 2017).

In Southeast Asia, a total of 1327 species are known to occur (Robson, 2015). The avifauna of Myanmar includes a total of 1,125 species. Of these 199 species are water birds, 289 species are winter visitors and 130 species are hibernate water birds. Eight of these species are endemic species, ten are critically endangered (CR) and 11 species are endangered (MBNS, 2018).

Hmanpya Hill environs are inhabited by numerous bird species. There are various habitats such as cultivated areas and forested areas. Therefore, food sources are abundant in this area for most of the birds. However, as not much research work has been done in the study area that the present work was pursued. The objectives were to identify and record the bird species in Hmanpya Hill Environs, to investigate the composition of bird species, to determine the species richness and to evaluate the relative abundance and average relative abundance of bird species recorded.

[1] Associate Professor, Department of Zoology, Myitkyina University
[2] Lecturer, Department of Zoology, Myitkyina University
[3] Demonstrator, Department of Zoology, Myitkyina Universit

Materials and Methods

Study area and study site

Waingmaw Township lies between 25˚7′ N, 25˚19′ N and 97˚17′, E 97˚25′ E. It is situated on the eastern bank of the Ayeyarwady River and 146.6 m above sea level.

Hmanpya Hill was selected as the study area. It is situated in Waingmaw Township, Kachin State. It is situated at 54 km away from Myitkyina and located at 25˚24′53.7″ N and 97˚28′04.1″ E. The height of the Hmanpya Hill is approximately 443.48 m. This site is composed of cultivated areas, bushy areas, small forested habitat areas and tall grasses.

Study period

The research was conducted from September 2018 to January 2019.

Data collection

Collection of data was made using point count method according to Bird Census Techniques (Bibby *et. al.*, 2001). Twelve points were allocated randomly in the study area. At each point, observation was made by standing and recording those seen within a radius of 50 m for 10 minutes. The distance between each point was 200m apart. Field surveys were conducted once every fortnight and commenced from 9:00 am to 11:00 am in the morning and from 2:00 pm to 5:00 pm in the afternoon. Observations were carried out with the aid of 8 × 40 binoculars and immediately taken on photographs by using Nikon coolpix P900.

Identification and classification of species

Identification of bird species in the field was done after Robson (2011 and 2015), and the classification followed after Bird Life International (2017).

Analysis of data

To assess species richness index the data accumulated was analyzed by using Margalef's (1958) method.

Species richness index (R) = $\frac{\text{S}-1}{\text{In N}}$ (Margalef's, 1958)

R = Margalef's index of species richness

S = Total number of species

N = Total number of individuals

This method incorporates the total number of individuals and is measure of the number of species present for a given number of individuals.

Relative abundance was also evaluated as follows:

Relative abundance = $\frac{\text{No.of individuals of a species}}{\text{Total no.of individuals of all species}}$

Relative abundance was categorized accordingly: (Bisht, *et. al.*, 2004)

uC (uncommon) = having relative abundance less than 0.01

C (common) = having relative abundance of 0.01 and above but less than 0.05

vC (very common) = having relative abundance of 0.05 and above

Results

From September 2018 to January 2019, a total of 30 species belonging to 26 genera, 17 families and seven orders were recorded in the study area (Table 1).

According to the monthly occurrence, the highest number of 18 species was recorded in December and the lowest number of 8 species in October. With respect to the monthly number of species and individuals recorded per species were taken into consideration it was found that, three species were encountered in every month of the five months study period and five species were encountered only once during a single month and represented by a single individual each and only one species although encountered only once during a single month is represented by two individuals. The remaining 21 species were encountered at least two months and the number of individuals ranged from 3 to 108 individuals (Table 2).

Among the bird species recorded the composition of bird species was the highest in order Passeriformes with 16 species (53%), followed by the three orders Coraciiformes, Falconiformes and Psittaciformes (3%) (Fig.1).

Richness and relative abundance of bird species

The results of Marglf's index (R) revealed that the highest richness index R = 3.19 was in December and the lowest R = 1.66 in October.

Relative abundance indicated that five species appeared as very common (vC), 11 species as common (C) and the remaining 14 species as uncommon (uC) species (Table 2).

Status of bird species

The residential status revealed that out of the 30 species recorded, 26 species were residents (R) and the remaining four species were winter visitors (WV), however, most are local migrants.

According to IUCN Red-List (2017), *Psittacula alexandri* Red-breasted Parakeet is catagorised as the Near Threatened (NT) and 29 species as in Least Concern (LC) (Table 2)

Table 1 List of recorded bird species in Mount Hman Pya during September 2018 to January 2019

Sr. No.	Order	Family	Genus	Species	Common Name
1.	Columbiformes	Columbidae	*Spilopelia*	*S. chinensis* (Scopoli, 1786)	Spotted Dove
2.			*Treron*	*T. curvirostra* (Gmelin, 1789)	Thick-billed Green-pigeon
3.				*T. phonicopterus* (Latham, 1790)	Yellow-footed Green-pigeon
4.			*Ducula*	*D. aenea* (Linnaeus, 1766)	Green Imperial Pigeon
5.	Accipitriformes	Accipitridae	*Pernis*	*P. ptilorhynchus* (Temminck, 1821)	Oriental Honey-buzzard
6.			*Spilornis*	*S. cheela* (Latham, 1790)	Crested Serpent-eagle
7.			*Accipiter*	*A. badius* Gmelin, 1788	Shikra
8.			*Buteo*	*B. refectus* Portenko, 1929	Himalayan Buzzard
9.	Coraciiformes	Meropidae	*Nyctyornis*	*N. athertoni* (Jardine & Belby, 1830)	Blue-beared Bee-eater
10.	Piciformes	Megalaimidae	*Psilopogon*	*P. lineatus* (Vieillot)	Lineated Barbet
11.				*P. asiaticus* (Latham, 1790)	Blue-throated Barbet
12.		Picidae	*Chrysocolaptes*	*C. quttacristatus* (Tickell, 1833)	Greater Flameback
13.	Falconiformes	Falconidae	*Microhierax*	*M. caerulescens* (Linnaeus, 1758)	Collared Falconet
14.	Psittaciformes	Psittacidae	*Psittacula*	*P. alexandri* (Linnaeus, 1758)	Red-breasted Parakeet
15.	Passeriformes	Eurylaimidae	*Psarisomus*	*P. dalhousiae* (Jameson, 1835)	Long-tailed Broadbill
16.		Oriolidae	*Oriolus*	*O. traillii* (Vigors, 1832)	Maroon Oriole
17.		Campephagidae	*Pericrocotus*	*P. flammeus* (Rorster,1781)	Scarlet Minivet
18.		Dicruridae	*Dicrurus*	*D. aeneus* Vieillot, 1817	Bronzed Drongo
19.				*D. hottentottus* (Linnaeus, 1766)	Hair-crested Drongo
20.		Cisticolidae	*Prinia*	*P. polychroa* (Temminck, 1828)	Brown Prinia
21.		Locustellidae	*Megalurus*	*M. palustris* Horsfield, 1821	Striated Grassbird
22.		Sturnidae	*Gracupica*	*G. contra* (Linnaeus, 1758)	Asian-pied Starling
23.				*G. nigricollis* (Paykull, 1807)	Black-collared Starling
24.			*Acridotheres*	*A. albocinctus* Godwin-Austen & Walden, 1875	Collared Myna
25.			*Saroglossa*	*S. spilopterus* (Vigors, 1831)	Spot-winged Starling
26.			*Gracula*	*G. religiosa* Linnaeus, 1758	Common Hill Myna
27.		Muscicapidae	*Eumyias*	*E. thalassinus* Swainson, 1838	Verditer Flycatcher
28.			*Cyornis*	*C. rubeculoides* Vigors, 1831	Blue-throated Flycatcher
29.		Nectariniidae	*Arachnothera*	*A. longirosta* (Latham, 1790)	Little Spiderhunter
30.		Emberizidae	*Emberiza*	*E. pusilla* Pallas, 1776	Little Bunting

Table 2 Monthly occurrence and relative abundance index of recorded bird species in study area during September 2018 to January 2019

Sr. No.	Scientific Name	Sept	Oct	Nov	Dec	Jan	Total	RA	IA	IUCN Status	Residential Status
1	*Spilopelia chinensis*	20	21	20	22	25	108	0.218	vC	LC	R
2	*Treron curvirostra*		7				7	0.014	C	LC	R
3	*Treron phonicopterus*	8			25		33	0.067	vC	LC	R
4	*Ducula aenea*				5	6	11	0.022	C	LC	R
5	*Pernis ptilorhynchus*				2	1	3	0.006	uC	LC	R
6	*Spilornis cheela*				1		1	0.002	uC	LC	R
7	*Accipiter badius*	1					1	0.002	uC	LC	R
8	*Buteo refectus*			1		1	2	0.004	uC	LC	WV
9	*Nyctyornis athertoni*				2		2	0.004	uC	LC	R
10	*Psilopogon lineatus*	2	8	4	3	5	22	0.044	C	LC	R
11	*Psilopogon asiaticus*	2	3	3	4		12	0.024	C	LC	R
12	*Chrysocolaptes quttacristatus*				3		3	0.006	uC	LC	R
13	*Microhierax caerulescens*	1	3			3	7	0.014	C	LC	R
14	*Psittacula alexandri*				3	4	7	0.014	C	NT	R
15	*Psarisomus dalhousiae*			6			6	0.012	C	LC	R
16	*Oriolus traillii*			4	4	3	11	0.022	C	LC	R
17	*Pericrocotus flammeus*	4	7	5	22		38	0.077	C	LC	R
18	*Dicrurus aneneus*		3		5	4	12	0.024	C	LC	R
19	*Dicrurus hottentottus*					3	3	0.006	uC	LC	R
20	*Prinia polychroa*	1	1				2	0.004	uC	LC	R
21	*Megalurus palustris*			2			2	0.004	uC	LC	R
22	*Gracupica contra*	13	14	15	12	14	68	0.137	vC	LC	R
23	*Gracupica nigricollis*	11		16	20	12	59	0.119	vC	LC	R
24	*Acridotheres albocinctus*				3		3	0.006	uC	LC	R
25	*Saroglossa spilopterus*				58		58	0.117	vC	LC	WV
26	*Gracula religiosa*				10		10	0.020	C	LC	R
27	*Eumyias thalassinus*					2	2	0.004	uC	LC	R
28	*Cyornis rubeculoides*	1					1	0.002	uC	LC	WV
29	*Arachnothera longirosta*				1		1	0.002	uC	LC	R
30	*Emberiza pusilla*			1			1	0.002	uC	LC	WV
	Total number of individuals	64	67	77	205	83	496				
	Total number of species	11	8	11	18	12					

LC (Least Concern) = 29 NT (Near Threatened) = 1 R (Resident) = 26 WV (Winter Visitor) = 4

(Source: MIMU1052v02 and Google Earth Pro 2019)

Plate 1 Location map of study area

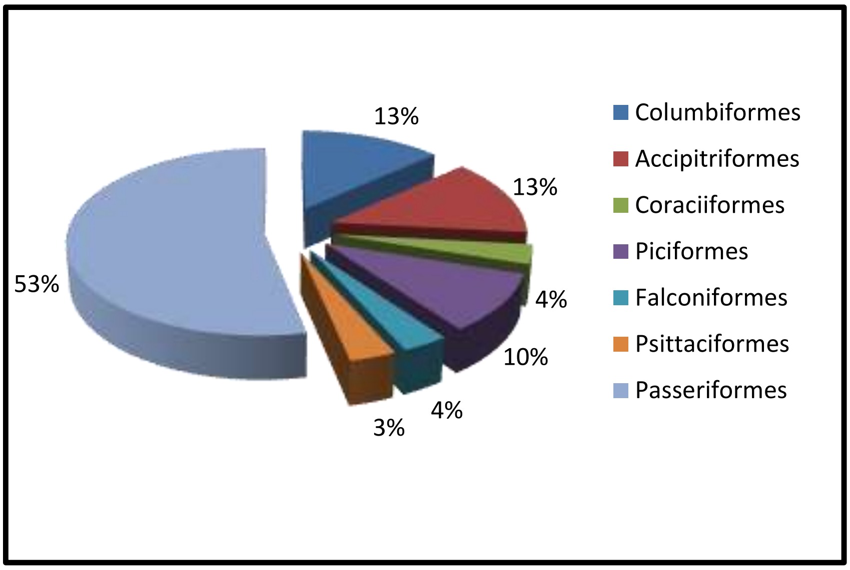

Figure 1 Orderwise relative species composition of bird species recorded during the study

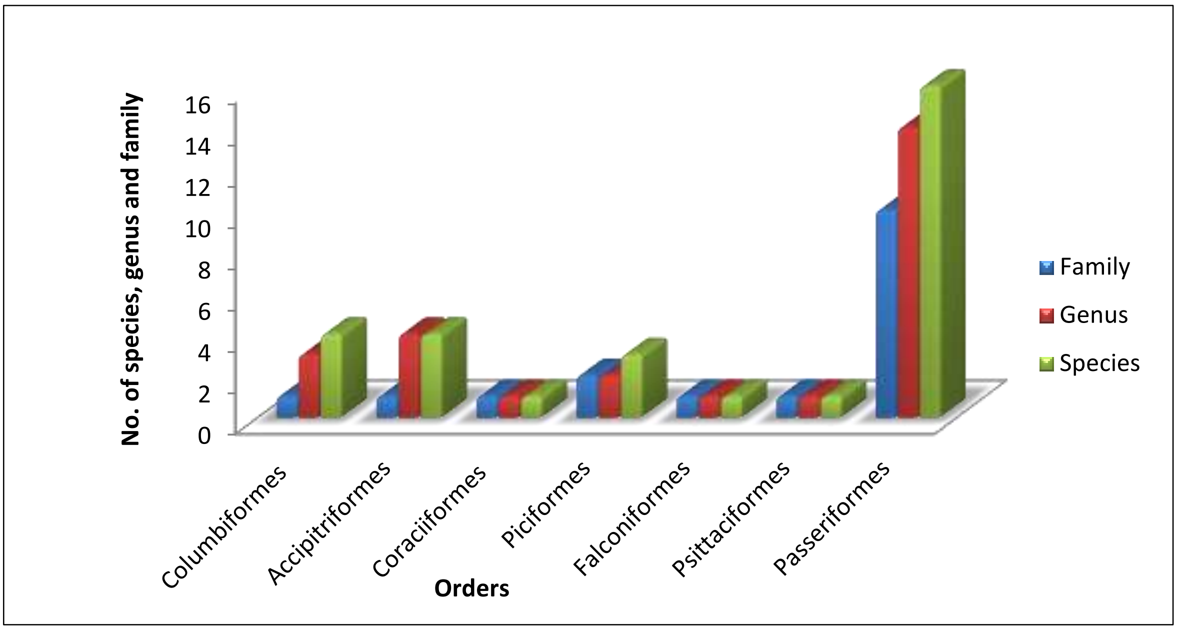

Figure 2 Relative number of species, genus and family of birds recorded during the study

A. *Spilopilia chinensis*

B. *Treron curvirostra*

C. *Treron phonicopterus*

D. *Ducula aenea*

E. *Pernis ptilorhynchus*

F. *Spilornis cheela*

G. *Accipiter badius*

H. *Buteo refectus*

I. *Nyctyornis athertoni*

J. *Psilopogon lineatus*

K. *Psilopogon asiaticus*

L. *Chrysocolaptes quttacristatus*

Plate 2 Bird species recorded

A. *Microhierax caerulescens*

B. *Psittacula alexandri*

C. *Psarisomus dalhousiae*

D. *Oriolus traillii*

E. *Pericrocotus flammeus*

F. *Dicrurus aeneus*

G. *Dicrurus hottentottus*

H. *Prinia polychroa*

I. *Megalurus palustris*

J. *Gracupica contra*

K. *Gracupica nigricollis*

L. *Acridotheres albocinctus*

M. *Saroglossa spilopterus*

N. *Gracula religiosa*

O. *Eumyias thalassinus*

P. *Cyornis rubeculoides*

Q. *Arachnothera longirosta*

R. *Emberiza pusilla*

Plate 2 Continued

Discussion

Hmanpya Hill is situated in Waingmaw Township, Kachin State and the surrounding environ is composed of cultivated areas, bushy areas, small forested habitat and tall grasses area.

During the study, a total of 30 species belonging to 26 genera, 17 families and seven orders were recorded in the study area. Among the bird species recorded, five species were considered as very common, 11 species were considered as common and 14 species were considered as uncommon species. Out of the 30 species, 26 species were residents and four species were winter visitors.

Ei Ei Phyu (2017) studied on species composition and abundance of birds in Myingyan Degree College Campus, Myingyan Township and recorded 50 species belonging to 40 genera, 29 families and 11 orders. In her study, the highest species composition was that of Passeriformes. Hkawn Seng (2017) recorded a total of 48 species under 35 genera, 26 families and 11 orders in Myitkyina University Campus, Myitkyina. In her study, the highest number was in order Passeriformes. According to Robson (2015), Passeriformes represent the largest order among all recorded orders in South-east Asia. In the present study, the order Passeriformes also represented the highest number (16) of bird species. Therefore, this finding agrees with Robson (2015).

Regarding the status, only one species *Psittacula alexandri* Red-breasted Parakeet was noted as near threatened species (NT) based on the IUCN Red-list (2017).

Comparing the data of the previous workers, Ni Ni Yin (2011) worked on community ecology of avian fauna in Meiktila environs and a total of 128 species belonging to 37 families and 12 orders were observed. Of these, two endemic species *Turdoides gularis* White-throated Babbler and *Mirafra microptera* Burmese Bushlark were recorded in her study. Robson (2015) also reported that *Turdoides gularis* White-throated Babbler was common resident in central and southern part of Myanmar and *Mirafra microptera* Burmese Bushlark was common resident in Central and North of Southern region, West of Eastern region. In this study, however, no endemic species was recorded and alluded to the differences in location and habitat.

Bauk Ra (2016) worked on species composition of birds in Waimaw Township, Kachin State with emphasis on foraging and richness of birds and a total of 182339 individuals and 113 bird species distributed under 80 genera, 50 families and 15 orders were observed from July 2013 to June 2016. In this research, only 496 individuals and 30 species were recorded from September 2018 to January 2019. The relative difference in terms of number of species and the number of individuals recorded was Bauk Ra (2016) did her study in four sites while the present study was centered on a single location and the disparity in the duration of the study periods. The present study was conducted only for duration of four months and Bauk Ra (2016) did her surveys for three consecutive years.

Soe Hein (2017) worked on species composition, richness and relative abundance of avifauna in Pyu Lake and environs, Tada-U Township. He recorded the highest richness index was R =6.94 in January. In the present study, the highest richness index was R = 3.19 in December. Since, it embodied a vast expanse of Pyu Lake and its environ where the arrival of winter visitors elevate the number of bird species already inhabited the area. In contrast, the present study area harbors no extensive surface body water. Therefore, it is alluded that abundance of bird species was due to arrival of local migrants with respect to the availability of food sources and season wise habitat compatibility and habitat heterogeneity during the cold season.

Conclusion

Hmanpya Hill is located within the Maina Reserve Forest harboured suitable habitats and support sufficiently number of bird species. Nevertheless, there is a need to maintain the eco-friendly nature of the study area in order to safeguard not only the avifauna that thrives in the environs of Hmanpya Hill but also the heterogeneity of the study area.

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MICROHABITAT UTILIZATION OF SOME ANURAN SPECIES IN MINBU ENVIRONS

Hla Moe Aye*

Abstract

Microhabitat utilization of some anurans were investigated during June 2018 to May 2019 in Minbu environs. Weekly data collection was carried out in the late evening. A total of 22 species inhabitated in ten microhabitats were recorded. Out of the recorded species, the highest numbers of species (15 species) were from paddy field while the lowest numbers of species (three species) were observed in temporary pool microhabitat. The anuran species were distributed in the study area and well-occupied in the different microhabitats located in different areas.

Keywords: anuran, microhabitat utilization, Minbu

Introduction

Most anuran lives in the tropical parts of the world than in the northern temperate climate. Most Asian tree frogs occur in south, southeast and east Asia from eastern India, Sri Lanka and Nepal through Myanmar, Thailand, Laos, Cambodia, and Vietnam, southeast along the Malay Peninsula onto the islands of Sumatra, Java, Borneo and Sulawasi, and throughout the Philippines. Many species of *Chiromantis*, *Polypedates*, and *Rhacophorous* also inhabit flooded rice fields and grasses or low shrubs between agricultural lands and forests (Duellman and Schlager, 2003).

Microhabitat loss is a significant cause of frog population decline, as are pollutants, climate change, the introduction of non-indigenous predators/competitors etc. A Canadian study conducted in 2006 suggested heavy traffic near frog microhabitats as a large threat to frog population. Natural fluctuations, in amphibian populations, from changing weather conditions and other natural environmental changes can be relatively large, potentially marking changes due to other factors (Mossman, *et al.,* 1998).

Minbu environs is one of the townships of Myanmar with suitable environment and microhabitats for existence of amphibian population. Its lies to the west of the Ayeyawady River. Environmental conditions are favourable for the occurrence of frogs and toads. However, due to changes of weather and human impacts such as over exploitation, indiscriminate uses of pesticides for agricultural purposes, over extraction of timber from forests, and extension of agricultural land and human settlements, the microhabitats for anurans become degraded and deteriorated. The information about anuran in Myanmar is scared and the works related to frogs and toads were very limited. With this background, present research was conducted to document the microhabitats of anurans in Minbu environs.

The main objectives of the study are to investigate the occurrence of the anuran species in different microhabitats of study area and to determine the microhabitats of anurans species in Minbu environs

Material and Methods

Study area

Minbu environs is existed between 19º52'16" to 20º18'54" N and 94º28'17" to 95º E. In the area, irrigated paddy lands substitute some dry farming land.

* Dr, Lecturer, Department of Zoology, University of Magway

Study sites

Three study sites were allocated Maungmakan village, Sabowit Chaung and Man Chaung in Minbu environs.

Study period

Field trips were made in Minbu environs during June 2018 to May 2019.

Study design and specimen collection

Specimen collections were made at the designated sampling sites and collected from the late evening till mid night. The data collection was made monthly in each study area based on visual encountered survey method. They were collected using hand net and by hand. Specimens were kept in plastic bags and the species, number of individual, collection sites, dates, microhabitat types and weather conditions were noted. Photographic records were taken instantly in fresh state in wild condition and were noted down. The specimens were preserved in 10% formalin for 24 hours and then transferred to 70 % ethanol for future use. The collected specimens were brought to the Department of Zoology, Magway University for species identification and measurements.

Identification and classification of species

The species identification and classification were made based on key characters and taxonomic descriptions given by Duellman and schlarger (2003), Zug (2010) and Frost (2015 and 2018).

Assigning status based on relative abundance

The status of anurans was assigned based on the value of relative abundance according to Bisht *et al*.(2004).

$$\text{Relative abundance} = \frac{\text{No.of individual of a species}}{\text{Total no.of individual of all species}}$$

uC = (uncommon) having relative abundance less than 0.0100

C = (common) having relative abundance of 0.0100 and above but less than 0.0500

vC = (very common) having relative abundance of 0.0500 and above

Plate 1 Map of study areas of Minbu environs (Source: from Google earth soft ware

Results

A total of 22 species of anuran belonging to five families confined to single order were recorded. Among the recorded species, four species of *Duttaphrynus melanostictus*, *Ingerophrynus macrotis*, *I. parvus* and *Phrynoidis aspera* are the representative of the family Bufonidae, 10 species namely, *Fejervarya cf. limnocharis, F. limnocharis, Hoplobatrachus crassus, H.tigerinus, H.ugulosus, Limnonectes grunniens, L. modestus, Occidozyga lima, Sphaerotheca breviceps* and *Zakerana greenii* belonged to the family Dicroglossidae and five species, *Calluella guttulata, Glyphoglossus molossus, Kaloula pulchra, K. p. pulchra* and *Microhyla ornata* represented the family Microhylidae, single species of *Humerana humeralis* is from the family Ranidae and the two species, *Chiromantis punctatus* and *Polypedates leucomystax* belonged to the family Rhacophoridae in Minbu environs. According to IUCN Red List, in the study area one near threatened (*Glyphoglossus molossus*), one endangered species (*Zakerana greenii*), two data deficiency (*Chiromantis punctatus* and *Polypedates leucomystax*) and the rest of 18 species in least concern were collected (Table 1).

Microhabitats analysis

Different environmental places where frogs and toads inhabited were analyzed and categorized into different microhabitats.

Distribution of microhabitat in different in Minbu environs

Ten microhabitats found were trees dominated area, grass ground, sandy area near water, swamp, pond, paddy field, ditch, human habitation, temporary pool and sandy field.

Occurrence of anuran species in different microhabitat types

In Minbu environs, *Duttaphrynus melanostictus*, *Kaloula pulchra* and *K. p. pulchra* was found in all microhabitats. *Fejervarya limnocharis* and *F.cf limnocharis* were found in all microhabitat types except temporary pool. *Ingrophrynus macrotis* and *I. parvus* was found in pond only. *Chiromantis punctatus* and *Polypedates leucomystax* were found in swamp only. *Calluella guttulata* was found in only sandy field. *Microhyla ornata* was found in three microhabitats. *Occidozyga lima* and *Glyphoglossus molossus* were found in five microhabitats. *Zakerana greenii* was collected in six microhabitats. Three species from temporary pool, five species from in each of human habitation, and trees dominated area, six species from grass ground, eight species from in each of ditch and sandy field, 10 species from pond, 11 species from in each of swamp and sandy area near water, 15 species from paddy field were recorded. The seven species are very common species, the anuran species well distributed in the study area and well-occupied in the different microhabitats located in different areas. Another seven species are uncommon species and eight common species were collected. Six microhabitat types such as sandy area near water, swamp, pond, ditch, paddy field and sandy field in very common species, three microhabitat types such as tree dominated area, grass ground and human habitation in common species and only temporary pool microhabitat type in uncommon species were collected (Table 2 and Fig.2).

A total of 22 species in Minbu environs, the highest species were in family Dicroglossidae (45.45%) followed by family Microhylidae (22.73%), family Bufonidae (18.18%), family Rhacophoridae (9.09%) and the lowest species in family Ranidae (4.55%) (Table 3, Fig 1). In the present study the highest species was found in paddy field microhabitat type (15 species) while the lowest in temporary pool microhabitat type (three species). In Minbu environs, *Duttaphrynus melanostictus*, *Kaloula pulchra* and *K. p. pulchra* was found in all microhabitats in all study sites.

Table 1 List of collected anuran species in Minbu environs

Sr. No	Family	Genus		Species	Common name	Venucular name	IUCN Status
1.	Bufonidae	*Duttaphrynus*	1.	*Duttaphrynus melanostictus* (Schneider, 1799)	Common toad	Hpar pyoke	LC
		Ingerophrynus	2.	*Ingerophrynus macrotis* Boulenger, 1887	Big-eye toad	Hpar pyoke	LC
			3.	*Ingerophrynus parvus* Boulenger, 1887	Dwarf toad	Hpar pyoke	LC
		Phrynoidis	4.	*Phrynoidis aspera* (Gravenhorst, 1829)	River toad	Hpar pyoke Chaung	LC
2.	Dicroglossidae	*Fejervarya*	5.	*Fejervarya cf. limnocharis* (Gravenhorst, 1829)	Paddy frog	Kyaw san kay	LC
			6.	*Ferjervarya limnocharis* (Gravenhorst, 1829)	Paddy frog	Kyaw san kay	LC
		Hoplobatrachus	7.	*Hoplobatrachus crassus* (Jerdon, 1854)	Brown edible frog	Hpar paung nyo	LC
			8.	*Hoplobatrachus rugulosus* (Wiegmann, 1834)	Chinese Edible frog	Sarr hpar kyie	LC
			9.	*Hoplobatrachus tigerinus* (Daudin, 1803)	Kaing land frog	Kaing hpar	LC
		Limnonectes	10.	*Limnonectes grunniens* (Latreille, 1801)	Nil	Sarr hpar	LC
			11.	*Limnonectes modestus* (Boulenger, 1882)	Grass frog	Nil	LC
		Occidozyga	12.	*Occidozyga lima* (Gravenhorst,1829)	Common floating frog	Hpar than lat	LC
		Sphaerotheca	13.	*Sphaerotheca breviceps* (Schneider, 1799)	Terrestrial frog	Kon hpar	LC
		Zakerana	14.	*Zakerna greenii* (Bloulenger, 1905)	Paddy frog	Hpar paung sinn	EN
3.	Microhylidae	*Calluella*	15.	*Calluella guttulata* (Blyth, 1855)	Myanmar squat frog	Nil	LC
		Glyphoglissus	16.	*Glyphoglossus molossus* Gunther, 1869	Balloon frog	Hpar ayne	NT
		Kaloula	17.	*Kaloula pulchra* Gray, 1831	Bull frog	Hpar kon nyin	LC
			18.	*Kaloula pulchra pulchra* Gray, 1831	Bull frog	Hpar kon nyin	LC
		Microhyla	19.	*Microhyla ornata* (Dumeril and Bibron, 1841)	Sand frog	Thae hpar	LC
4.	Ranidae	*Humerana*	20.	*Humerana humeralis* (Boulenger 1887)	Nil	Hpar paung shay	LC
5.	Rhacophoridae	*Chiromantis*	21.	*Chiromantis punctatus* (Wilkinson, 2003)	Myanmar plant Frog	Hpar pyan thae	DD
		Polypedates	22.	*Polypedates leucomystax* (Gravenhorse, 1829)	Tree Frog	Hpar pyan Kyi	DD

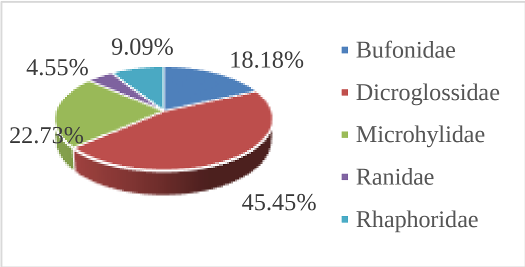
DD-Data Deficiency, NT-Near Threaten, EN-Endanger species, LC- Least Corncern

Table 2 Occurrence and abundance of anuran species in different microhabitat types in Minbu environs

Sr. No	Species	Trees dominated area	Grass ground	Sandy area near water	Swamp	Pond	Paddy field	Ditch	Human habitation	Temporary pool	Sandy field	Total	Relative abundance	Status
1.	*Duttaphrynus melanostictus*	5	10	25	30	60	80	40	10	5	20	285	0.15	vC
2.	*Ingerophrynus macrotis*	-	-	-	-	20	-	-	-	-	-	20	0.01	uC
3.	*Ingerophrynus parvus*	-	-	-	-	10	-	-	-	-	-	10	0.005	uC
4.	*Phrynoidis aspera*	-	-	15	-	-	-	-	-	-	10	25	0.013	uC
5.	*Fejervarya cf. limnocharis*	5	10	20	20	30	30	20	5	-	20	160	0.085	vC
6.	*Fejervarya limnocharis*	10	20	30	30	30	40	30	10		20	220	0.117	vC
7.	*Hoplobatrachus crassus*	-	-	-	-	-	50	-	-	-	-	50	0.026	C
8.	*Hoplobatrachus rugulosus*	-	-	-	-	-	50	-	-	-	-	50	0.026	C
9.	*Hoplobatrachus tigerinus*	-	-	-	-	-	100	-	-	-	-	100	0.053	vC
10.	*Limnonectes grunniens*	-	-	-	-	-	80	-	-	-	-	80	0.042	C
11.	*Limnonectes modestus*	-	-	-	-	-	50	-	-	-	-	50	0.026	C
12.	*Occidozyga lima*	-	-	20	20	30	40	30	-	-	-	140	0.074	vC
13.	*Spherotheca breviceps*	-	-	20	-	-	-	-	-	-	50	70	0.037	C
14.	*Zakerana greenii*	-	5	5	5	10	20	5	-	-	-	50	0.026	C
15.	*Calluella guttulata*	-	-	-	-	-	-	-	-	-	10	10	0.005	uC
16.	*Glyphoglossus molossus*	-	-	5	5	10	15	5	-	-	-	40	0.021	C
17.	*Kaloula pulchra*	15	20	30	30	40	60	20	10	5	10	240	0.127	vC
18.	*Kaloula pulchra pulchra*	5	10	15	10	20	20	10	5	5	10	110	0.058	vC
19.	*Microhyla ornata*	-	-	5	5	-	10	-	-	-	-	20	0.01	uC
20.	*Humerana humeralis*	-	-	-	-	-	30	-	-	-	-	30	0.016	C
21.	*Chiromantis punctatus*	-	-	-	10	-	-	-	-	-	-	10	0.005	uC
22.	*Polypedates leucomystax*	-	-	-	10	-	-	-	-	-	-	10	0.005	uC
	Total number species	5	6	11	11	10	15	8	5	3	8	22		
	Total number of individual	40	75	190	175	260	675	160	40	15	150	1880		

Table 3 Composition of anuran species in different families in Minbu environs

Sr.No.	Family	Genus	Species	% Composition
1.	Bufonidae	3	4	18.18
2.	Dicroglossidae	6	10	45.45
3.	Microhylidae	4	5	22.73
4.	Ranidae	1	1	4.55
5.	Rhacophoridae	2	2	9.09



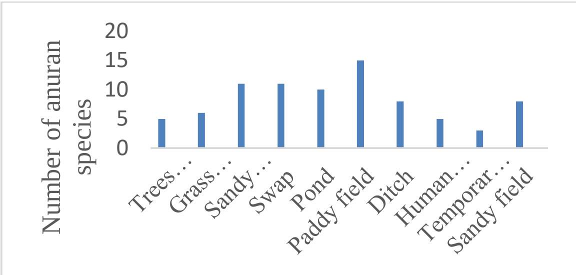


Figure 1 Composition of anuran species

Figure 2 Number of anuran species among different microhabitats

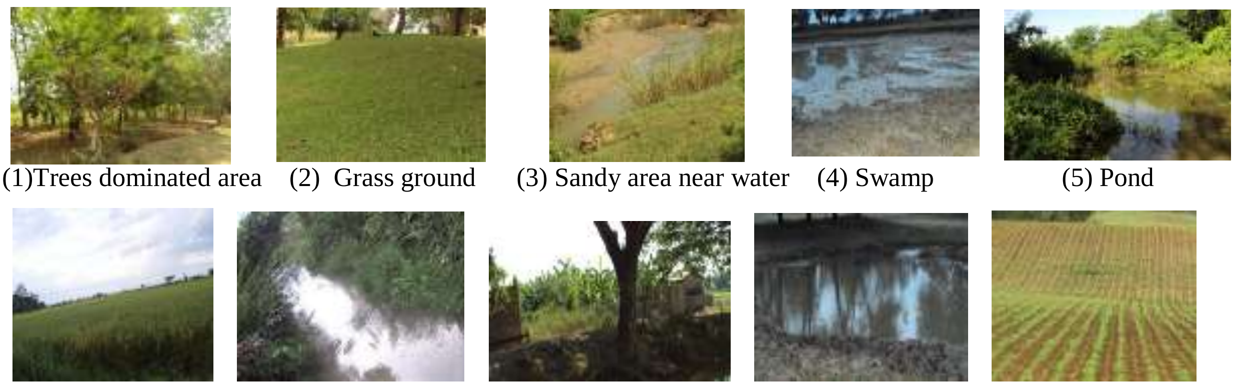
(1)*D. melanostictus* (2) *I. macrotis* (3)*I. parvus* (4) *P. aspera* (5) *F. cf. limnocharis* (6)*F.limnocharis*

(7)*H. crassus* (8) *H.rugulosus* (9) *H. tigerinus* (10) *L. grunniens* (11) *L. modestus* (12)*O. lima*

(13) *S. breviceps* (14)*C. guttulata* (15)*Z. greenii* (16)*G. molossus* (17) *K. pulchra* (18)*K.p pulchra*

(19)*M. ornata* (20) *H. humeralis* (21)*C. punctatus* (22) *P.leucomystax*

Plate 2 Recorded species of anuran in Minbu environs



(1)Trees dominated area (2) Grass ground (3) Sandy area near water (4) Swamp (5) Pond

(6) Paddy field (7) Ditch (8) Human habitation (9)Temporary pool (10) Sandy field

Plate 3 Microhabitat types of anuran species in Minbu environs

Discussion

Three species in temporary pool, five species in each of trees dominated area, human habitation, six species in grass ground, eight species in sandy field and ditch, 10 species in pond, 11 species in each of sandy area near water and swamp and 15 species in paddy field were recorded in Minbu environs.

Simon (1987) identified microhabitat types with key symbols such as the microhabitat frequented, the life microhabits, and certain biological characteristics. Different species use different microhabitats and also the same microhabitat. In the present study, when consideration was made on occurrence of each species in different microhabitats, *D. melanostictus, K. pulchra* and *K.p pulchra* was recorded in all observed microhabitats. Thus this species may be considered very cosmopolitan in their distribution and tolerable species. *F. liminocharis* and *F.cf liminocharis* are also considered very wide spread species because these two species were recorded in every microhabitat except temporary pool. *Z. greenii* was found distributed in six microhabitat types, whereas *O.lima* and *G.molossus* in five microhabitats. These species are regarded as a fairly distributed species in the study area. *M.ornata* in three microhabitats, and *P. aspera* and *S. breviceps* in two microhabitat types were observed. These species may be regarded as less adjusted species.

Eleven species are considered very specific for inhabitation because they were noted only in one microhabitat type.

Regarding with microhabitats occupied by different species of anuran, microhabitat type of paddy field may be considered very good and suitable microhabitat for anurans. Because 15 species of anurans among 22 species observed in this study were recorded in this microhabitat. Paddy fields are always provided with water during their growing season and hence, moist and damp soil attracts frogs and toads suitable for feeding, sheltering and breeding sites. Other good microhabitats preferred by anurans are sandy area near water, swamp and pond microhabitats. The less preferred microhabitats is temporary pool because each of these microhabitat was occupied with least number of three species. Temporary pool is existed only in limited period especially during rainy time.

Win Mar Khaing (2007) stated that a maximum of 11 species were found at water edge and paddy field and minimum three species were observed at ground. In this work most species (15 species) were found at paddy field microhabitat and less number of species (three species) was recorded temporary pool microhabitat types.

Krebs (2001) described that species may adapt to temperature, moisture, or light levels phenotypically, or genotypically. Therefore, some species are restricted to particular microhabitats type and some are widespread in their distribution. The distribution of many species can be affected by human changes to microhabitats. In the present study, under the family Bufonidae, the species of *D. melanostictus* under Dicroglossidae, the species of *F. limnocharis* under Microhylidae and the species of *K. pulchra* were found in the Minbu environs. They are regarded as the widely distributed and adjustable frog and toad species. Kathy Htun (2012) stated that the lowest of *Occidozyga lima* was found in Pathein environs. Her finding is different from present study. *O. lima* was observed in five microhabitat types. Minbu environs is suitable for this species.

Nwe Nwe Win (2012) reported that they were observed in cropland, grassland, shrub, human habitation, aquatic environs and especially in rice field with larger number in Yangon environs. Thus, the present finding was in agreement with those of the above authors. All recorded species except *I. parvus, P. aspera, F. cf. Limnocharis, S. breviceps, C. guttulata, C. punctatus* and *P.leucomystax* were collected from paddy field microhabitat type.

There is a need to conserve the tropical moist microhabitats including Minbu environ, since local and regional microhabitat loss, degradation and large-scale environmental changes are causal factors associated with decline amphibian population. Thus, microhabitats and communities must be conserved. In addition; since anurans are sensitive to environmental degradation and pollution are good biological indicators, so that these living assess need to be sustained. Nevertheless, anurans need to be sustained simply because, as living assess they have the inborn right to live and thrive. As a result, Minbu environs is inhabited with more number of anuran species and the microhabitat types of these areas are considered more suitable for existence of anurans. Therefore, it is required to determine the status of current amphibian population and their microhabitats in Minbu environs.

Conclusion

During the study period, 22 species of frogs and toad belonging to 16 genera under five families were recorded from Minbu environs. *Duttaphrynus melanostictus*, *Kaloula pulchra* and *K.p. pulchra* were observed to be habitat generalist. Among 22 species of anuran recorded from the 15 species were found in paddy field very common species in microhabitat types and three species were found in temporary pool uncommon species in microhabitat types. Therefore the species occurrence in two microhabitats was quite different in the Minbu environs.

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STATUS OF MERCURY CONTAMINATION IN MUSCLE OF WALLAGO CATFISH FROM AYEYARWADY RIVER SEGMENT BETWEEN SINGU AND SAGAING TOWNSHIPS, MANDALAY REGION, MYANMAR

Soe Soe Aye[1], Khin Khin Lay[2], Ni Ni Ko[3], Ei Ei Ko[4]

Abstract

The Impact of mercury pollution has been a critical issue worldwide. The present study focused on assessment of mercury contamination status in Ayeyarwady River using adult Wallago catfish as bio-indicator during June 2017 to December 2019. Mercury concentration in the muscle of fish was analysed by mercury analyser (MA-3 Solo NIC). Mean mercury concentration in fish muscle was recorded as 0.62±0.23 ppm (dry weight basis), and 0.14±0.05 ppm (wet weight basis). Average mercury concentration in the fish muscle in wet weight basis showed lower than the WHO (1990) standard (<0.5µg/g), while higher than this standard in dry weight basis. Mercury concentration in fish muscle of the present study was about five times lower than last five years record in Ayeyarwady River. Illegal gold mining is the major source of mercury contamination to the aquatic ecosystem of the Ayeyarwady River, so as education program to local people and regular monitoring about the hazard of mercury are effective tools for the conservation of vulnerable aquatic organisms and health safety of the people. This finding would be provided the information for assessing the impact of the mercury pollution on biodiversity, especially Irrawaddy Dolphin in the Ayeyarwady River of Myanmar.

Keywords: Mercury contamination, *Wallago*, bio-indicator, Ayeyarwady River.

Introduction

Nowadays, the hazard of mercury pollution, especially in aquatic environment, has been a critical issue in environmental management, conservation of wildlife and public health around the world. An estimation of the level of contamination in a particular environment can be revealed by the assessment of the status of aquatic organisms such as algae, macrophyte, zooplankton, bivalve mollusks, seabirds and fish (Manickavasagam *et al*., 2019). Many researchers recommended the fish as a bio-indicator to detect the mercury contamination in the environment because of highly bio-accumulation in its body (Olaifa *et al*. 2004).

Methyl mercury toxicity can cause a neurological disorder called Minamata disease in humans. This mercury-related disease occurred in Japan round about 1950-1960 when mercury pollution occurred in Minamata Bay due to the wastewater discharges of the chemical industry (Kyaw Myint Oo, 2010). People ingested the fish and shellfish contaminated with mercury developing neurological symptoms as loss of consciousness and sometimes death. This disaster pointed out the importance of mercury management and alarmed to developed countries. Recently, mercury pollution has become a serious problem not only in developed countries but also in developing countries, since excessive use of mercury in artisanal and small-scale gold mining has increased in developing countries (Harada, 1995).

In Myanmar, some research works on mercury contamination in fishes from some segments of Ayeyarwady River were carried out by Wildlife Conservation Society and Whale and Dolphin Conservation Society (Smith, *et al*. 2003), Khin Myint Mar (2011), Soe Soe Aye and Khin Ni Ni Win (2015). However, regular and localized studies of mercury contamination in fish muscles are still needed to conduct for assessing the mercury pollution along the Ayeyarwady River. Therefore,

[1] Pro-Rector, West Yangon University
[2,3] Lecturers, Department of Zoology, Kyaukse University
[4] Lecturer, Department of Zoology, Yadanabon University

the present study was focused on mercury contamination status in Ayeyarwady River using Wallago catfish as bio-indicator with following objectives:

- to detect the mercury concentration in muscle of Wallago catfish collected from the study area in relation to well-being of fish
- to evaluate the safety consumption to Wallago catfish in the study area using WHO (1990) standard.
- to assess comparatively the status of mercury contamination in the Ayeyarwady River using the recorded data of present and previous studies.

Materials and Methods

Study Area and Study Period

Study area is the Ayeyarwady River segment between Singu and Sagaing townships, Mandalay Region, Myanmar, and located between $22^{\circ}33'$ N, $95^{\circ}58'$ E and $21^{\circ}52'$ N, $95^{\circ}59'$ E (Figure 1). It is included the portion of Irrawaddy Dolphin protected area (from Kyauk Myaung to Mingon) where a biologically unique human-dolphin cooperative fishery is famous. Study period lasted from June 2017 to December 2019.

Studied Fish Species

Fish species for analysing mercury contamination was chosen *Wallago attu* (Bloch & Schneider, 1801) of Family Siluridae. It is carnivore, bottom dweller and potamodromous fish. *Wallago attu* was chosen as target fish species because it is sensitive indicator of environment according to the finding of Singh and Tandon (2009).

Figure 1 Location map of the study area

Specimen Collection

Adult fish specimens were purchased from local fishermen in the study area. Specimen collection was conducted as three specimens per month. A total of 30 specimens including 9 samples in the rainy season, 12 samples in the cold season, and 9 samples in the dry season were collected during the study period. Fish specimens were not available in June and July when the fishing activities were legally prohibited due to the spawning season. Collected specimens were kept in the ice box and bring to the laboratory of Zoology Department, University of Mandalay for preparing mercury analysis.

Preparation for Mercury Analysis

Collected fish specimens were washed with distil water, skinned and cut out approximately 50g of the axial muscles. Then, fish muscle was cut into slices for dry rapidly and weighted in wet condition. Consequently, flesh slices were dry in drying oven at 60˚C until reaching the constant weight. Each dry specimen was weighted again and kept in separate polyethylene bag and stored in the refrigerator at 20˚C before mercury analysis. Code number of each specimen, collection date, wet weight and dry weight were labelled on the respective specimen bag.

Method of Mercury Analysis

Each specimen was homogenized by using electric blander before conducting mercury analysis. And then, 5g each of three specimens collected in the same month were composed into one sample and mix thoroughly for mercury analysis.

Digestion procedure was conducted followed after Hajeb *et al*. (2009). Firstly, 0.1g of dry powder of fish muscle was weighted using analytical balance and put into 100ml digestion tube, then, 5ml of analytical grade nitric acid (HNO_3) was added. After that the mixture was digested at 40-90ºC in water bath for 3hs till getting clear solution. The digested sample was then cooled at room temperature about 30mins and subsequently diluted 40ml volume with deionized water. Blank solutions were prepared at the same time. Consequently, mercury concentrations in samples were detected duplicating for each sample using mercury analyser (MA-3 Solo NIC) at Nanova Laboratory, Yangon.

The unit of mercury concentration was expressed as microgram per gram (µg/g).

The mercury concentration in fresh fish was calculated according to the method of Sanders, *et al*. (2008).

$$\text{Moisture on dry weight basis } = \frac{\text{Concentration dry weight}}{1 + \text{ moisture on dry weight basis}}$$

$$\text{Hg concentration (wet weight) } = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Dry weight}}$$

Condition Factor

Condition factor (K) was calculated according to Bagenal (1978) as follows:

$$K= 100\ W/L^3$$

Where W is the total body weight in grams and L the standard length in centimeters, the factor 100 is used to bring K close to a value of one. The number 1 indicates a "normal" fish in good condition.

Statistical Analysis

Recorded data were statistically analysed using Microsoft Excel 2010 and IBM SPSS Statistics Version 22. Variation of the mercury concentration among the seasons was analysed by "t" test. Relationship between mercury concentration in the fish muscle and condition factor of fish was analysed by Pearson's correlation coefficient and regression tests.

Mercury Level Limit for Human Consumption

Safety guideline consumption of fish by WHO (1990) was <0.5 µg/g of mercury.

Results

Analysis of mercury concentration in the muscle of Wallago Catfish based on 30 specimens in the study area indicated that mercury concentration in fish muscle varied seasonally (Table 1). Mean mercury concentration in fish muscle was found to be significantly highest in the rainy season (0.85±0.01 µg/g dry weight basis and 0.19±0.03 µg/g wet weight basis) ($t=9.798$, $p<0.001$), decreased in the cold season (0.60±0.13 µg/g dry weight basis and 0.13±0.03 µg/g wet weight basis), and slightly increased again in the dry season (0.66±0.25 µg/g dry weight basis and 0.16±0.06 µg/g wet weight basis).

Table 1 Mercury concentration in the muscle of Wallago catfish in different seasons

Season	No. of specimens	Mercury concentration (µg/g)			
		Dry Weight Basis		Wet Weight Basis	
		Mean±SD	Range	Mean±SD	Range
Rainy	9	0.85±0.01	0.84-0.85	0.19±0.03	0.18-0.22
Cold	12	0.60±0.13	0.44-0.75	0.13±0.03	0.10-0.17
Dry	9	0.66±0.25	0.37-0.85	0.16±0.06	0.09-0.21

The condition factor was analyzed to detect the well-being of fish in the study area during the study period (Figure 2). Monthly variation of condition factor value was observed, the lowest value (K=0.33) was recorded in August 2017 while the highest value (K= 0.96) was recorded in May 2018. In all studied fish samples, the condition factor values were found to be less than one indicating poor condition of fish in all seasons. During the study period, condition factor values of studied fish samples were significantly negative correlation with mercury concentrations in their muscle ($r=-0.65$, $p<0.05$) (Figure 2).

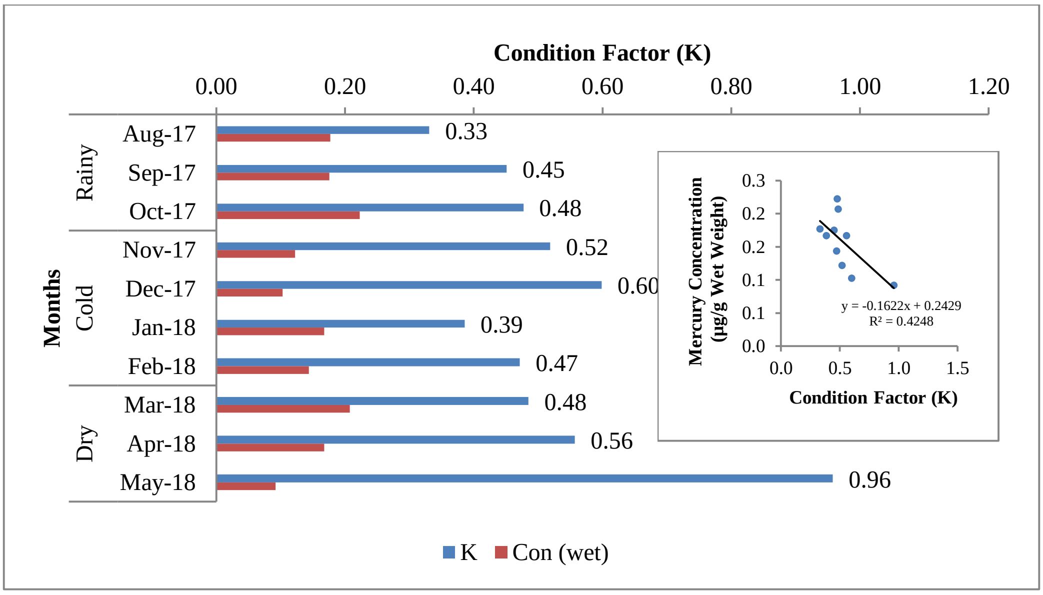

Figure 2 Relation of monthly condition factor value and mercury concentration in the muscle of Wallago catfish in the study area

WHO mercury level limit for human consumption is noted as <0.5 µg/g. Mercury concentrations (wet weight basis) of all studied fish samples were detected to be lower than the permissible limit of safety consumption (<0.5 µg/g) in all seasons. However, mercury concentrations (dry weight basis) in most of samples were found to be exceeding the permissible limit of safety consumption (>0.5 µg/g) in all seasons as 100% of fish in the rainy season, 75% of fish in the cold season and 67% of fish in the dry season (Figure 3).

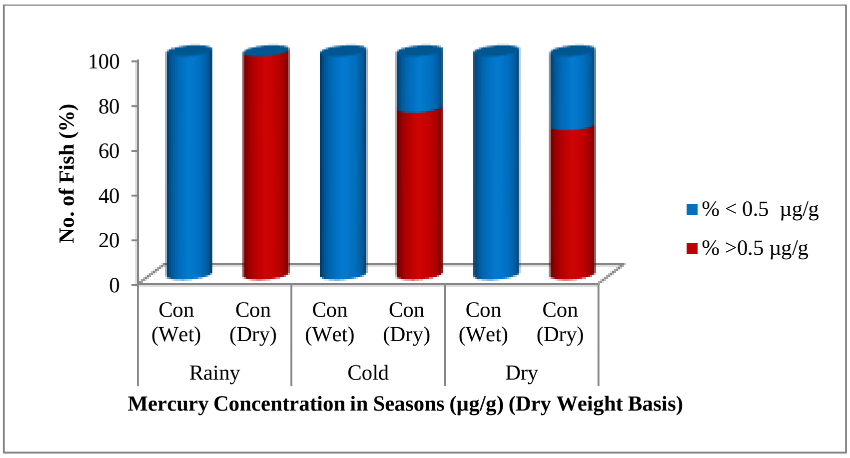

Figure 3 Percentage of fish exceeding WHO Hg level limit in different seasons

The comparison of the present and previous studies analyzed on mercury concentration in the muscle of Wallago Catfish indicated that the mercury concentration increased significantly in different segments of the Ayeyarwady River from 2003 to 2015, while mercury concentration decreased remarkably at the present (Table 2).

Table 2 Comparison of mercury concentration (µg/g wet weight) in muscle of Wallago catfish between present study and previous studies of Myanmar

Location		Mercury Concentration (µg/g)	Reference	Remarks
Region	Ayeyarwady River Segment			
Mandalay	Bamaw and Kyauk Myaung	0.317	Smith *et al*. (2003)	<WHO Standard
Mandalay	Mandalay	0.674	Khin Myint Mar (2011)	> WHO Standard
Magway	Magway	0.978	Khin Myint Mar (2011)	> WHO Standard
Magway	Pakokku	0.671	Soe Soe Aye & Khin Ni Ni Win (2015)	> WHO Standard
Mandalay	Singu and Sagaing	0.142	Present Study (2019)	< WHO Standard

Mercury concentration in the present study was about five times lower than the data of last five years ago at Pakokku Segment (Soe Soe Aye and Khin Ni Ni Win, 2015) and the data of last nine years ago at Mandalay Segment (Khin Myint Mar, 2011). The highest mercury concentration (0.978 µg/g) in the muscle of Wallago catfish was noted in Magway Segment (Khin Myint Mar, 2011), it is nearly seven times higher than the Hg concentration of the present study. These previous records were higher than the WHO permissible limit of human consumption (>0.5 µg/g).

Discussion

Ayeyarwady River, one of the largest free-flowing rivers in Southeast Asia, is not only unique and special, but also the lifeline of Myanmar and majority of the people is dependent on the river for their daily life. The water quality of Ayeyarwaddy River has been in decline for many years especially due to mining operations, deforestation, and lack of soil protection and other human activities (Bowles, 2013). The ecosystem of the Ayeyarwady River is vulnerable especially due to the artisanal and small-scale gold mining by using mercury (Yousafzai *et al.*, 2010).

In the present study, Wallago catfish was used as bio-indicator to detect the mercury contamination in the Ayeyarwady River, since it is carnivorous, bottom dweller and potamodromous. They have more chances to contact both water and sediment ran off from gold mining and mercury used from artisanal and small-scale gold mining. In the rainy season, high and torrent water drags mercury contaminated sediment from upstream to the downstream. The more mercury-contaminated sediment deposited the more chance to contact the fish, so as mercury concentration in fish muscle was detected to be higher in the rainy season. This finding is similar to the finding of Soe Soe Aye and Khin Ni Ni Win (2015) who studied the Ayeyarwady River Segment in Pakokku Township, Magway Region. Therefore, mercury contamination can be predicted not only in the study area but also in the upstream of the river by analysing of Wallago catfish. Gupta (2015) also documented Wallago catfish as a good bio-indicator for heavy metal pollution in the river.

The previous authors stated that very low-levels of pollution may have no apparent impact on the fish itself, which would show no obvious signs of illness. However, fecundity of fish population may decrease gradually, and fish population may decline in long term leading to extinction of these important natural resources (Dupuy *et al*., 2014). In the present study, condition factor values of studied fish samples were detected to be less than one indicating poor condition of these fishes. Besides, these values were negatively correlated with the concentration of mercury in their muscles. Although these fishes have not showed serious symptoms of mercury toxicity at the moment, they may suffer gradually weakness of their health by mercury toxicity in their lifetime and this impact may lead to their next generations as stated by previous authors. It is the warning for conservation of the endemic and endangered species inhabiting in the study area especially Irrawaddy Dolphin since they can also suffer long term impact of mercury toxicity the same as the case of the studied Wallago catfish.

Methyl mercury is an intensely toxic developing neurological symptom and can enter into the human body by various ways. One of the ways to enter mercury into the human body is ingesting such polluted fish resulting in mercury toxicity (Harada, 1995). Kyaw Myint Oo (2013) suggested that it is a good choice to eat fish once or twice a week for getting cancer fighting fats, but beware the hazard of mercury which is a contaminant accumulated in many fish species. In the present study, average mercury concentration in the muscle of fish samples as wet weight basis was lower than the permissible limit of WHO standard (0.5µg/g) in all seasons, while in the muscle of fish as dry weight basis was higher than this standard. Therefore, it should be caution that consuming a large amount of dried and salted fish made in the study area can be dangerous to regular fish consumers in all seasons.

The data of the present study and previous studies (Smith *et al*., 2003; Khin Myint Mar, 2011; Soe Soe Aye and Khin Ni Ni Win, 2015) indicated the effect of the gold mining operations as the source of mercury contamination in the Ayeyarwady River by using Wallago catfish as bio-indicator. In 2002, mercury concentration in fish muscle was low (0.317µg/g) and within the WHO standard, when gold mining operations were few in the Ayeyarwady River (Smith *et al*., 2003). After 2005, although the government has tried to reduce the gold mining operations, these operations have been gradually growing (Smith and Mya Than Tun, 2006), and Ayeyarwady River is more and more polluted. It is clearly seen in the data of Khin Myint Mar (2011) in Mandalay Segment (0.674 µg/g), Khin Myint Mar (2011) in Magway Segment (0.978 µg/g), and Soe Soe Aye and Khin Ni Ni Win (2015) in Pakokku Segment (0.671 µg/g). These mercury concentrations are exceeding the WHO standard for human consumption. In 2015, the government initiating the action plan to control the gold mining operations (Kawakami *et al*, 2019), as a result, the numbers of mining operations were relatively decreased (Bates *et al*, 2015). In the present study, mercury concentration in fish muscle of wet weight basis (0.142 µg/g) was nearly five times lower than the last five years report of Soe Soe Aye and Khin Ni Ni Win (2015), and becoming within the WHO standard of human consumption. Therefore, it is assumed that illegal gold mining is the major source of mercury contamination to the aquatic ecosystem of the Ayeyarwady River.

Conclusion

The Ayeyarwady River plays an important role as a niche to a large diversity of fish and aquatic animals, including Irrawaddy Dolphin which is the endemic of Myanmar and endangered species in IUCN Red List. Recently, the health of the Ayeyarwady River become the importance issue, since mercury is a globally threaten contaminant in both wildlife management and public health, so as sustainable awareness programs to mining companies and local workers should be conducted to safely handle mercury and eliminate or reduce its toxic effects. So far, Ayeyarwady River seems to be recovered from mercury toxicity according to the comparative data of the present and previous studies. Therefore, sustainable monitoring is recommended to assess the toxic pollution for the conservation of biodiversity, especially Irrawaddy dolphin and health safety of people who depend on the Ayeyarwady River in Myanmar.

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BREEDING BIOLOGYOF BURMESE BUSHLARK, *MIRAFRA MICROPTERA* HUME, 1873 IN BAGAN ENVIRONS

Hele Swe Po[1], Pwint Thu Aye[2], Khin Gyee Maung[3]

Abstract

Study on the breeding biology of avian species is the key to understand their behavior, demographics, and population dynamic. Thus, the present study was intense on this field in little know of Myanmar endemic species, Burmese Bushlark (*Mirafra microptera*) to fulfillthe regional data gap. The monthly field survey was conducted from March 2017 to September 2019. Fifty-one nests were found in three years study periods. The breeding seasonof *Mirafra microptera* was from the beginning of March to the mid of September with 1- 2 eggs in clutch size. Twenty-six eggs out of 100 eggs hatched with the hatching success (26%),fledgling successes (81%), breeding success (41%), and crude breeding success (21%) recorded for three years study periods. Breeding success was a significantly negative relationship with temperature and a positive relationship with humidity.

Keywords: Burmese Bushlark, breeding season, clutch size, hatching, fledgling and breeding success

Introduction

Information on breeding biology and performance is an important part of the population ecology of birds and is essential in effective conservation measures for endemic, threatened, and declining species (Martin *et al.*,2000).The breeding ecology of larks being mainly monogamous and territorial, they breed either as isolated pairs or in loose colonies, they nest on the ground, females are solely responsible for nest construction and incubation, clutch sizes are usually small but vary considerably, nestling growth and development is extremely fast, breeding success is generally poor and post-fledging dependence lasts relatively long (De Juana *et al.*2004). Larks breed either seasonally or particularly amongst nomadic species, opportunistically after irregular rain has fallen in semi-arid and arid regions. Breeding in larks is triggered by different environmental conditions for different habitats. In general, larks breed during the wet season or after irregular rains in arid areas when primary production is greatest and invertebrates are more abundant (Dean, 2004).

Bagan is one of the Key Biodiversity in Myanmar Central Dry Zone which is locatedin Nyaung U Township, Mandalay Region with an annual rainfall of 621.73 mm (24.47inches) and temperature ranges (25 – 39.2 °C). The natural vegetation includes semi-desertplant species, thorn bushes, and shrubs and scatters dwarf plants. Thus, the distribution ofBurmese Bushlark (*Mirafra microptera*) has a very large range in Myanmar central dry zoneonly. Moreover, a detailed study on reproductive performance of Burmese Bushlark in thestudy area has not been yet. Therefore, the present study has been focused on the breedingecology of *Mirafra microptrea* in Bagan environs. The specific objectives of the study were to record the breeding activity of the study species, to examine the breeding success of thestudy species and to determine the relationship between some environmental factors and breedingsuccess.

[1] Lecturer, Department of Zoology, Loikaw University
[2] Lecturer, Department of Zoology, University of Mandalay
[3] Associate Professor, Department of Zoology, University of Yangon

Materials and Methods

Study area and study period

The present study was conducted in Bagan environ which is situated in Nyaung U Township, Mandalay Region (21˚7'N to 21˚12'N and 94˚52' E to 94˚56' E) from March 2017 to September 2019.

Identification

Identification of study bird species was followed by Alstrom (1998), Smythies (2001),and Robson (2011). Identification of plant species were referred to Hundley and Chit Ko Ko, 1987.

Field survey method and data collection

Weekly nest observation was conducted with the help of field assistants. Nests searching were predicted by habitat types and parental behavior cues of the target species. When a nest was found, a thorough observation was done by two days per week to collect thedata and to search for breeding activities: adults carrying nesting materials. Nest diameter,cup depth, clutch size, the length (l), and width (w) of the eggs were measured by ruler and measuring tape to the nearest centimeters. The nest micro-habitat structure was recorded within 1 m^2 quadrant of the nest. Nest orientation was determined with the aid of a compass. The growth and development of individual nestlings were recorded every two days. Nestling development was recorded concerning plumage development and growth. Parental care was observed by the aid of a camera trap (Nikon 300 and Meizu) from a hiding place (2–3 meters away).

Analysis of environmental factors

Temperature and humidity were measured by using a thermohygrometer. Rainfall wastaken from the Department of Meteorology, Nyaung U Station, Mandalay Region.

Data analysis

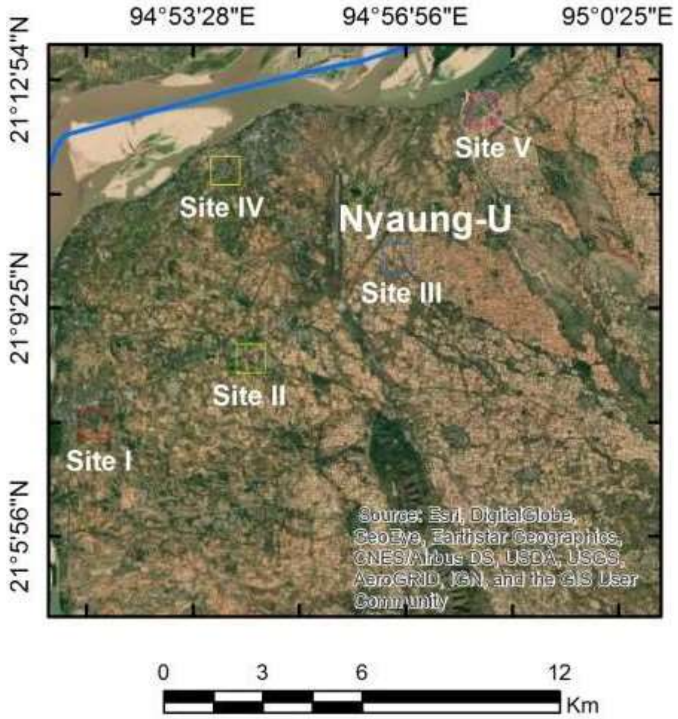
From the time of scraping ground of the base of the host plant to the nest built was regarded as the nest construction period. The incubation period was started from the egg- laying period to the hatching period. Hatching success, fledgling success, and breedingsuccess were estimated by Mayfield’s (1975).

$$\text{Hatching success (\%)} = \frac{\text{Number of birds young}}{\text{Total number of eggs}} \times 100$$

$$\text{Fledgling success (\%)} = \frac{\text{Number of birds fledgled}}{\text{Number of hatching}} \times 100$$

$$\text{Breeding success (\%)} = \frac{\text{Nest with fledling sucess}}{\text{Total recorded nests}} \times 100$$

$$\text{Crude breeding success (\%)} = \frac{\text{Number of fledlings}}{\text{Total recorded of eggs}} \times 100$$



(**Source:** Goggle Earth, 2019)

Figure 1 Map of the Study Area

Results

Breeding season of *Mirafra microptera*

The breeding season of the study birds started from the onset of March to mid- September. In early March of the study periods, the male birds initiated the display flight songs. In mid-September, the reproductive process was successful (Plate 1A, B).

Nesting period and nest of *Mirafra microptera*

The present study witnessed the nesting behavior of *M. microptera* at the commencementof April and continued to the mid of September during the three-year study periods. Fifty-oneactive nests (12 nests in the year 2017, 19 nests in the year 2018, and 20 nests in the year 2019)were recorded. Nesting was the peak in the onset in June, July, and the second peak in May andthen a few nests in April 2017 (Table 1 and 2).

Nest construction of *Mirafra microptera*

The nest construction was fairly deep scrape in the ground first and then lined with fine grass blades and rootlets. *M.microptera* used only one kind of material (Grass, *Sarga* sp.)with a length of 30cm for nest. *M. microptera* spent three days on the construction of the nest.The nest of *M. microptera* was dome shape. The majority of nests faced to the south (32%) and the rest to north-east (23%), south-east (16%), north and south-west (10% each), north- west (6%) and east (3%). The nest site selection of *M. microptera* was the cropland without crop that has the base of herbs for the nest. Most nests (74%, n= 30) constructed at the baseof the forbs plant (*Sida cordofolia*).The remaining nests were on the grass tuft (*Sarga* sp.) (26%, n= 30) (Plate 1 C, D)

Clutch size of *Mirafra microptera*

A total of 100 eggs were recorded from 51 nests with the mean clutch size (1.96±0.19 eggs). Clutch size ranged from 1–2 eggs per nest was recorded during the study period. The mean inter-annual differences in clutch size were 1.83±0.3eggs in the year 2017, 2±0.00 eggs in the year 2018 and 2±0.00 eggs in the year 2019 (Table 1).

The mean nest diameter of *M. microptera* was 10.41±1.67cm in length and 5.5±1.58cm in width. The mean nest depth was 3.66 ± 0.6 cm (Table 1).

The mean dimension of egg size (2.22±0.48 cm in length and 1.26± 1.2cm in width) was within the range of 2-3 cm in length and 1-2 cm in width (Table 1).

Incubation period of *Mirafra microptera*

The incubation commenced upon lying of the first egg, and the incubation period was 12 to 13 days (12.42± 0.85 days) (Table 2).

Hatching success of *Mirafra microptera*

Twenty-six eggs out of 100 eggs hatched with the hatching success (26%) for three years study periods. For the year 2017, the hatching success was 18%, 21% in the year 2018, and 35% in the year 2019 (Table 2).

Breeding success of *Mirafra microptera*

For breeding success, *M. microptera* laid a total of 100 eggs in 51 nests, and from which 26 hatched, and 21 fledged successfully. The breeding success rate was 39%. As the yearly breeding success 25%, 37%, and 55% were recorded in the years 2017, 2018, and 2019 respectively (Table 2).

Crude breeding success of *Mirafra microptera*

The crude breeding success of *M. microptera* was 14% in the year 2017, 18% in the year 2018, and 28% in the year 2019.Overall crude breeding success was 21% (Table 2).

Some behaviors of parental care

Some behaviors in parental care: guiding the nest, polishing the nest, cleansing the nest, feeding, and flying were found during the study periods. Before *M. microptera* entered the nest, she guided and checked 2 meters around the nest for 2 minutes. Nestlings were fed caterpillar and arthropods by both parents (Plate 3A, B).

Relationship of weather parameters and breeding success

The environmental condition of the study area was within the range of 26.5˚C to 35˚Cfor minimum temperature, 36.5˚C to 45.8˚C for maximum temperature; 37% to 72% for minimum humidity, 93% to 100% for maximum humidity; 0 to 121 mm for rainfall during the study periods. Detail environmental weather conditions is shown in Table 3.

The relationship between studied weather parameters and breeding success of the *M. microptera*, the breeding success of the study bird species was a strong negative co relationship to the temperature ($p<0.01$). When the environmental temperature of the study area was high, the breeding and hatchability of the egg were low. In contrast, the breeding commenced after the first rain. Spearman’s rho correlation test revealed that the breeding success of the study bird species was a strong positive relationship with the humidity ($p<0.01$) (Fig.2 and Table 3).

Table 1 Measurement of nests, eggs and clutch size of *Mirafra microptera* in the study area

Measurement	Number	Year 2017 Mean±SD (Range)	Year 2018 Mean±SD (Range)	Year 2019 Mean±SD (Range)	Total Mean±SD (Range)
Clutch size (no.)	51	1.83±0.3 (1 – 2)	2±0 (2)	2±0 (2)	1.96±0.19 (1 – 2)
Nest length (cm)	30	8.54±0.59 (8 – 9.5)	11.05±1.6 (8 – 13)	11.5±0.7 (10 – 12)	10.41±1.67 (8 – 13)
Nest width (cm)	30	3.7±0.3 (3 – 4)	6.33±1.6 (3.4 – 8)	6.33±0.5 (5.5 – 7)	5.5±1.58 (3 – 8)
Nest depth (cm)	30	3.11±0.54 (2.5 – 4)	4.05±0.53 (3.5 – 5)	3.79±0.27 (3.5 – 4)	3.66±0.6 (2.5 – 5)
Egg length (cm)	60	2.24±0.15 (2 – 2.25)	2.24±0.13 (2 – 2.5)	2.18±0.1 (2 – 2.4)	2.2±0.48 (2 – 2.5)
Egg width (cm)	60	1.51±0.18 (1.2 – 1.7)	1.31±0.16 (1 – 1.8)	1.24±0.07 (1.1 – 1.4)	1.26±1.2 (1 – 1.8)

A. Pairing behavior

B. Male singing

C. Nest built at the base of the ForbOrientation: East Clutch size (One egg)

D. Nest built at the base of the grass Orientation: South-eastClutch size (Two egg)

Plate 1 Some courtship behavior and nest condition of *Mirafra microptera*

Table 2 Breeding success of Mirafra microptera in the study area

Year	Months	Nest observed	Eggs laid	Incubation days	Hatching	Fledgling	HS%	FS%	BS%	CBS%
2017	April	2	4	12	0	0	0	0	0	0
	May	3	4	13	0	0	0	0	0	0
	June	4	8	13	3	2	37.5	66.66	50	25
	July	3	6	12	1	1	16.66	100	33.33	16.66
	Total	**12**	**22**	**12.5±0.57**	**4**	**3**	**18.18**	**75**	**25**	**13.64**
2018	April	0	0	11	0	0	0	0	0	0
	May	3	6	13	0	0	0	0	0	0
	June	7	14	13	3	3	21.42	100	42.85	21.42
	July	9	18	12	5	4	27.77	80	44.44	22.22
	Total	**19**	**38**	**12.25±0.95**	**8**	**7**	**21.05**	**87.5**	**36.84**	**18.42**
2019	April	1	2	11	0	0	0	0	0	0
	May	2	4	12	0	0	0	0	0	0
	June	2	4	14	0	0	0	0	0	0
	July	3	6	13	2	2	33.33	100	66.66	33.33
	August	5	10	12	4	3	40	75	60	30
	September	7	14	13	8	6	57.14	75	85.71	42.85
	Total	**20**	**40**	**12.6±0.81**	**14**	**11**	**35**	**78.57**	**55**	**27.5**
	Grand Total	**51**	**100**	**12.42±0.85**	**26**	**21**	**26**	**81**	**41**	**21**

HS= Hatching success, FS=Fledgling success, BS=Breeding success, CBS= Crude breeding success

Table 3 Relationship between some environmental factors and breeding success of *Mirafra microptera* in the study area

		HS		FS		BS		CBS	
		r	P	r	p	r	P	r	P
Spearman's rho	Temperature (˚C)	-0.749**	0.002	-0.836**	0	-0.730**	0.003	-0.730**	0.003
	Humidity (%)	0.846**	0	0.843**	0	0.865**	0	0.865**	0
	Rainfall (mm)	0.157	0.591	0.187	0.523	0.134	0.648	0.134	0.648

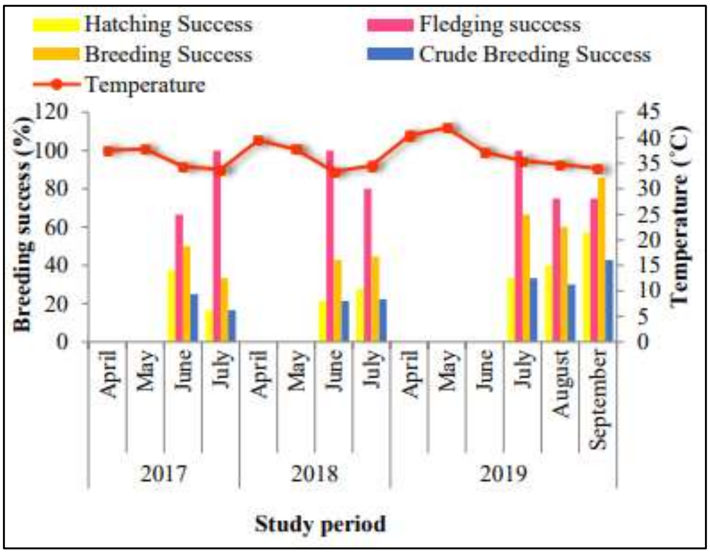


Figure 2A Temperature and breeding success

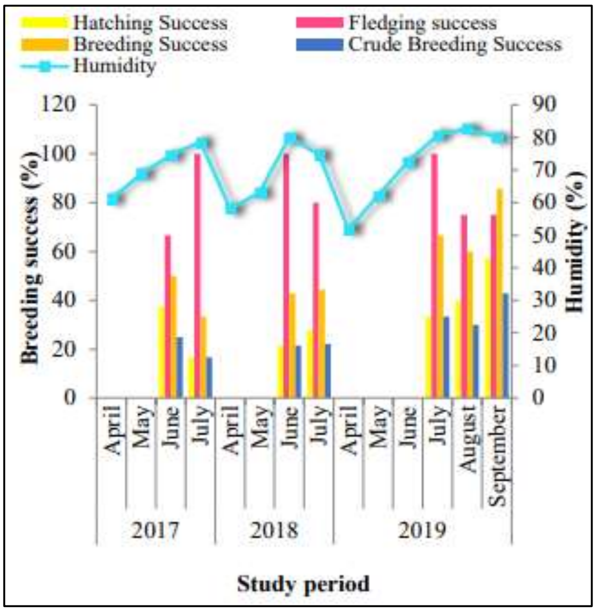


Figure 2B Humidity and breeding success

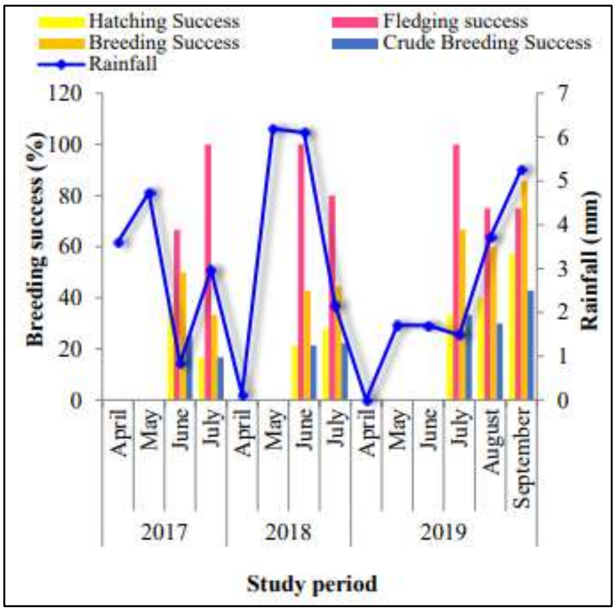


Figure 2C Rainfall and breeding success

Figure 2 Relationship between some environmental factors and breeding success of *Mirafra microptera* in the study area

A. Incubation

B. Two day after hatching

C. Three days after hatching

D. Four days after hatching

E. Six days after hatching

F. Seven days after hatching

Plate 2 Breeding success of *Mirafra microptera* in the study area

A. Fecal cleaning by parents

B. Food carrying and feeding behaviorof both parents

Plate 3 Some behaviors of parental care by *Mirafra microptera* in the study area

A. Grazing of cows and goats in studied area

B. Nest destruction by cows and goats

C. Threaten by dogs

C. Threaten by dogs

Plate 4 Some impacts on *Mirafra microptera* in the study area

Discussion

The breeding season of *Mirafra microptera* in Bagan environs was from March to September during the study periods. The present result coincided with the previous researcher, Hockey *et al.* (2005). They said that several lark species may avoid breeding season in winter as the low ambient temperatures and in mid-summer when the ambient temperature at a peak may make breeding risky. However, the present study on the breeding season of Burmese Bushlark in Bagan environs differed from Kyaw Nyunt Lwin and Khin Ma Ma Thwin (2003) who reported in Birds of Myanmar that breeding seasons of the Burmese Bushlark is from June to October.

The present study of the nest construction period was similar to Thabo (2013) who reported the mean nest construction period of 2.5 days (range 1–3 days) in the Pink billed Lark. In the present study shorter than the 4–9 days reported for other larks (Maphisa *et al.*, 2009) and of Maclean (1970). De Juana *et al.* (2004) stated that both sexes continued to add material to the structure and lining of the nest throughout the incubation period, a common phenomenon in many lark species. Similar behavior was recorded in the study species. *Mirafra microptera* was completely constructed their nest three days. After two days ago, they laid eggs and incubation took 12.5 days and fledglings lasted 7.5 days.

Vickery *et al*. (1999) believed that the grassland bird community also relies on heterogeneity in nesting substrates. Grassland birds make their nests on the ground of forbs, grasses, or litter. The nest shape of Eastern meadowlarks (*Sturnella magna*) is dome-shaped and nest constructing materials are live and dead grasses. The present study was agreed with the statements due to the same types of nest material and nest shape.

According to Maclean (1970) and Tarboton (2011), most Pink-billed Lark nestsorientation face either south or east. During the present study period, the majority of nests faced to the south but also found to north, north-west, and east directions. It is clear that nest orientation may be correlated with the thermoregulatory function and other environmental factors e.g. wind directions which were important determinants of nest orientation on a local landscape level.

According to Morel and Morel 1984, the clutch sizes are 2-3 eggs on average in tropical or arid zone species of family Alaudidae but the clutch size of *Mirafra microptera* in the present study area was 1-2 during the study period. The small clutch size is necessary to ensure the nestlings get the most benefits from the transient. Every increase in the clutch size by one egg means an extra day for the breeding cycle and an extra day that nestlings are potentially exposed to nest predations and the possibility that the favorable conditions may besurpassed.

Relating to the breeding success, the mean incubation period (12.5 days) of present studied species *Mirafra microptera* matched with the 11-13 days in Black Eared sparrow Lark reported by Maclean (1970) and Lloyd (1999). In the present study, the nestling period of 7.5 days of *Mirafra microptera* is similar to African sparrow-larks which generally range from 7-10 days (Lloyd, 1999). Larks are faster growth and can walk or run well and even flutter-fly in some instances upon fledging. This allows the brood to disperse, thus reducing the likelihood of the entire brood being lost to predation (Donald, 2006).

Donald (2006) also observed that ground-nesting larks suffer very high nest depredation rates and crude breeding success is estimated less than 30% for larks. It is similarto the present study bird of crude breeding success (21%) in the present study bird.

Nonetheless, the percentage of crude breeding success of *Mirafra microptera* in the study area was decreased. Depending on the low breeding success and habitat destruction, thepresent study could be suggested that Myanmar endemic species, *Mirafra microptera* should be concentrated for conservation.

Finally, the male and female of the *Mirafra microptera* were slightly different only in the breeding period in the present study area. Moreover, the two sexes of birds examined showed very similar patterns throughout the year.

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INFLUENCE OF POTASSIUM SOLUBILIZING BACTERIA ON GERMINATION AND DEVELOPMENT OF MAIZE (*Zea mays* L.) SEEDLING

Yamin Kyaw[1] Wai Zin Min[2] Sandar Moe[3] and Kyaw Myo Naing[4]

Abstract

Totally five potassium solubilizing bacteria (KSB) were isolated from rhizosphere of grass (*Brachiaria mutica* Staf.) and maize (*Zea mays* L.). To know the density of bacterial inoculum, plate count method was used. To identify the isolated bacteria, basic staining methods and biochemical tests of KB003Hi25™ Identification Kit were used. In order to evaluate the effects of potassium solubilizing bacteria on the growth of maize, five replicates of laboratory experiment with five treatments KSBGR-1(T1), KSBGR-10(T2), KSBMS-1(T3), KSBMR-1(T4), KSBMR-2(T5) and one control were carried out in Microbiology Laboratory, Zoology Department, Pathein University during May 2019 to January, 2020. Seeds were inoculated with solution of 10^8 CFU/mL of KSB and control seeds were not inoculated. The isolated bacterial strains were identified as *Bacillus* sp. The germination percent of maize increase over control at different treatments were 94.4%, 89.6%,85.6%, 82.4%, 78.4%, and 72.8%, respectively in the order T3>T1>T5>T2>T4> Control. In this study, root length and shoot length of treated seedlings increased significantly ($p<0.05$) over control at 4DAS, 6DAS and 7DAS. These results suggested that inoculation of isolated KSB can be considered as efficient alternative biofertilizers to promote maize seed germination and development.

Keywords: Potassium Solubilizing Bacteria, Rhizosphere, biofertilizer, grass, maize

Introduction

Potassium (K) is considered as an essential nutrient and a major constituent within all living cells. Naturally, soils contain K in larger amounts than any other nutrients (Zang and Kong, 2014). Highest proportions of potassium in soils are insoluble rocks and minerals such as micas, illite and feldspar. Potassium involved in the adjustment of plant cellular osmotic pressure and the transportation of compounds in plants. Moreover, potassium promotes the activation of enzymes, the utilization of nitrogen and the syntheses of sugars and protein. Microbes can release soluble K from K-bearing minerals such as K-feldspar, mica and illite.

The use of certain microbes in agricultural soils can assist the solubilization of K in addition to physical and chemical weathering of K minerals (Masood and Bano, 2016). Using K-solubilizing microbes to increase the concentration of available K ions in the soil may mitigate K deficiency (Barker *et al*., 1998). Microbial inoculants that are able to dissolve potassium from minerals and rocks have influence on plant growth and have both economic and environmental advantage (Jabin and Ismail, 2017).

Some bacteria like *Bacillus*, *Thiobacillus*, *Pseudomonas*, *Acidothiobacillus* have been found to simplify and secrete potassium from potassium- bearing minerals in soils (Sheng, 2005 and Liu *et al*., 2012). Some of potassium solubilizing bacteria that capable in dissolving potassium in the soil such as *Paenibacillus* sp., *Bacillus* spp., *B. mucilaginosus* and *B. edaphicus* (Muralikannan, 1996, Sheng, 2005, Sugumaran and Janarthanam, 2017and Liu *et al*., 2012). Berthelin (1983) demonstrated that potassium is solubilized from precipitated forms through production of inorganic and organic acids by *Thiobacillus*, *Clostridium* and *Bacillus*. Microorganisms like *Aspergillus niger*, *Bacillus extroquens*, and *Clostridium pasteurianum* were

[1] Lecturer, Department of Zoology, University of Mandalay
[2] Assistant Lecturer, Department of Zoology, Hinthada Universiy
[3] Assistant Lecturer, Department of Zoology, Pathein University
[4] Lecturer, Department of Zoology, Pathein University

found to grow on muscovite, biotite, orthoclase, microclase and mica in vitro (Archana *et al*., 2013).

Minerals potassium solubilization by microbes which enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble K (Rajan *et al*., 1996). Inoculation of maize and wheat plants with *Bacillus mucilaginosus*, *Azotobacter chroococcum* and *Rhizobium* were used to motilize potassium from waste mica, which in turn acted as a source of potassium for plant growth (Singh *et al*., 2010). Therefore, there are immense possibilities for further increasing the production of crop by application of potassium solubilizing bacteria. Therefore, the present study was conducted with the following objectives are to isolate potassium solubilizing bacteria from the rhizosphere of maize and grass, to investigate the cell characters and staining reactions of isolated bacteria species and to evaluate whether inoculum bacteria could enhance growth parameters of maize.

Materials and Methods

Experimental site and study period

The experiment was conducted at Microbiology Laboratory, Department of Zoology, Pathein University during May, 2019 to January, 2020.

Collection of samples

Maize plants (*Zea mays*) were collected from the cultivated fields of Dawwar Village (17° 2′ 39.08″ N and 95° 27′ 08.06″ E), Pantanaw Township, Ayeyarwady Region and Grasses (*Brachiaria mutica*) were collected from Pathein University Campus (16˚ 48' 19" N and 94˚ 45' 17" E).

Isolation of Potassium Solubilizing Bacteria

One gram of rhizospheric soil or one gram of grinded root pieces was thoroughly mixed with sterilized distilled water and then 10 fold dilution was made. A 20 μL of each dilution was inoculated on Aleksandrov medium (Himedia, India). The plates were incubated at the incubator (30°C) for 7 days and clear zone forming colonies on Aleksandrov medium were selected as potassium solubilizing bacteria (KSBs). Streak plate method was used to purify these selected bacteria. Clear zone formations of isolated bacteria were rechecked by dropping 10μL of bacteria suspension onto Aleksandrov medium.

Biochemical Tests of Isolated Bacteria

Biochemical characters were recorded using KB003 Hi25TM identification Kit (Himedia, India). Selected isolates were added to the wells of kit. Biochemical test characters were recorded and interpretation of the results was following the instructions supplied by the manufacturer. Identification of bacteria was carried out the methods defined in Bergey’s Manual of Determinative Bacteriology (Holt *et al.*, 1994).

Differential Staining Techniques

Gram’s staining (Bradshaw, 1992), capsule staining, endospore staining and acid- fast staining (Cruickshank, 1960) were used to identify the bacteria species.

Catalase Test

Catalase test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh culture of pure isolate was taken on glass slide and one drop of hydrogen peroxide was added (Babu *et al*., 2017).

Detection of Motility

Motility of the isolated bacteria can be detected in semi-solid agar medium (Atlas, 2010). Ten milliliter of semi-solid agar was dispensed in test tubes. The tubes containing the medium were inoculated by stabbing with straight wire. After incubation, motile bacteria will spread into the medium and non- motile will confine to the stab.

Enumeration of Bacteria by Standard Plant count method

All isolated bacteria species associated with the rhizosphere of grass and maize were enumerated. The isolated bacteria from slant cultures were placed and grown in peptone water about 24 h and then streaked on Aleksandrov medium plates. After growing, these were inoculated into the test tubes containing 10mL of peptone water. These tubes were incubated at 37˚ C in the incubator for 24 h. Ten fold dilution were then prepared with sterile distilled water and 20µL of each dilution was spread on the surface of plate count agar plates with three replicates. The agar plates were incubated at 37˚C for 24 h.

After incubation, the number of colonies was counted with the aid of colony counter. And the broth culture of viable cell per mL was calculated as suggested by Reynolds and Farinha (2005). Colony forming unit per milliliter or gram of sample=number of colonies/dilution×amount plated

Inoculum preparation

Isolated bacteria, KSBGR -1 (T1), KSBGR-10 (T2), KSBMS-1(T3), KSBMR-1 (T4) and KSBMR-2(T5) were used as inoculums. Bacteria were grown in Aleksandrov's medium. Selected bacteria were grown in 10 mL peptone water for 24 hours at 37ºC. Final concentrations of inoculums were made to 10^8CFU/mL.

Inoculation of seeds

Maize seeds were sterilized with 0.1 % NaOCl for 2 to 3 minutes. The seeds were washed four times with sterilized distilled water. The surface sterilized seeds (25 seeds per each treatment) for treatments were immersed in each inoculum (10^8 CFU/mL) for 3 hrs. Control seeds were only immersed in diluted peptone water without bacteria.

Experimental design

Five treatments and one control with five replications were considered for the experiment: T1= treated with KSBGR-1, T2= treated with KSBGR-10, T3= treated with KSBMS-1, T4= treated with KSBMR-1, T5= treated with KSBMR-2 and Control = without inoculation of bacteria.

Germination condition

Petri dishes with inoculated seeds (25 seeds per dish) were covered with sterilized wet towel and kept in the dark for two days. After two days, seeds were sown in petri dishes containing 1% agar solution (water agar) supplemented with mica source (0.2%). These were kept under light condition at room temperature. Bacterial suspension was added according during the exposure to light.

Germination parameters

One seedling was randomly selected from each petri dish. Measuring of shoot and root lengths were taken from 1 to 7 days after sowing. Percentage of seed germination was calculated by the following equation (Krishnaswami and Sheshu, 1890);

Germination percent (%) = Number of seed germinated / Total number of seeds × 100

Statistical analysis

Data of the experiment were subjected to statistical analysis using IBM-SPSS software (version 25). The differences between the treatment and control means were determine by using One-way ANOVA with LSD, post-hoc test at 0.05 level.

Results

Total five bacterial isolates were selected as potassium solubilizers. Gram staining, acid-fast staining, endospore staining and capsule staining were carried out to identify cell characters (Table 1). Biochemical tests were recorded using KB003 Hi25^{TM} Identification Kit (Himedia, India). All the isolates were Nitrate reduction, Saccharose and Glucose positive, and Phenylalanine Deamination, H_2S production, Voges Proskauer's, Indole, Adonitol, Rhamnose, Raffinose, Trehalose and Oxidase negative (Table 2 and 3). The percentage increase of germination percent have followed the order T_3 (94.4%) > T_1 (89.6%) > T_5 (85.6%) >T_2 (82.4%) > T_4 (78.4%) and control (72.8%) (Table 4). The individual treatment at 1DAS and 2DAS increased over control but the differences are not significant. ANOVA result for root lengths at 3DAS increased significantly while shoot lengths are not significantly different. At 4DAS, root lengths of T2, T3 and T4 treated seedlings were significantly longer than control. Shoot length of all treated seedlings increased over control except T4. At 5DAS root lengths of T2 and T4 inoculated seedlings were significant while shoot lengths of all treatments increased significantly over control at $p<0.05$. At 6DAS, root and shoot lengths of inoculated seedlings were significantly increased over control ($p<0.05$). At 7DAS, root length of T2, T3, T4 and T5, shoot length of all treatments were increased significantly ($p<0.05$) over control (Table 10 and 11).

Table 1 Morphology and Staining reaction of KSB isolates

Isolates	Motility	Cell size (μm)	Cell shape	Arrangement	Gram	Acid-fast	Endospore	Capsule	Catalase
KSBGR-1	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	+
KSBGR-10	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	+
KSBMS-1	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	-
KSBMR-1	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	-
KSBMR-2	+	0.9-1.35	Short rod	Singly/ pair	+	-	+	+	-

Table 2 Biochemical Test results of selected isolates (StripI)

No	Strip I Test	KSBGR-1	KSBGR-10	KSBMS-1	KSBMR-1	KSBMR-2
1	ONPG	-	-	+	-	-
2	Lysine utilization	-	-	+	+	+
3	Ornithine utilization	-	-	+	+	+
4	Urease	-	-	+	-	+
5	Phenylalanine Deamination	-	-	-	-	-
6	Nitrate reduction	+	+	+	+	+
7	H_2S production	-	-	-	-	-
8	Citrate utilization	-	-	+	+	+
9	Voges Prokauer's	-	-	-	-	_
10	Methyl red	+	+	+	+	+
11	Indole	-	-	-	-	-
12	Malonate utilization	-	-	+	+	+

Table 3 Biochemical Test results of selected isolates (StripII)

No	Strip II Test	KSBGR-1	KSBGR-10	KSBMS-1	KSBMR-1	KSBMR-2
1	Esculin hydrolysis	-	+	-	-	-
2	Arabinose	+	+	-	-	-
3	Xylose	+	+	-	-	-
4	Adonitol	-	-	-	-	-
5	Rhamnose	-	-	-	-	-
6	Cellobiose	-	-	+	+	+
7	Melibiose	+	+	-	-	-
8	Saccharose	+	+	+	+	+
9	Ralfinose	-	-	-	-	-
10	Trehalose	-	-	-	-	-
11	Glucose	+	+	+	+	+
12	Lactose	-	-	+	+	+
13	Oxidase	-	_	-	-	-

Table 4 Germination percent of maize at 7 DAS

Treatment	Germination percent (7 DAS)	Percentage of germinated seeds (%)
Control	18.2 ±2.38 [a]	72.8%
KSBGR-1 (T1)	22.4± 2.07 [b]	89.6%
KSBGR-10 (T2)	20.6±2.19 [a]	82.4%
KSBMS-1 (T3)	23.6±1.34 [b]	94.4%
KSBMR-1 (T4)	19.6±2.40 [a]	78.4%
KSBMR-2 (T5)	21.4±2.60 [a]	85.6%

Table 5 ANOVA result for Germination percent (7DAS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.167	5	18.833	3.870	.010
Within Groups	116.800	24	4.867		
Total	210.967	29			

Table 6 Mean Root length of maize plant at 1 to 7days after sowing in control and treatments (n=5)

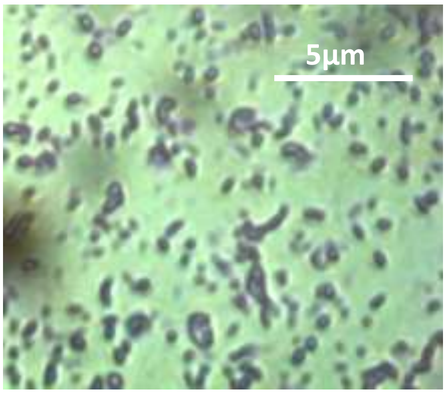
	Root Length (Mean ±SD)						
Treatments	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control	2.06 ± 0.74^{a}	2.92 ± 0.62^{a}	1.70 ± 0.31^{a}	2.12 ± 0.73^{a}	2.00 ±0.51^{a}	2.58 ± 0.29^{a}	2.80 ± 0.49^{a}
T1	3.40 ± 1.42^{a}	3.18 ± 1.26^{a}	1.92 ± 0.94^{a}	2.78 ± 0.77^{a}	3.50 ± 1.75^{a}	3.74 ± 1.65^{a}	4.3 ± 1.65^{b}
T2	3.30 ± 1.04^{a}	2.64 ± 0.96^{a}	2.64 ± 0.47^{a}	3.42± 0.58^{b}	3.78 ± 0.80^{b}	5.56 ±0.87^{ab}	4.56 ± 1.09^{b}
T3	4.18 ± 1.93^{b}	4.30 ± 2.12^{a}	3.62 ± 1.43^{a}	3.90 ± 0.79^{ab}	3.58 ± 2.06^{a}	5.18 ± 2.44^{b}	5.92 ± 1.92^{ab}
T4	4.48± 1.76^{b}	4.98 ± 2.33^{b}	4.32 ± 2.77^{b}	4.54 ± 1.13^{ab}	4.06 ± 0.97^{b}	4.94 ± 1.49^{b}	4.98 ± 1.12^{b}
T5	3.36 ± 1.13^{a}	3.04 ± 0.97^{a}	4.14 ± 1.89^{b}	2.62± 0.90^{a}	3.58 ± 0.93^{a}	4.56 ± 1.20^{b}	4.96 ± 1.50^{b}

Means followed by a common letter in the same column are not significantly different at 5% level by LSD
T1= KSBGR-1, T2= KSBGR-10, T3= KSBMS-1, T4= KSBMR-1, T5= KSBMR-2, Control= without bacteria

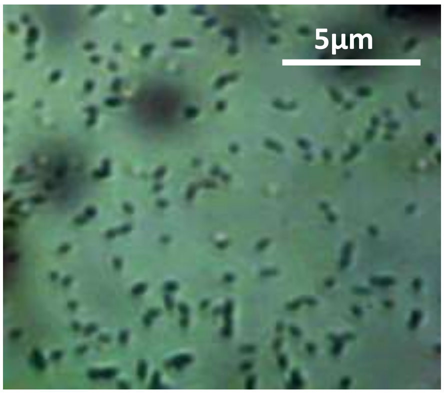
Table 7 Mean Shoot length of maize plant at 1 to 7 days after sowing in control and treatments (n=5)

	Shoot Length (Mean ±SD)						
Treatments	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
Control	1.92± 0.39^{a}	2.38± 0.33^{a}	2.42± 0.34^{a}	4.5± 0.49^{a}	4.20± 0.36^{a}	4.58± 0.62^{a}	6.74± 0.78^{a}
T1	2.00± 0.54^{a}	3.00± 0.72^{a}	3.38± 0.95^{b}	5.98± 1.02^{ab}	7.84± 1.57^{ab}	8.76± 1.48^{b}	11.54± 1.94^{ab}
T2	2.40± 0.65^{a}	2.82± 0.66^{a}	3.24± 0.92^{b}	5.56± 1.08^{b}	6.92± 0.96^{b}	8.50± 1.33^{b}	11.90± 3.75^{ab}
T3	2.14± 0.22^{a}	2.88± 0.76^{a}	3.4± 0.56^{b}	5.62± 0.76^{ab}	7.68± 1.15^{ab}	8.48± 0.76^{b}	10.14± 1.73^{b}
T4	2.00± 0.50^{a}	2.78± 1.03^{a}	2.88± 0.28^{a}	4.42± 0.41^{a}	5.30± 0.90^{a}	7.02± 1.10^{b}	9.64± 2.40^{a}
T5	2.30± 0.76^{a}	3.04± 1.49^{a}	3.08± 0.29^{a}	5.18± 0.46^{b}	6.98± 0.58^{b}	9.20± 2.71^{b}	11.62± 3.35^{ab}

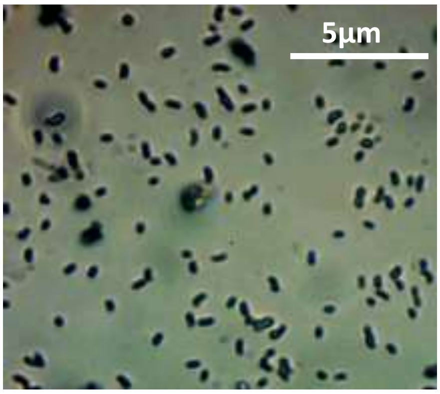
Means followed by a common letter in the same column are not significantly different at 5% level by LSD
T1= KSBGR-1, T2= KSBGR-10, T3= KSBMS-1, T4= KSBMR-1, T5= KSBMR-2, Control= without bacteria



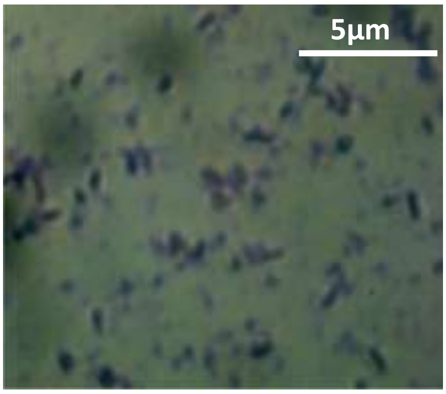
A. Gram Positive Short Rod



B. Not Acid -Fast



C. Endospore



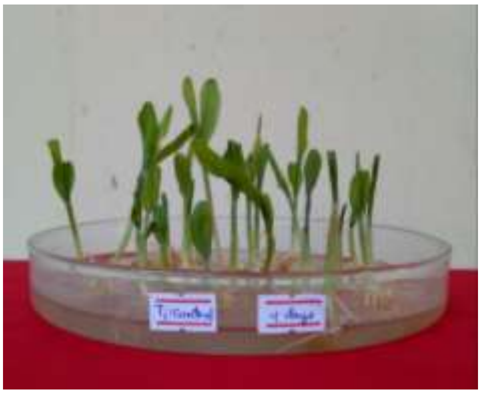
D. Capsulate

Plate 1 Sample of Staining Reaction of KSBGR-10 (0.9- 1.35 µm)

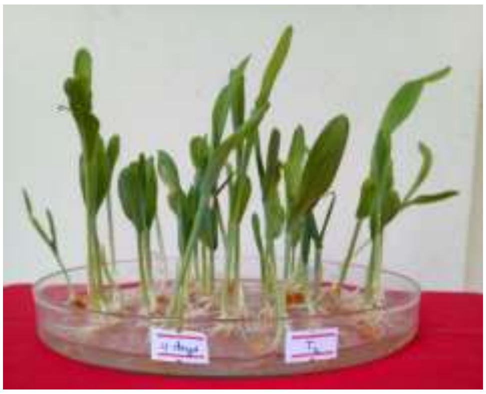
A. Biochemical Test of KSBGR-10

B. Biochemical Test of KSBMS-1

Plate 2 Sample of Biochemical Test of KSBGR-10 and KSBMS-1



A. Control (4DAS)



B. Treatment (4DAS)

C. Maize Seedlings (1DAS)

D. Maize Seedlings (7DAS)

Plate 3 Sample of Maize Seedlings at 1 to 7days after sowings

Table 8 ANOVA result for Root Length (1 DAS)

	Sum of Squares	**Df**	**Mean Square**	**F**	**Sig.**
Between Groups	17.790	5	3.558	1.816	.148
Within Groups	47.020	24	1.959		
Total	**64.810**	**29**			

Table 9 ANOVA result for Shoot Length (1 DAS)

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	.899	5	.180	.618	.687
Within Groups	6.980	24	.291		
Total	**7.879**	**29**			

Table 10 ANOVA result for Root Length (7 DAS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.490	5	5.298	2.978	.031
Within Groups	42.700	24	1.779		
Total	**69.190**	**29**			

Table 11 ANOVA result for Shoot Length (7 DAS)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	94.834	5	18.967	2.967	.032
Within Groups	153.396	24	6.392		
Total	**248.230**	**29**			

Discussion

In the present study isolated strains of KSBGR-1 and KSBGR-10 from the rhizosphere of grass, and KSBMS-1, KSBMR-1 and KSBMR- 2 from the rhizosphere of maize were used as treatments for maize germination. All selected bacterial strains were gram positive, motile, endospore and capsule forming bacteria. KSBGR-1 and KSBGR-10 were catalase positive and KSBMS-1, KSBMR-1 and KSBMR-2 were catalase negative. All the isolates were Nitrate reduction, Methyl red, Saccharose, Glucose positive and Phenylalanine Deamination, H_2S production, Voges Proskauer’s, Indole, Adonitol, Rhamnose, Raffinose, Trehalose and Oxidase negative. In Bergey’s Manual of Determinative Bacteriology, the characteristics of genus *Bacillus* were rod shape, 0.5-2.5 µm, motile, gram positive, endospore produced, catalase positive and oxidase different reaction in different species (Holt *et al*., 1994).

Prajapati and Modi (2012) expressed that *Bacillus* sp. were Glucose, Arabinose, positive and Urease, Phenylalanine deaminase, Adonitol negative. Parmar *et al*. (2016) stated that *Bacillus* sp. were urea hydrolysis and catalase test positive, and Voges Proskauer and H_2S production negative.

In this investigation, selected isolates were similar with the above observations except in catalase test. Thus, KSBGR-1 and KSBGR-10 may be *Bacillus* species because they were catalase positive as describe in Bergey’s Manual. KSBMS-1, KSBMR-1 and KSBMR-2 may be other species because they were catalase negative. Noumavo *et al*. (2013) used 10^8 CFU/mL of rhizobacteria to treat for the growth on maize seed germination and seedling development. In this research, 10^8CFU/mL of KSB was also used to inoculate in the growth experiment of *Zea mays*.

In this research, the percentage of maize germination increased significantly over control ($p<0.05$). The highest germination percent (94.4%) was observed in KSBMS-1 (T3) inoculated seedlings. Noumavo *et al*. (2013) reported that highest germination rate in maize was observed in the treatment with the combination of *Pseudomonas fluorescens* and *P. putida*.

All treatments with potassium solubilizing bacteria (KSB) of this investigation increased significantly ($p<0.05$) over control in root and shoot lengths of maize. In general, seed inoculation with potassium solubilizing bacteria was found to have positive effects on aerial biomass and root biomass in maize plants. This growth promoter effect could be attributed to the potential of these strains to increase the availability of nutrients, such as phosphorus, and siderophore and phytohormone production (Viruel *et al*., 2014), as well as to their capacity to colonize the root system and interact positively with the plant.

In this observation, some isolated strains were *Bacillus* species and inoculation of these strains with mica resulted the significant effect on the germination percent and growth of maize. The findings by Ahmed (2016) and Sheng (2005) on the maize, cotton and rape respectively corroborated the results obtained this study. They also used the mica and inoculated with potassium solubilizing microorganisms (*Bacillus edaphicus*) to investigate the effect on the root and shoot growth. Similar increase in plant growth parameters due to inoculation of KSB have been reported by several researchers in sudan grass (Basak and Biswas, 2009), and in ground nut (Sugumaran and Janarthanam 2007) when treated with K solubilizing *Bacillus* strains.

Conclusion

The rhizosphere of maize and grass samples were used in the study for isolation of potassium solubilizing bacteria. A total of 5 KSB isolates are isolated on Aleksandrov's medium. All the isolated bacteria were found to be capable of solubilizing K from insoluble K-bearing minerals source. KSBGR-1 and KSBGR-10 may be *Bacillus* species because they were catalase positive as describe in Bergey's Manual while KSBMS-1, KSBMR-1 and KSBMR-2 may be other species. These isolated bacteria were used to examine their influence on the growth of maize seedlings. Currently, the use of chemical fertilizers and manures cannot be refused without avoiding a consequent of abruptly decline in food production. Hence, there is an urgent need for alternative nutrients of plant in agriculture to reduce the adverse environmental effects of chemical fertilizers. The screening method used in laboratory is an available technique to select the effective bacterial strain for the growth and development of particular crop. This study confirms the influence of potassium solubilizing bacteria on germination and development of seedlings. These results suggested the possibility to use these potassium solubilizing bacteria as initial culture of biofertilizer to increase the output of maize.

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WING MORPHOLOGY AND ECOLOCATION CALLS OF *Taphozous melanopogon*, Temminck 1841 (Chiroptera: Emballonuridae) FROM CHAE-DAW-YAR CAVE

Khin Moh Moh Tun[1], Sai Sein Lin Oo[2] and Khin Mya Mya[3]

Abstract

The wing morphology of black-bearded tomb bat, *Taphozous melanopogon* (Chiroptera: Emballonuridae) collected from the Chaedawyar Monastery cave, Madaya Township. The study of wing morphology, echolocation frequency of *T. melanopogon* analyzed during June 2018 to February 2019 involved 20 males and 20 females of *T. melanopogon*. The aim of this study was to examine whether wing morphology of bat have relationship with feeding habits. The wing parameters were measured onto one centimeter square graph paper in the field. Evaluation was focused on three major parameters; wing loading (WL), aspect ratio (A) and wing tip shape index (I) between males and females by using t-test. The values of wing loading ($t = 0.881$, $df = 38$, $p = 0.219$), aspect ratio ($t = -196$, $df = 38$, $p = 0.478$) and wing tip shape index ($t = 0.843$, $df = 38$, $p = 0.072$) were calculated. Echolocation frequency of *T. melanopogon* revealed that the peak frequency was 29.85 kHz. The study results concluded that *T melanopogon* wing morphology were not significant differences in nonreproductive female and male on intraspecific.

Keywords: wing morphology, echolocation calls, chaedawyar cave, Madaya Township

Introduction

In ecomorphological analysis, one seeks to establish the nature and strength of the relationship between morphology and ecology. The morphology of bats relates to their ecology, with special attending to the structure of the feeding apparatus, particularly the morphology of the wing in relation to flight performance. In bat ecomorphology, the feeding and flight apparatus have been the subjects of much study (Swartz *et al*., 2003). The food types of bats exploit a wider range than any other mammalian order.

Bats have wings of different shape and sizes. The differences are largely a reflection of the foraging strategy of the bat-where they feed, how they feed, and what they feed on. There are two main ways in which wings can vary. First, wing area can be large or small relative to the size of the bat and second, wings can be short and broad or long and narrow. The different structures of wing shape are also important the relative lengths and areas of the arm-and hand wing vary considerably. The shape of wing tip may be broad and rounded or narrow and pointed. All of these measurements were regarded as a bat's flight style and they can be related to its foraging strategy (Altringham, 1996).

The flight behavior and feeding niches of bats can be known by measuring the wing morphology. Generally, three factors of bats characterized are (i) high wing loading, high aspect ratio, and high wingtip shape indices fly fast in open area, (ii) high wing loading, average aspect ratios and average wingtip shape indices fly in and around the edges of vegetation in background-cluttered space, and (iii) low wing loading, average or low aspect ratios and pointed wing tip shape indices fly in highly-clutter space (Norberg and Rayner, 1987). Wing morphology is really important in shaping the flight style eg: aerial - hawking, trawling, gleaning or perch hunting. Most insectivorous bats catch their prey in flight, therefore flight style and wing morphology are very important in prey selection. Some morphological characteristics can be used to quantify diets to

[1] Dr, Lecturer, Department of Zoology, University of Mandalay
[2] Dr, Associate Professor, Department of Zoology, University of Mandalay
[3] Dr, Professor (Retd.), Head of Department of Zoology, University of Mandalay

some degree, other external variables are also important in determining the diets of insectivorous bats eg: season, local insect community composition or geographic range (Moosan *et al*., 2012).

Wing characteristics which were measured body weight, forearm length showed the strongest relationship with hard insects followed by longest cranial length. The content of soft insects in bat diets was negatively related to body weight, forearm length and longest cranial length (Weterings & Umponstria, 2014).

In insectivorous bats, echolocation call structure and wing morphology is a primary determinant of foraging strategy (Norberg and Rayner 1987). The echolocation calls of microchiropteran bats have been categorized as constant frequency (CF: single tones which remain at one frequency for a time) or frequency modulated (FM: sweeping up or down in frequency). Calls can be made up of one or more of these components, and are consequently described as FM calls, CF/FM calls (with a CF components followed by an FM component (Jennings *et al*. 2004). Emballonurid bats foraging in edge space near vegetation and over water used higher frequencies, shorter call durations and shorter pulse intervals compared with species mostly hunting in open, uncluttered habitats (Jung *et al*. 2007).

Emballonurid bats produce shallow-modulated and multi-harmonic echolocation calls with most energy concentrated in the second harmonic. Although overall call structure is rather similar within the family, there are species-specific differences in call parameters, namely peak frequency, call duration, pulse interval, direction of call modulation, and presence or absence of short, frequency-modulated (FM) components. Echolocation call libraries are frequently developed over large geographic areas. Species that passes similar calls over broad geographic scales may however have allopatric distributions and hence may be identifiable from their echolocation calls if geographic range can be predicted accurately (Hughes *et al*. 2010).

In Upper Myanmar, there has been few research conducted on ecomorphology of some insectivorous bats. Nyo Nyo Tun (2007) worked on ecomorphology of five rhinolophids bats, three hipposiderids bats and one vespertilionid species. *Taphozous melanopogon* does not include in her study. The ecomorphology need to be studied as feeding ecology and flight performance was related. The aim of this study was to examine whether wing morphology of bat have relationship with feeding and foraging habitat. It is expected that wing shape will reflect the feeding habitat and feeding habit. Therefore, the present study has been undertaken with the following objectives:

- to assess the ecomorphology of *Taphozous melanopogon* from the study area
- to analyze the relationship between wing morphology of males and females by using multivariate statistical analysis
- to analyze the echolocation calls of *T melanopogon* in the study area

Materials and Methods

Study site

The study was conducted at Maha-bawdi-chaedawyar monastery cave (22˚10″45.481″N, 096˚15'17.770″E) in the vicinity of Taungpulu village, Madaya Township, Mandalay. The colony resides in a limestone cave in the vicinity of the monastery. The cave is surrounded by a thick vegetations bamboo grove and woody plants (such as, Thityar, Ingyin, Dahat, Yinkhat etc.). Moreover, bananas plantations, mangoes trees, betel leaves, guava orchards and cultivated plots surround the adjacent areas (Fig 1). The study was carried out from June 2018 to February 2019.

Measurements and analysis of wing morphology

Bats were captured by hand nets inside the cave. Wing measurements were taken on alive and anaesthetized adult males and non-pregnant females. Body mass (M) was determined using a spring balance, Pesola (600 g) and forearm length was measured using digital calipers in accuracy 0.01 mm. The analysis of wing morphology followed after (Norberg and Rayner, 1987).

Body mass (M), was taken fresh specimens from which wing dimensions were also measured. Wingspan, (B), is the distance between the wingtip of a bat with wing extended so that the leading edge is straight. Wing area, (S), is the combined area of the two wings, the entire tail membrane and the portion of the body between the wings. Aspect ratio, (AR), calculated is the square of wing span divided by wing area, $A= B^2/S$ whereas, Wing loading, (WL), is mass (M) times gravitational acceleration ($g= 9.81$ m/ s²) divided by wing area, $WL = Mg / S$. Tip length ratio, (Tl), is calculated the ratio of the length of the hand wing, L_{hw}, to the length of the arm wing, L_{aw}. $Tl = L_{hw}/L_{aw}$ and the tip area ratio, (Ts), is the ratio between the hand wing area (chiropatagium) S_{hw} to the arm wing area (plagiopatagium plus propatagium) S_{aw}. $Ts = S_{hw}/S_{aw}$. Tip shape index, (I), was evaluated as $I = Ts / Tl – Ts$ (Fig 2).

Wing tracing

In tracing the wing of *Taphozous melanopogon*, first each specimen was placed onto one centimeter square graph paper with the dorsal up, then the left wing the fully stretch to get a maximum span and fully stretched of the wing membrane. The uropatagium was also fully stretched. Then, the wing was carefully traced along the edges including one half of the head and the uropatagium.

Recording of echolocation calls

Echolocation calls were recorded for bats using an ultrasound detector (D1000X, Pettersson Electronic AB, Uppsala, Sweden) at a distance about 1 m the bat flew freely in dark after released from the cave entrance. Time-expanded calls were digitized to a sampling rate of 44.1 kHz with 16 bits precision and analyzed using the software Batsound (version 4.2.1). Start-frequency (Start-F, kHz) and end frequency (End-F, kHz) of each harmonic, pulse duration (PD, ms); DF was measured from power spectra, Start-F and End-F from spectrograms, and PD and IPI from oscillograms following Zhang *et al*. (2007).

Statistical analysis

The student t-test was used to analyze the difference ecomorphology of bat between males and females. Results were considered significant at $P < 0.05$. The Statistical Analysis for Social Sciences SPSS (version 22) was used for analysis.

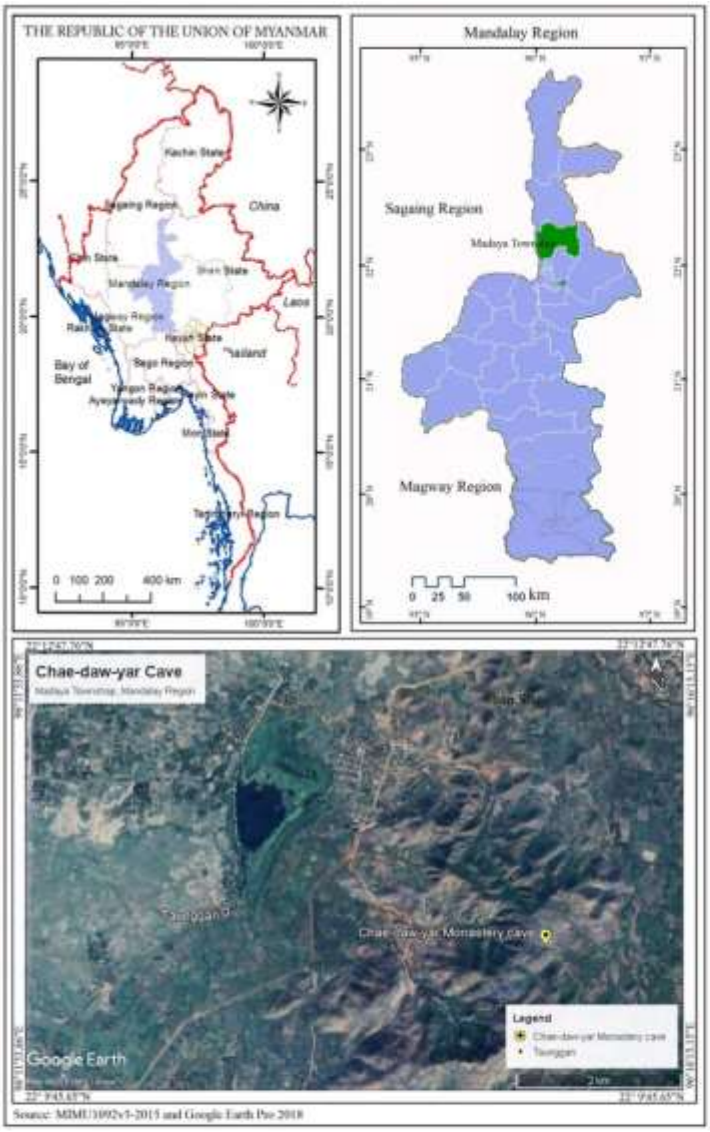

Figure 1 Location: Chae-daw-yar Monastery Cave, Madaya Township.

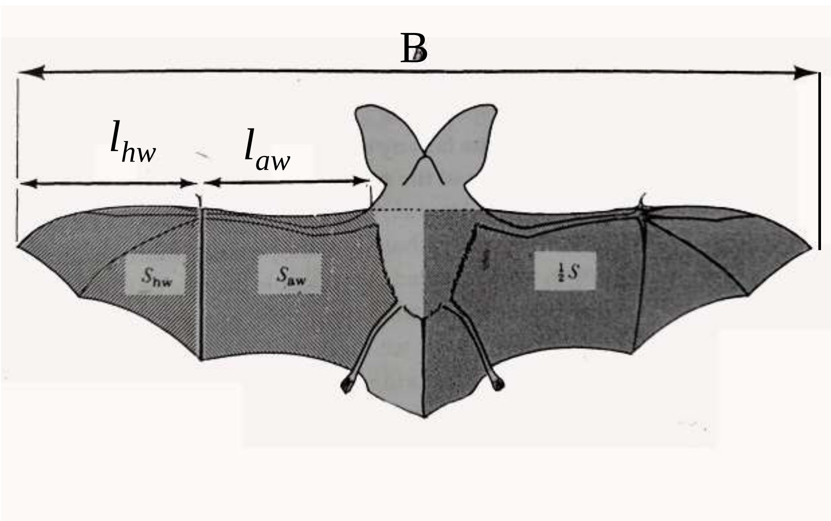

Figure 2 Wing trace of *Taphozous melanopogon* illustrating the flight morphology measurements used (After Norberg and Rayner 1987)

Results

A total of 40 specimens of *Taphozous melanopogon* (20 males and 20 females) were involved to assess the relationship between wing morphology. Morphometric data on the 13 factors to the ecomorphological aspects of the bats wing were examined and mean taken for statistical analysis and evaluation of ecomorphology of wings (Appendix 1).

The results of average body mass, (64.95 kg); forearm, (0.027 mm); wing span, (0.16 m); wing area, (0.018 m^2); aspect ratio, (9.06); wing loading, (15.21 m^2); length of hand wing, (0.182); length of arm wing, (0.168); hand wing area, (0.0058); arm wing area, (0.0092); tip length ratio, (1.09); tip area ratio, (0.65) and tip shape index (1.56) were calculated in males while the average of body mass, (65.5 kg); forearm, (0.0264 mm); wing span, (0.164 m); wing area, (0.018 m^2); aspect ratio, (9.10); wing loading, (14.53 m^2); length of hand wing, (0.176); length of arm wing, (0.163); hand wing area, (0.0060); arm wing area, (0.0092); tip length ratio, (1.09); tip area ratio, (0.63); and tip shape index, (1.44) were calculated in female (Table 1).

Evaluation of was pointed on three major parameters; wing loading (WL), aspect ratio (A) and wing tip shape index (I) between males and females by using t-test. The values of wing loading ($t = .881$, $df = 38$, $p = 0.219$), aspect ratio ($t = -196$, $df = 38$, $p = 0.478$) and wing tip shape index ($t = .843$, $df = 38$, $p = 0.072$) were calculated (Appendix 2). Wing parameters were used to compare body mass (M), forearm (FA), wing span (B), wing areas (S), aspect ratio (A), wing loading (WL), tip length ratio (Tl), tip areas ratio (Ts)and tip shape index (I) between males and females, yielding no significant differences sexes.

The present study, echolocation calls of *T. melanopogon* were recorded in start frequency, 21.62 kHz and end frequency value 35.12 kHz while the FMAXE, 29.85 kHz. The value of maximum frequency 32.55 kHz, minimum frequency 25.53 kHz and duration 4.8 ms while the average frequency value 29.36 kHz were recorded in this study (Figure 3). According to the results, the wing morphology and skull structures were not significantly different in both sexes.

Table 1 Morphometric measurements of males and females of *Tahpozous melanopogon*.

Wing Parameters	Male (N=20)				Female (N=20)			
	Mean	SD	Min	Max	Mean	SD	Min	Max
FA(mm)	0.027	± 0.0017	0.025	0.03	0.0264	± 0.0031	0.021	0.032
Mass(kg)	64.95	± 1.0501	62	66	65.5	± 1	64	68
B^2(m)	0.159	± 0.0215	0.24	0.3	0.1637	± 0.0279	0.11	0.187
S(m^2)	0.0176	± 0.0023	0.0126	0.0204	0.0180	± 0.0023	0.0122	0.0216
A	9.06	± 0.7842	7.77	11.02	9.10	± 1.9354	8.2424	10.3889
WL(N m^2)	15.21	± 2.7825	12.50	21.02	14.5256	± 2.1177	10.9579	19.62
L_{hw}	0.1824	± 0.0107	0.162	0.204	0.1757	± 0.0427	0.002	0.204
L_{aw}	0.1680	± 0.0135	0.13	0.184	0.1632	± 0.0404	0.0021	0.188
S_{hw}	0.0058	± 0.0007	0.0042	0.0066	0.0060	± 0.0008	0.0042	0.0068
S_{aw}	0.0092	± 0.0016	0.0056	0.0112	0.0092	± 0.0015	0.0056	0.012
I	1.5575	± 0.5269	0.9117	2.6875	1.44	± 0.3458	0.91	1.95
T_l	1.0907	± 0.0862	0.9674	1.2769	1.09	± 0.0965	0.95	1.3333
T_S	0.6474	± 0.1009	0.5098	0.8214	0.63	± 0.0734	0.5510	0.82

Abbreviations used are: n, numbers of bats; M, body mass; FA, forearm length; B, wing span; S, wing areas; A, aspect ratio; WL, wing loading; Shw, hand wing area; Saw, arm wing area; L_{hw}, length of hand wing; Law, length of arm wing; Ts, S_{hw}/S_{aw}; Tl, L_{hw}/L_{aw}; I, wing tip shapeindex $I = Ts/(Tl – Ts)$.

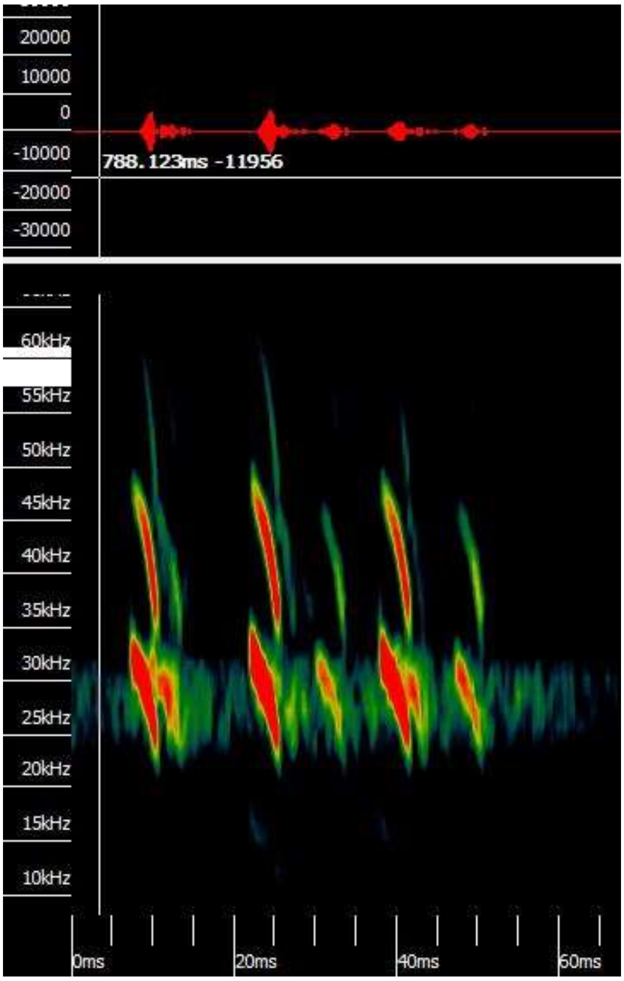

Figure 3 Waveform and sonogram of echolocation pulse from *Taphozous melanopogon*

Discussion

The present study was carried out on wing parameters taken on forty specimens and skull structures of twenty specimens of black-bearded tomb bat in males and females from June 2018 to February 2019. *Taphozous melanopogon* (Emballonuridae) is a medium-sized, bat with the wings moderately long, narrow and tip pointed. Based on the three parameters values of wing loading ($t = 0.881$; $df = 38$, $p = 0.219$); aspect ratio ($t = -196$; $df = 38$, $p = 0.478$) and tip shape index ($t = .843$; $df = 38$, $p = 0.072$) were recorded in male and female. Adams, (1997) stated that wing morphology varies not only with body size but also between sexes and among developmental stages within single species. The wing loading, aspect ratio and mass influence the variation of flight performance, feeding niche and speed. Altringham, (1996) reported that the best examples are perhaps some *Taphozous* species from the emballonurids and other mollossids. They feed in the open, catching insects on the wing. Their fast flight means that they cannot turn tight circles. They would not be good at hunting among trees, or at hovering to pick insects of foliage.

The present study of females, the average wing loading was lower than in males. The females and males of wing loading were (14.53 $m^2 \pm 2.1177$ and 15.21 $m^2 \pm 2.7824$) and while the average wing area of females and males were (0.0180 $m^2 \pm 0.0023$ and 0.0176 $m^2 \pm 0.0023$). But the value of body mass in females is not different with males. The average body mass in females was (0.264 ± 0.0031) while the average body mass in males was (0.0269 ± 0.0017) respectively. Stern *et al.* (1997) studied that wing loading of adult females fluctuated greatly with seasonal changes in body mass. Wing loading of females, a factor of their greater wing areas and lower body mass, was significantly lower than in males. Norberg and Rayner, (1987) stated that the bat with short, rounded wingtips are found to be slow flying, maneuverable bats, in association with low AR and low WL. To be maneuverable, that is to have a small turning circle and fly slowly. The fast, efficient flyers hawking on insects in the open, high WL, high AR species are not very maneuverable. However, they often have pointed wingtips, which increase their agility. Agile bats have the ability to rapidly initiate a roll, altering their flight path. The relationship between wing morphology and agility is therefore complex, involving some important adaptations. Findley (2016) disclosed the wing morphology for 136 species of bats representing 15 families. It is noted that wing area and wing loading were positively correlated with overall size but that wing length was negatively related to the length of hand wing and the length of arm wing variables.

Echolocation calls of *Taphozous melanopogon* were appeared long broadband multi-harmonic frequency-modulated (FM) call and low intensity. Hughes *et al.* (2011) reported that *T. melanopogon* showed the longest narrowband component in its call, and if the curvature of the call could be quantified then species identification within this group may be improved. Norberg and Rayner (1987) reported that *T. melanopogon* was used narrow band echolocation calls at relatively lower frequency (usually less than 40 kHz) in more open area when they were searching for prey.

The study revealed that the value of start frequency was 21.62 kHz and end frequency 35.12 kHz and the peak frequency was 29.85 kHz. Wei *et al* (2008) also recorded 30.10 kHz Guangxi Province in China. Nang Aye Aye Shein (2007) also recorded that the peak frequency value was 28.75 kHz in Patheingyi Township and these results indicated that these features the ability to fly fast in open area or detecting relatively large prey. Lei Lei Thin (2012) recorded that 29.56 kHz at Mandalay University and also stated that start frequency of *T. melanopogon* was 30.67 kHz, end frequency value was 21.94 kHz, and pulse duration value was 6.34 ms respectively. Therefore, the result of the present was quite similar to the above authors.

Conclusion

In the present study, it was observed that the wing span was moderate long, narrow and pointed wingtips while wing loading and aspect ratio were high. Therefore, it is assumed that the bat will have an ability to fast fly in open area and hawk insects at high altitudes. Similarly, Wei *et al*, (2008) also pointed out that aspect ratio and wing loading of *T. melanopogon* were high while wing span was long but tip shape index was found to be low. These features suggest an ability to fly fast in open areas, over treetops, and along the edge of forest or semi-cluttered habitats. Norberg and Rayner (1987) provided an overall view on the ecomorphology of flight and feeding and serve as important foundations. However using the three flight parameters, wing loading, aspect ratio and tip shape index appeared not enough to classify habitat segregation. The present study of wing morphology and echolocation calls may be aid the bats to fly various flight performances to catch the captured prey.

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Appendix 1

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Mass	Equal variances assumed	3.973	.053	.562	38	.577	.00045	.00080	-.00117	.00207
	Equal variances not assumed			.562	29.538	.578	.00045	.00080	-.00119	.00209
FA	Equal variances assumed	.306	.584	-1.696	38	.098	-.55000	.32424	-1.20639	.10639
	Equal variances not assumed			-1.696	37.910	.098	-.55000	.32424	-1.20644	.10644
B^2	Equal variances assumed	.015	.904	-.622	38	.537	-.00411	.00660	-.01748	.00926
	Equal variances not assumed			-.622	37.841	.537	-.00411	.00660	-.01748	.00926
S	Equal variances assumed	.045	.833	-.497	38	.622	-.00036	.00072	-.00183	.00111
	Equal variances not assumed			-.497	37.985	.622	-.00036	.00072	-.00183	.00111
A	Equal variances assumed	.513	.478	-.196	38	.845	-.04291	.21841	-.48506	.39923
	Equal variances not assumed			-.196	35.063	.845	-.04291	.21841	-.48628	.40045
WL	Equal variances assumed	1.561	.219	.881	38	.384	.68898	.78188	-.89385	2.27182
	Equal variances not assumed			.881	35.482	.384	.68898	.78188	-.89755	2.27552
L_{hw}	Equal variances assumed	2.191	.147	.396	38	.695	.00390	.00986	-.01605	.02385
	Equal variances not assumed			.396	21.386	.696	.00390	.00986	-.01657	.02437
L_{aw}	Equal variances assumed	1.764	.192	.504	38	.617	.00479	.00952	-.01447	.02406
	Equal variances not assumed			.504	23.220	.619	.00479	.00952	-.01488	.02447
S_{hw}	Equal variances assumed	2.338	.135	-.164	38	.871	-.00004	.00024	-.00053	.00045
	Equal variances not assumed			-.164	35.110	.871	-.00004	.00024	-.00054	.00046
S_{aw}	Equal variances assumed	1.376	.248	-.268	38	.790	-.00013	.00049	-.00111	.00085
	Equal variances not assumed			-.268	37.709	.790	-.00013	.00049	-.00111	.00085
I	Equal variances assumed	3.423	.072	.843	38	.405	.11877	.14092	-.16651	.40406
	Equal variances not assumed			.843	32.808	.405	.11877	.14092	-.16800	.40555
Tl	Equal variances assumed	.179	.674	-.059	38	.953	-.00172	.02892	-.06026	.05683
	Equal variances not assumed			-.059	37.526	.953	-.00172	.02892	-.06028	.05685
Ts	Equal variances assumed	2.667	.111	.469	38	.641	.01309	.02789	-.04337	.06956
	Equal variances not assumed			.469	34.722	.642	.01309	.02789	-.04355	.06974

Appendix 2

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
A	Equal variances assumed	.513	.478	-.196	38	.845	-.04291	.21841	-.48506	.39923
	Equal variances not assumed			-.196	35.063	.845	-.04291	.21841	-.48628	.40045
WL	Equal variances assumed	1.561	.219	.881	38	.384	.68898	.78188	-.89385	2.27182
	Equal variances not assumed			.881	35.482	.384	.68898	.78188	-.89755	2.27552
I	Equal variances assumed	3.423	.072	.843	38	.405	.11877	.14092	-.16651	.40406
	Equal variances not assumed			.843	32.808	.405	.11877	.14092	-.16800	.40555

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INVESTIGATION OF WATER PARAMETERS AND FISH STATUS FROM PAUK INN AND PADONMILAY INN, CHAUNG U TOWNSHIP, SAGAING REGION

Khin Thandar Latt[1], Htay Khaing[2], Myint Kyaw[3]

Abstract

In the present study, a total of 10782931 individuals, 40 species belonging to 30 genera, 17 families and eight orders from Pauk Inn (site I) and 780133 individuals, 33 species under 26 genera, 16 families and eight orders from Padonmilay Inn (site II) were recorded during October 2018 to September 2019. The total production fish weight was recorded over 283581 kg/yr from Site I and over 15802 kg/ yr. from site II. The water sample was collected from both study sites for analyzing some water parameters during planting and harvesting period of *Melon* spp. and other crops were examined by the laboratory of water supply and sanitation department of Mandalay. Some parameters of water temperature, pH and total alkalinity from both sites were fall within the EPA limit and the rest of the water parameters were unsuitable limit for fish fauna in the study area. This study was aimed to focus the limnology and sustainable utilization of floodplain for fisheries development.

Keywords: Wetlands (Inn), Water parameters, Fish, EPA limit

Introduction

Wetlands are ecosystems or units of the landscape that are found on the interface between land and water. Wetlands are covered approximately 5% - 8% of the global land area. These are important part of the ecosystem and are among the most threatened of all environmental resources. Wetlands are one of the most productive ecosystems and play crucial role in hydrological cycle. Wetland ecosystems are associated with a diverse and complex array of direct and indirect use depending on the type of wetlands, soil water characteristics and associated biotic influences. Direct uses include water supply source and harvesting of wetland products such as fish and plants resources. Indirect benefits are derived from environmental functions such as floodwater retention, groundwater recharge/ discharge, climate mitigation and nutrient abatement (Abraham, 2015).

Fish are cool blooded aquatic vertebrates. They are keystone species which determine the distribution and abundance of other organism in the ecosystem they represent and good indicators of water quality and health of the ecosystem (Malakar and Boruah, 2017). Fish is sensitive to change in water chemistry due to different antropogenic activities from their catchment. Fish assemblages have widely been used as ecological indicators to assess and evaluate the level of degradation and health of water bodies at various spatial scales. However, the impact of the anthropogenic activities, habitat degradation, exotic species introduction, water diversions, pollution and global climate change are main causative agents for the aquatic species rapid decline (Basavaraja *et al*., 2014).

In Myanmar, fisheries can be classified into inland fisheries and marine fisheries. The inland fisheries are mainly flood fisheries made possible by the vast river system and heavy raindfall. There are also leasable fisheries, which cover a large area. Leasable fisheries operate in streams, lakes and ponds during the monsoon. A fishery is leased through a bidding process, and winning bidder is granted the right to harvest the fisheries resources in demarcated "Inns" from September to April. Revenue from the lease is collected by the Department of Fisheries listed throughout the country, of which about 3800 are being operate at present (Win Aung, 2019).

[1] Dr, Lecturer, Department of Zoology, Monywa University
[2] Dr, Lecturer, Department of Zoology, Monywa University
[3] Lecturer, Department of Zoology, Monywa University

In Chaung U Township, there are ten flooded plains (Inns). Among these, Pauk Inn is the largest and highest production of nutritious fish resources area and Padonmilay is the second largest Inn, which provides fish resources for local people. During the last decades, agricultural activities have been expanded in the wetland area of the Chaung U Township very rapidly which has affected the wetland ecosystem. According to Sabbir *et al*., 2010, water quality focuses on the various aspects of the physicochemical parameters of water that detect the status of pollution and suitability of a particular water body for various aquatic organisms as well as fisheries. Thus, the investigation of water parameters and status of fishes from two flooded plains in Chaung U Township is importance. So, the present study was carried with the following objectives:

- to investigate the water quality in water from Pauk Inn and Padonmilay Inn and
- to assess the fish status in the two study sites.

Materials and Methods

Study sites and study area

Chaung U Township is situated in Sagaing region within the Dry Zone of Central Myanmar, which has dry and hot climate. It is located at the southwestern part of Monywa Township. It lies between Latitudes 21° 57′ 0″ North and Longitudes 95° 16′ 0″ East. Two study sites were chosen in the present study.

Site I - Pauk Inn lied Eastern bank of Chindwin River, Chaung U Township, Sagaing Region and Central Myanmar. It is situated between Taw Chaung Gyi and Makyi Gwa Village. It lies between 21°48' 54.20" N-21°48'50" N and 95°11'34" E-95°13'11.55" E. It extends approximately 12.88 kilometers in length and covers an area of 180.63 hectares. The water body of Pauk Inn is drained from Chindwin River in June. The depth of water in this area is around 7 meters in the rainy season but the water level is very low in summer. Some crops were cultivated in southern part of the study area. There are many houses near the southern part area. *Melon* spp. was cultivated around the northern part of Inn. The water body of the northern part of the Pauk Inn is derived by Chindwin River via from southern part of the Pauk Inn.

Site II – Padonmilay Inn is situated between Makyi Gwa Village and Thone Pan Hla Village, Chaung U Township. It is located at a distance of approximately 5km from Chindwin River. The water body of the Padonmilay Inn is drained by Chindwin River via from Pauk Inn. The water level in the study area is around 6 meters in the rainy season but very low in summer. It lies at Latitudes 21° 50' 12.71" N and Longitude 95° 12' 24.84" E. It measures 10.6 km from north to south and 4.83 km) from east to west and covers an area of 150.61 hacteras. *Melon* spp. was cultivated extensively along the both sides of the Padonmilay Inn.

Study period

The study period was conducted from October 2018 to September 2019.

Sample collection

The present study is an attempt to study of fish fauna from Pauk Inn and Padonmilay Inn, Chaung U Township. Samplings were done twice a month. Collections were made from both study sites caught by fishermen. The physical appearance of fish was noted down and the photo was also taken immediately after capturing the fish. Small specimens were preserved in 5% formalin for future examination. The medium-sized specimens were injected with 10% formalin on the side of abdomen for total fixation before they were preserved.

Identification and classification

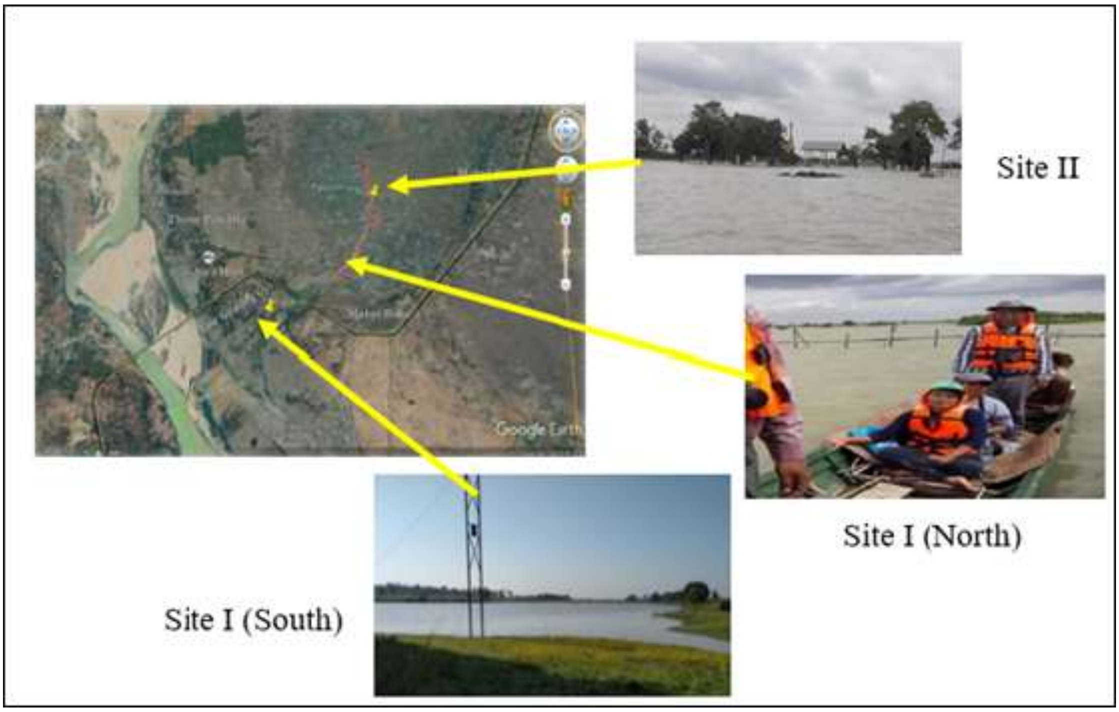
Genus and species were identified according to Lagler *et al*., (1977), Talwar Jhingram (1991), FishBase and 2013 Jayaram (2013). The identified specimens were arranged and presented according to the system of classification given by Jayaram (2013).

Collection of water sample and analysis

For water quality analysis, random samples of water were collected from the study site in the morning during planting period and harvesting period of *Melon* spp. around the study area. Planting period of *Melon* spp. started from December to March and harvesting period started from April to June. Water samples were collected from 50 cm depth in each location. Water samples were collected in clean plastic bottles. Bottles were rinsed with distilled water and then the water from the Inn (wetland) before sample collection. The water parameters such as water temperature, pH, nitrites, alkalinity, dissolved oxygen (DO), biochemical oxygen demand (BOD) was estimated in the laboratory of water supply and sanitation department, Mandalay Region.

Collection of data

In the present study, fish catch statistics has been recorded at monthly interval by visiting the fish landing sites. The fishermen families residing in these areas have been interviewed. Interview from the leaseholder, the cultured fish species such as *Hypophthalmichthys molitrix, Ctenopharyngodon idellus, Cyprinus carpio, Barbonymus gonionotus, Gibelion catla, Labeo rohita* and *Colisa labiosus* were seeded only in Northern part of Pauk Inn in May. The productivity rates of fish from the study area were taken by fishery lessee and local fishmonger that were selling in the market.



(**Source:** Google earth 2018)

Plate 1 Location map of the study area with study sites

(A) (B) (C)

(D) (E) (F)

Plate 2 Environmental Impacts to study area (A)Farming near the study area (B) washing medium cups (C) Pesticides on farming (D) Plastic Mulch waste (E) Feeding ground for birds (F) Pasturing of Domesticated animals

(A) Fishing activities (B) Fish collecting (C) Fish selling

Plate 2 Fishing activities and fish selling

(A) *Channa striata* (B) *Puntius sarana* (C) *Glosogobius giuris*

Plate 4 Some skin infected fish species in the study area

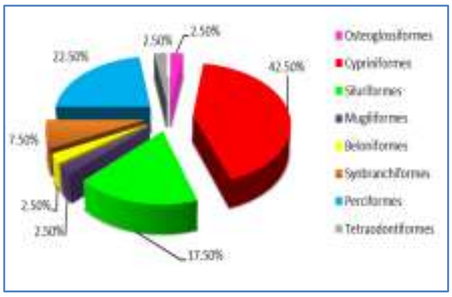


Figure 1 Percentage composition of fish species in different orders from Site I Pauk Inn

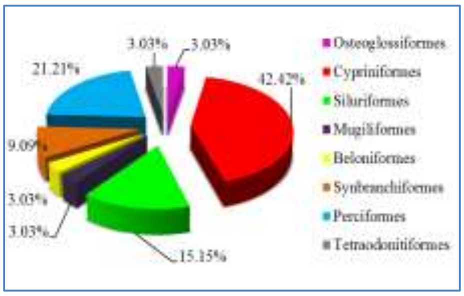


Figure 2 Percentage composition of fish species in different orders from Site II Padonmilay Inn

Result

Species composition of recorded fish species

A total of 40 fish species of fishes belonging to 30 genera, 17 families and eight orders were recorded from Site I Pauk Inn, Chaung U Township. Of these eight orders, the order Cypriniformes with (17 species, 42.5%), followed by Perciformes with (nine species, 22.50%), Siluriformes with (seven species, 17.5%), Synbranchiformes with (three species, 7.50%) whereas each of Osteoglossiformes, Mugiliformes, Beloniformes and Tetraodontiformes with (one species, 2.50%). In the study period, the maximum numbers of 37 species were recorded in February, while the highest number (1545180 individuals) was recorded in December. While the minimum numbers of three species and the lowest number of (1871 Individuals) was recorded in May. *Puntius sarana* was the highest total number of 5883946 individuals while *Johnius coitor* was the lowest number of four individuals (Table 1 and Fig.1).

A total of 33 fish species belonging to 26 genera, 16 families and eight orders were recorded from Site II Padonmilay Inn (Lake), Chaung U Township. Among the eight orders, order Cypriniformes with (14 species, 42.42%), followed by Perciformes with (seven species, 21.21%), Siluriformes with (five species, 15.15%), Synbranchiformes with (three species, 9.09%) whereas each of Osteoglossiformes, Mugiliformes, Beloniformes and Tetraodontiformes with (one species, 3.03%). During the study period, the maximum numbers of 32 species were recorded in February (2019) while the highest number of (445897) individuals were recorded in November. While the minimum numbers of three species and the lowest number of (24) individuals were recorded in May. *Puntius sarana* was the highest total number of 286108 individuals while *Labeo boga* and *Xenentodon cancila* were the lowest number of one individual (Table 1 and Fig.2).

Production of fishes during the study period

In the present study, total production of fishes was over (283581.39 kg/yr) from Pauk Inn. The highest catch weight was found in December (70483.2 kg/yr) while the lowest in May (38.4 kg/yr). There were 40 fish species were collected from the Site I. Total production of fishes was over (15802.56 kg/ yr) from Padonmilay Inn. According to the recorded data, the highest catch weight was found in November (7406.4 kg/yr) while the lowest in May (1.2 kg/yr). There were 33 fish species were collected from the Site II. All fishes were directly sold to traders, wholesalers and retailer. The majority of fish traders from the study area were retailers from local market and wholesalers from near Township such as Monywa and Chaung U. Often fishes were sold nearest villages in the study area due to local demand.

Physico-chemical parameters of water from two study sites

Physico-chemical parameters of water were observed in Pauk Inn and Padonmilay Inn (Lake) during planting period and harvesting period from the study area (Table 2).

Table 1 List of fish species recorded from Pauk Inn and Padonmilay Inn

No.	Order	Family	Scientific Name	S.I	S.II
1	Osteoglossiformes	Notopteridae	*Notopterus notopterus*	+	+
2	Cypriniformes	Cyprinidae	*Hypophthalmichthys molitrix*	+	-
3			*Esomus danricus*	+	+
4			*Amblypharyngodon atkinsonii*	+	+
5			*Ctenopharyngodon idellus*	+	+
6			*Puntius carnaticus*	+	+
7			*Puntius sarana*	+	+
8			*Puntius chola*	+	+
9			*Barbonymus gonionotus*	+	+
10			*Cyprinus carpio*	+	+
11			*Osteobrama belangeri*	+	+
12			*Osteobrama cunma*	+	-
13			*Cirrhinus mrigala*	+	+
14			*Catla catla*	+	+
15			*Labeo boga*	+	+
16			*Labeo calbasu*	+	+
17			*Labeo rohita*	+	+
18			*Labeo stoliczkae*	+	-
19	Siluriformes	Bagridae	*Sperata aor*	+	-
20			*Mystus cavasius*	+	+
21			*Mystus pulcher*	+	+
22		Siluridae	*Ompok bimaculatus*	+	-
23			*Wallago attu*	+	+
24		Clariidae	*Heteropneustes fossilis*	+	+
25		Loricariidae	*Pterygoplichthys pardalis*	+	+
26	Mugiliformes	Mugilidae	*Rhinomugil corsula*	+	+
27	Synbranchiformes	Mastacembelidae	*Macrognathus aral*	+	+
28			*Macrognathus zebrinus*	+	+
29			*Mastacembelus armatus*	+	+
30	Perciformes	Ambassidae	*Parambassis ranga*	+	+
31		Cichlidae	*Oreochromis mossambicus*	+	+
32		Sciaenidae	*Johnius coitor*	+	-
33		Gobiidae	*Glossogobius giuris*	+	+
34		Anabantidae	*Anabas testudineus*	+	+
35	Beloniformes	Belonidae	*Xenentodon cancila*	+	+
36		Channidae	*Channa striatus*	+	+
37			*Channa punctatus*	+	+
38		Belontidae	*Colisa fasciatus*	+	+
39			*Colisa labiosus*	+	-
40	Tetraodontiformes	Tetraodontidae	*Tetradon cutcutia*	+	+

Table 2 Comprison of water parameters of water recorded from Pauk Inn and Padonmilay Inn with EPA standard

Water parameters	Pauk Inn (South)		Pauk Inn (North)		Padonmilay Inn		EPA standard
	P (I)	P (II)	P (I)	P (II)	P (I)	P (II)	
Temperature (HC)	24	35	24	35	24	35	25
pH	7.5	7.6	7.5	7.6	7.4	8.2	6.0-9.0
DO (mg/L)	3.58	1.42	2	1.5	1.45	0.97	≥7
BOD(mg/L)	35	32	28	25	25	30	≤5
TDS (mg/L)	549	697	493	599	192	660	500
Total Alkalinity (mg/L)	280	308	240	300	392	1170	50-300
Nitrite (mg/L)	0.1	0	0.25	0	0.1	1.2	≤0.03
Salinity (%)	0.5	0.7	0.5	0.6	0.4	0	0.05-0.5
TSS (mg/L)	283	384	266	382	1354	750	≤25

EPA = Environmental Protection Agency
P(I) = Planting period (Dec. to Feb.),
P(II) = Harvesting period (March to May)

Discussion

In the present study, a total of 40 fish species belonging to 30 genera, 17 families and eight orders were recorded from Pauk Inn and 33 fish species, 26 genera, 16 families and eight orders were recorded from Padonmilay Inn. Among the eight orders of fish species, the order Cyrpiniformes represented as the highest species composition of both study sites (42.5 %, 17 species) from Pauk Inn and (42.42%, 14 species) from Padonmilay Inn. According to the result, it was concluded that the fish species in the order Cypriniformes was a dominant. The result from the present study was agreed with Kay Zin Thet (2016), and Nwe Nwe Aye (2018), they also recorded from Pauk Inn, Chaung U Township. Their results stated that the order Cypriniformes was the largest species composition. It may be concluded that the tropical weather condition is preferred to order Cypriniformes and this order contains more fish species than the other orders.

Kay Zin Thet (2016) reported that a total of 44 species from Pauk Inn, Chaung U Township. Compare with the present study, 32 fish species were similar and 12 species such as *Gudusia variegata*, *Tenulosa tilo*, *Salmophasia sardinella*, *Aspidoparia mora*, *Osteobrama feae*, *Puntius chola*, *Botia histrionica*, *Lepidocephalus thermalis*, *Mystus bleckeri*, *Hemibagrus menoda*, *Eutropiichthys vacha* and *E. burmanicus* were not found in the present study. It may be assumed that weather condition was drought in this year and the entrance of water from the river was very low. So, these fish species may not be entered in the study area from river or it may possible that there may be gradual scarcely presence of natural fishes in this Inn.

Compared with the two study sites, the recorded 33 fish species from Padonmilay Inn were also recorded from the Pauk Inn. However, seven fish species such as *Hypophthalmichthys molitrix*, *Osteobrama cunma*, *Labeo stoliczkae*, *Sperata aor*, *Ompok bimaculatus*, *Johnius coitor* and *Colisa labiosus* were found only in Pauk Inn. Except *H. molitrix*, the rest six species were natural fish. This may be assumed that these natural fishes entered from Chindwin River to the adjacent site of the Pauk Inn.

Lasne *et al*., 2007 stated that environmental variables could be mainly determined by the slope, temperature and depth of the water body. This statement agreed with the present finding. During the course of study, the highest water temperature of both study sites were 35 HC and the lowest was 24 HC. According to the EPA (2001), the standard limit of water temperature is 25 HC. The water temperature from both study sites during the planting period were fall within the limit and those for harvesting period were higher than the standard limit. The catching fish weight was gradually higher than in the months of October to January. This may be due to the presence of favorably large water body and suitable water temperature for fishes. The fish catching weight of both study sites were gradually decline and the lowest fish caught weight was recorded in the month of May. Since the water levels from both study sites were very low in May.

The highest TDS content (1170 mg/L) was recorded during the harvesting period and the lowest (392 mg/L) during the planting period from Padonmilay Inn. The TDS content from Pauk Inn was more or less acceptable for fish. The standard limit of TDS in water is 500 mg/L (EPA, 2001). In addition all of the TSS contents from two study sites were much higher than the standard limit of ≤ 25mg/L (EPA, 2001). The contents of TDS and TSS value from Padonmilay Inn were very higher than the standard limit. This may be due to deposit of waste materials such as plastic mulch and other materials from the farmlands of *Melon* spp. and other crops around the study area and this lead to unfavorable water for fish species.

In the present study, DO contents of both study sites were much lower than the desired limit of ≥7 mg/L (EPA, 2001). Moreover, the content of BOD limit from two study sites during the planting and harvesting period were much higher than the standard EPA, 2001 limit of ≤ 5mg/L. The water qualities of two study sites were degraded and it was unsuitable for fisheries and aquatic organisms. This might be due to the concentration of waste pollutants and much of seeded fish to the study area. American Publish Health Association, 1992 stated that BOD indicates a potential for reducing DO content in water and this result could be stress for fish and even death. However, no fish was observed or seen under these conditions in both study sites.

Water is the home of fish and its quality is one of the most overlook aspects of fish culture until its affect fish production. Comparison with EPA, 2001 standard limit for fish, almost all of the recorded pH value and salinity from two study sites were suitable for fish fauna. Except the content of alkalinity during the harvesting period of Padonmilay Inn, the rest content were fall within the standard limit 50-300 mg/L (EPA, 2001). However, some parameters of DO, BOD, TDS and nitrites were unsuitable range for fish fauna.

In the present study, the high concentration of nitrites (0.25 mg/L) from northern part of the Pauk Inn and (0.1 mg/L) was recorded from the southern part of Pauk Inn and Padonmilay Inn during the planting period. There is no nitrite content was observed in two study sites during the harvesting period of cultivated plants. The standard EPA limits was (≤0.03 mg/L) and the values of nitrite content in harvesting period were lower than the standard limit. This may be due to the utilization of fertilizer from the farmland, the materials that contain organic matters and other excreta around the study area. These two extreme results revealed that the water parameters of two study sites were not impoverished with nitrite containing substances as well as not satisfactory level for fish production.

Hussian, 2018 stated that wetlands and waterbirds are inseparable elements and waterbirds are an important of most of the wetland ecosystem in the food web of wetland nutrient cycle. Htay Khaing *et al*., 2018 stated that 35 waterbird species from Pauk Inn. Thus, Pauk Inn supported breeding, feeding and spawning ground for most waterbird species. Crossing in water of grazing animals and pasture for domesticated animals can also affected water quality both positively and negatively. Thus, these factors may cause major surface water quality problem for fish associated with pathogens by other animals.

Noga (2000) stated that skin ulcer on fish are one of the most well recognized indicators of polluted or otherwise stress aquatic environments. Some fishes from the study area such as *Chana* *striata*, *Puntius* spp., *Glosogobius giuris* and *Osteobrama belangeri* suffered skin infection on the fish body. It is observed that the Pauk Inn is encroached by environmental impacts such as agricultural land, overfishing, construction of house around the study area, pastures of domesticated animals, deposit of waste materials and visiting wild animals. Encroachment of wetland will also be harmful in near future. If the encroachment will be continue there will be disappeared of wetland. It will create imbalance in the nature.

Conclusion

From the overall discussion, it can be concluded that water parameters of both study sites were poor for aquatic environment as well as for fish production. There were several human made impacts exist that may affect the water quality in future. For these reasons the study recommended to converse the water quality of both study sites and their environment by regular monitoring of water quality with standard limit, keep record about fish species and their status, building awareness among the local people to conserve the study areas with local participation.

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HISTOLOGICAL CHANGES IN THE GILLS OF *OREOCHROMIS* SP. GUNTHER, 1889 FROM NYAUNG KAING IN (LAKE), MONYWA TOWNSHIP

Thandar Tun[1], Khin Myat Maw[2], Thant Zin[3]

Abstract

The histological changes found in the gills of the *Oreochromis* sp. were investigated from June 2018 to July 2019, in Nyaung Kaing In (Lake), Monywa Township. The seasonal histological investigation in the gills of *Oreochromis* sp. revealed abnormalities such as curling and club shaped of lamellae, incomplete and complete fusion of lamellae, shortening of lamellae, degeneration and desquamation of lamellae, dilation of gill filaments and arches, hyperplasia and hypertrophy of gill filaments, separation of gill filaments, elastic cartilage, haemorrhage of gill filaments**,** edema of gill arch, blood congestion of gill filaments and arches, cysts with parasites in gill filaments, aneurysms and epithelial lifting were observed. Physicochemical parameters of water quality analyzed seasonally revealed that parameters such as dissolved oxygen, total alkalinity, and ammonia nitrogen were beyond the acceptable limits in some seasons of study period .The other parameters showed within the normal range. Biochemical oxygen demand was higher than the permissible limit during three seasons. Heavy metals such as arsenic, copper, cadmium and lead values analyzed seasonally within the permissible limits.

Keywords: *Oreochromis* sp., gills, histological changes, Nyaung Kaing In (Lake)

Introduction

All living organisms attain ability to adapt themselves to change in the environment such as temperature, humidity, oxygen supply or toxicant exposure. There are wide varieties of toxicants present in the environment in the form of metals, nanoparticles, pesticides, insecticides etc. Such toxicants may reach water bodies such as freshwaters, rivers, lakes or streams in a variety of ways. Thus, the pesticides on reaching the aquatic ecosystem greatly influences the non-target organisms, especially fish (Helfrich, 2009).

Fish gills comprise a large part of fish body that contacts the external environment and play an important role in the gas and ion exchange between the organism and environment (Oliva *et al.*, 2009). The gill surface is more than half of the entire body surface area. In fish the internal environment is separated from the external environment by only a few microns of delicate gill epithelium and thus the branchial function is very sensitive to environmental contamination (Cengiz, 2006).

Fishes are considered to be the most significant biomonitors in aquatic systems for the estimation of metal pollution level; they offer several specific advantages in describing the natural characteristics of aquatic systems and in assessing changes to habitats. In addition fish were located at the end of the aquatic food chain and may accumulate metals and pass them to human being through food causing chronic or acute diseases. Studies from the field and laboratory works accumulation of heavy metals in a tissue on water concentration, pH, hardness, salinity, alkalinity and dissolved organic carbon may affect significantly into fish (Authman, 2015).

Fish are relatively sensitive to change in their surrounding environment including an increase in pollution. Fish health may reflect and give a good indication of the health status of a specific aquatic ecosystem. Fish are widely used to evaluate the health of aquatic ecosystem and

[1] Dr, Lecturer, Department of Zoology, Monywa University
[2] Dr, Associate Professor, Department of Zoology, Banmaw University
[3] Dr, Professor and Head, Department of Zoology, University of Mandalay

their physiological changes serve as biomarkers of environmental pollution (Kock *et al.*, 1996).

Along the connecting channel of Chindwin River into the Nyaung Kaing In (Lake), cultivated paddy fields appeared during the hot season. Pesticide usage on the paddy fields may reach the water body and have impact on aquatic organisms including fish. Therefore, it is required to examine whether the pollution of water has affected on the organs of some fish. Because, the quality of water may be considered very important to maintain the health of aquatic biota and human population health. The present work was undertaken with the following objectives:

- to determine the histological changes in the gill tissues of selected fish species
- to investigate the physicochemical parameters and heavy metal contents in the water of Nyaung Kaing In (Lake)

Materials and Methods

Study area

Fish specimens were collected from Nyaung Kaing In (lake), Monywa Township and situated between 22°5'22"- 22°6'2"N and 95°8'45"- 95°9'24"E and covered an area of 27.44 hectares, (Plate 1).

Study period

The study period was from July 2018 to June 2019.

Collection of fish specimen

The *Oreochromis* sp. was selected for this study based on the availability throughout the years. Fishes were collected seasonally and at least five specimens of selected species were preserved in 10% formalin solution for identification and histological studies.

Measurement of fishes

Total length and body weight of fish specimens were measured in the range of (17.8-26.5 cm) and (200 - 280 g) respectively during the study period.

Histological procedures

Collected fishes were dissected directly in the field. After dissection of fish samples, parts of gill were carefully taken out. Gill tissues were preserved in Bouin's solution in glass bottle for histological studies. The collected fish were brought to the Department of Zoology, University of Mandalay for further studies. Histological procedures generally include the following steps; (1) tissue processing, (2) embedding into paraffin wax, and (3) sectioning by microtome, (4) mounting onto the glass slide and (5) staining. The gill tissues were sectioned at 5-7µm thickness by a rotary microtome.

Water analysis

The water samples were collected in dark bottle (one-liter capacity) with no airspaces. The collection was made on three seasons (rainy season, cold season and hot season) during the study period.

The analysis of water was conducted seasonally at Laboratory of Water and Sanitation Department, Mandalay City Development Committee (MCDC) for determination of physicochemical parameters such as pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total alkalinity, ammonia nitrogen, nitrite nitrogen and nitrate were analyzed in Freshwater

Aquaculture Research Yangon, Department of Fisheries. Heavy metals such as copper, arsenic, lead, and cadmium were examined in Department of Research and Innovation, Yangon. Water temperature was examined directly at field by thermometer.

(**Source:** Google Earth, 2020)

Plate 1 Location map of the study area

Results

In the present study, *Oreochromis* sp. from Nyaung Kaing In (Lake), Monywa Township was examined to determine the histological changes of gills (Plat 2).

Seasonal histological changes in the gill tissues of *Oreochromis* sp.

Histological changes observed during the rainy season in the gill tissues of *Oreochromis* sp. included curling of lamellae, club-shaped lamellae, incomplete and complete fusion of lamellae, shortening of lamellae, degeneration and desqumation of lamellae, dilation of gill filament and gill arch, hyperplasia and hypertropy of gill filament, separation of lamellae, elastic cartilage of lamellae, haemorrhage in gill filament, edema of gill arch, blood congestion of filaments, epithelial lifting, aneurysms were observed (Table 1, Plate 3).

Histological changes in the gill tissues of *Oreochromis* sp. during the cold season showed the same as found in the rainy season and the hypertropy of gill filament and epithelial lifting were not observed in the cold season. Cyst with parasites in gill filament occurred during this season only (Table 1, Plate 3).

Histological changes in the gill tissues of *Oreochromis* sp. during the hot season are the same as observed in the rainy and cold season. The complete fusion of lamellae, cyst with parasites in gill filament were not observed in the hot season. Epithelial elastic cartilage was mostly found during the hot season and edema of gill filaments at the top of epithelial lifting was also found during this season (Table 1, Plate 3).

Water parameters of Nyaung Kaing In (Lake)

The water parametrs and heavy metal concentrations of Nyaung Kaing In (Lake) was analysed seasonally during July 2018 to June 2019.

Water parameters value of standard for Aquacecltūre (Bhatnagar and Devi, 2013) and permissible limits of heavy metals concentration of WHO (2011) standards were also described (Table 2, Fig. 4.1).

Table 1 Seasonal histological change in the gill tissues of *Oreochromis* sp. from Nyaung Kaing In (Lake)

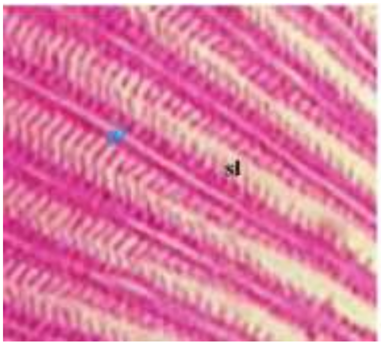
Sr no.	Histological changes	Rainy	Cold	Hot
1	Curling of lamellae	+	+	+
2	Club shaped lamellae	+	+	+
3	Incomplete fusion of lamellae	+	+	+
4	Complete fusion of lamellae	+	+	-
5	Shortening of lamellae	+	+	+
6	Degeneration of lamellae	+	+	+
7	Desquamation of lamellae	+	+	+
8	Dilation of gill filaments	+	+	+
9	Dilation of gill arch	+	+	+
10	Hyperplasia of gill filaments	+	+	+
11	Hypertropy of gill filaments	+	-	+
12	Separation of lamellae	+	+	+
13	Elastic cartilage of lamellae	+	+	+
14	Haemorrhage in gill filaments	+	+	+
15	Edema of gill arch	+	+	-
16	Blood congestion of filaments	+	+	+
17	Aneurysms (telangiectasia)	+	+	-
18	Cyst with parasites in gill filaments	-	+	-
19	Edema of gill filaments at the top	+	+	+
20	Epithelial lifting	+	-	+

+ = observed, - = Not observed

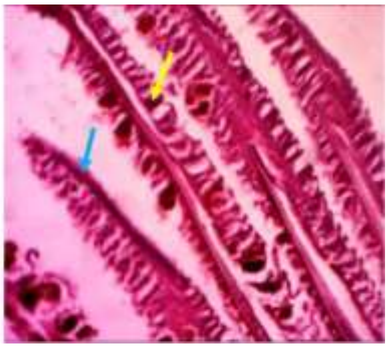
Table 2 Water parameters and heavy metal contents of water from Nyaung Kaing In (Lake) during study period

Physicochemical parameter and heavy metals	Rainy season			Cold season			Hot season			Standard for Aquaculture (Bhatnagar and Devi, 2013)			WHO (2011)
	Aug	Sep	Mean	Dec	Jan	Mean	Apr	May	Mean	Acceptable range	Desirable range	Stress range	
Water temperature (ºC)	32°C	30°C	31°C	22°C	17°C	19.5°C	34°C	35°C	34.5°C	15-35	20-30	<12, >35	
pH	7.1	7.1	7.1	7.1	7.5	7.3	8.1	8.1	8.1	7-9.5	6.5-9	<4, >11	
Dissolved oxygen (DO) (mg/L)	4.35	2.62	3.485	5.83	3.94	4.885	0.94	2.45	1.695	3-5	5	<5, >8	
Biochemical oxygen demand (BOD) (mg/L)	9.80	12	10.9	25	28	26.5	28	30	29	3-6	1-2	>10	
Total alkalinity $CaCO_3$ (mg/L)	160	160	160	296	328	312	420	400	410	50-200	25-100	<20, <300	
Ammonia nitrogen (mg/L)	0.136	0.110	0.123	0.069	0.032	0.05	0.144	0.370	0.26	0-0.05	0-<0.025	>0.3	
Nitrite nitrogen (mg/L)	0.399	0.370	0.38	0.048	0.016	0.03	0.033	0.107	0.07	0.02-2	<0.02	>0.2	
Nitrate nitrogen(mg/L)	0.136	0.122	0.13	0.036	0.044	0.04	0.101	0.25	0.18	0-100	0.1-4.5	>100, 0.01	
Arsenic (mg/L)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01				0.05
Copper (mg/L)	0.018	0.014	0.016	0.012	0.010	0.011	0.040	0.022	0.031				2.0
Cadmium (mg/L)	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008	<0.0008				0.01
Lead (mg/L)	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015	<0.015				0.05

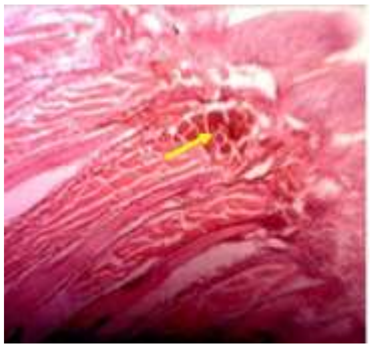
Plate 2 *Oreochromis* sp.



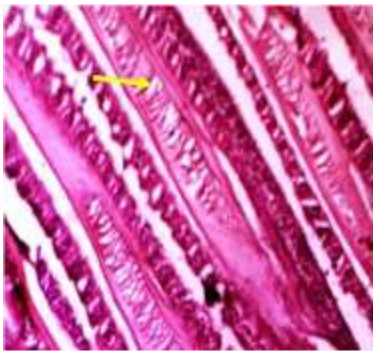
A. Normal gill structure
(pl = primary lamellae)
(sl = secondary lamellae)



B. Aneurysm (yellow arrow) and degeneration lamellae (blue arrow) (100x)

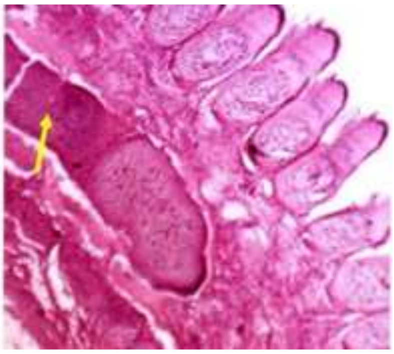


C. Blood congestion at the base of filament (400x)



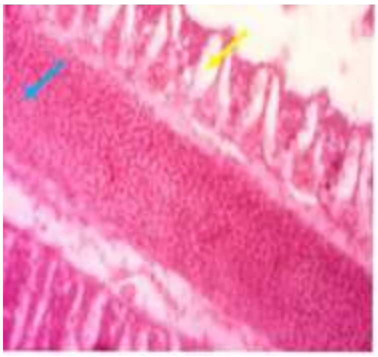
D. Elastic cartilage of gill filament (100x)

E. Complete fusion at the top lamellae (blue arrow) and elastic cartilage (yellow arrow) (100x)

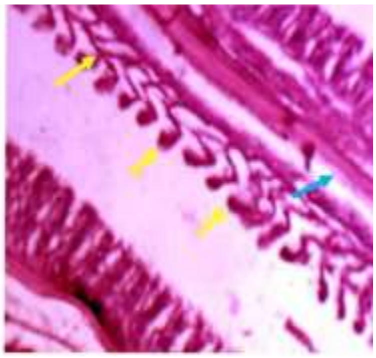


F. Haemorrahage in gill arch (400x)

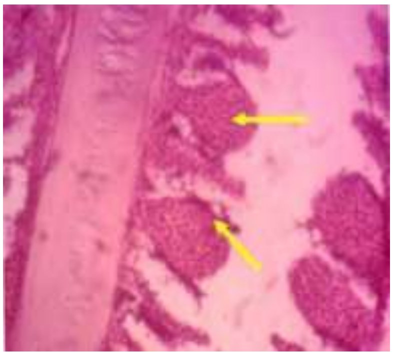
Plate 3 Histological changes in the gill tissues of *Oreochromis* sp



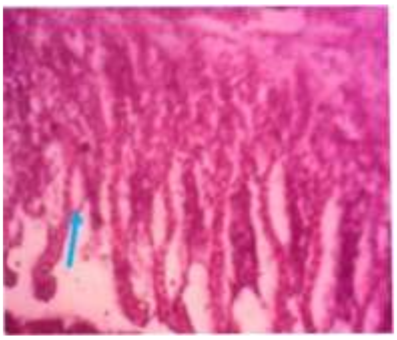
G. Blood congestion (blue arrow) and dilation of gill filament (yellow arrow) (400x)



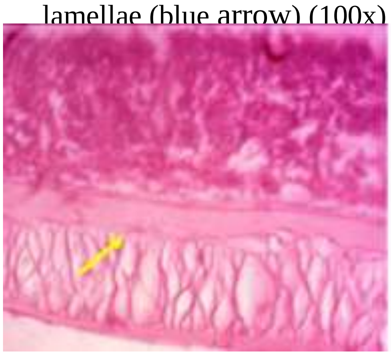
H. Club-shaped lamellae (yellow arrows) and separation of lamellae (blue arrow) (100x)



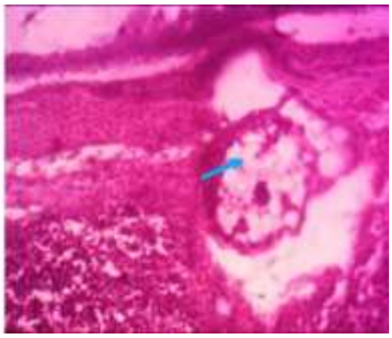
I. Aneurysms (telangiectasia) (400x)



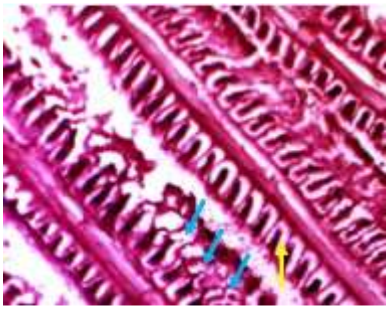
J. Epithelium lifting (400x)



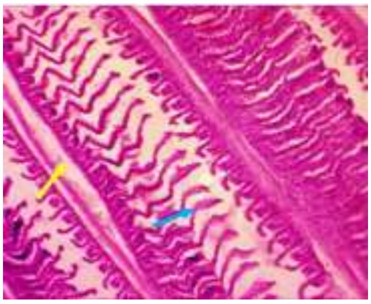
K. Elastic cartilage of primary lamella (400x)



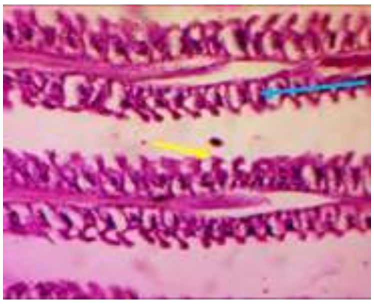
L. Cyst with parasite in gill filament (400x)



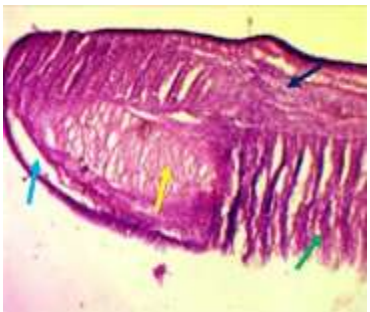
M. Hyperplasia of lamellae (blue arrows) and edema of lamellae (yellow arrow) (100x)



N. Desquamation of lamellae (yellow arrow) and curling of lamellae (blue arrow) (100x)



O. Club shaped lamella (yellow arrow) and curling (blue arrow) (100x)



P. Bulging at the tip of lamellae (black arrow) and elastic cartilage (yellow arrow) and lifting (blue arrow) (400x)

Plate 3 Continued

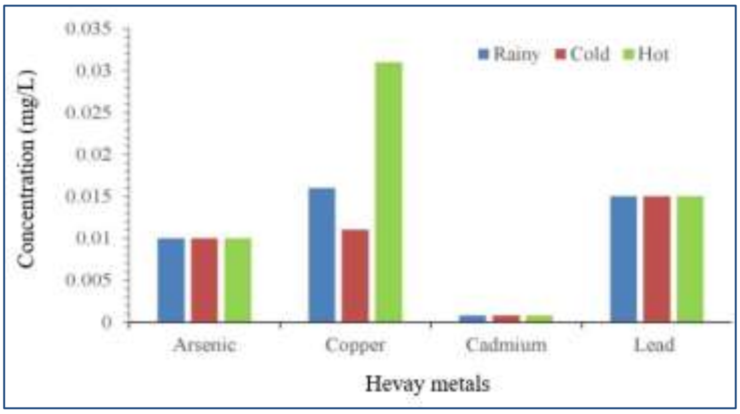


Figure 1 Heavy metals contents in the water of Nyaung Kaing In (Lake), Monywa Township during different seasons

Discussion

Histological changes in fish gills have been increasingly studied as bioindicators for assessing aquatic contamination in environmental monitoring studies (Fricke *et al.,* 2012). In the present study histological changes in the gills of *Oreochromis* sp. was investigated during July 2018 to June 2019 in Nyaung Kaing In (Lake), Monywa Township.

Histological changes in the gill tissues of *Oreochromis* sp. during three seasons were observed. The present findings are in agreement with Fernandes and Mazon (2003) who reported that, the major changes in fish are hypertorpy and hyperplasia of the epithelial cells, partial fusion of some secondary lamellae, lamellar aneurysm, beside epithelial lifting and edema. This may be early responses of the gill to the harmful substances. These alterations are examples of defense mechanisms because the lifting lamellar epithelium and edema increased the distance between external environment and the blood, thus serving as a barrier to the entrance of contaminants. Lwin Mar Oo (2017) studied the histological changes in the gills of *Oreochromis* sp. Tilapia was infected with myxosporean parasites in Taungthaman in (Lake), Amarapura Township. In the present study cyst with parasites in the gill filament occurred during the cold season only. The present histological changes characters were similar with myxosporean parasites studied by Lwin Mar Oo (2017).

Hughes and Perry (1976) reported the mild hyperplastic condition of the cell which is an indication of stress due to soap and detergent contamination. In the present study, the most occurrences of characters were hyperplasia of secondary and primary lamellae in examined fish species during the whole study period. This may be assumed that the study area is situated nearest the urban dwelling.

The water sample analysis was made on three seasons (rainy season, cold season and hot season) during the study period. Based on the water quality of Nyaung Kaing In (Lake) analyzed with respect to seasons, pH can affect fish health. According to standard of Bhatnagar and Devi (2013), the results of observation range (7.1, 7.3, 8.1) during three seasons are the suitable pH values for freshwater fishes.

Dissolved oxygen affects the growth, survival, distribution, behavior and physiologyof shrimps and other aquatic organisms (Solis, 1988). In the present study, mean values of the dissolved oxygen (3.485, 4.885, 1.695) mg/L were observed according to seasons. According to results of Bhatnagar and Devi, 2013, the hot season was also stressed but the rainy and the cold seasons are in the desirable range. In the present result of BOD based on season, (10.9, 26.5, 29) mg/L were observed. According to Bhatnagar and Devi (2013), the present results of BOD are higher than the permissible limit during the three seasons. Mallat (1985) recorded several alterations are non-specific and may be induced by different types of contaminant. As a consequence of the increased distance between water and blood due to epithelial lifting, the oxygen uptake is impaired. The present study agreed with Mallat (1985) according to the results of DO and BOD. In the present study, the seasonal mean values of total alkalinity (160, 312, 410) mg/L were recorded. The rainy season of total alkalinity was attained within the acceptable range and in cold and hot seasons were higher than the permissible limit according to Bhatnagar and Devi (2013).

In the present study, the seasonal mean values of ammonia nitrogen (0.123, 0.05, and 0.280) mg/L were recorded. According to Bhatnagar and Devi (2013), the values of ammonia are higher than the permissible limit in the rainy and hot season. The ammonia concentrations of cold season was attained within the acceptable range. The nitrite nitrogen and nitrate mean values (0.38, 0.03, 0.07) mg/L and (0.13, 0.04, 0.16) mg/L respectively were recorded seasonally and these values were within the permissible limit during the seasons of study period. Pereira *et al.* (2017) reported that chronic exposure to nitrate also affects the swimming behavior as well as the health of fish. The important histopathological effects of ammonia on the gill were hyperplasia and fusion of the lamellae. Several authors have reported similar alterations to the gills of a range of fish species when exposed to ammonia. Therefore, in the present study the histological changes in the gill tissues of *Oreochromis* sp. similar to the characters exposed to ammonia. Thus, the present results of histological changes may be due to ammonia.

Arsenic, a naturally occurring element, is a worldwide contaminate that is found in rock, soil, water, air and food. Drinking water but for most people, the major exposure source is the diet, mainly fish and seafood (Castro-Gonzales, and Mendez-Armenta, 2008). In the present study, the mean values of Arsenic analyzed seasonally were observed (<0.01, <0.01, <0.01) mg/L respectively. In accordance with the results, the value of Arsenic is within the permissible limit according to WHO (2011). Toxicity induced by high concentrations of copper in the surrounding is responsible for growth reduction and negative effects on survival and reproduction (Sorensen, 1991). In the present study the mean values of copper (0.016, 0.011, 0.031) were observed seasonally. In accordance with the result, the values of copper was variable values during three seasons and these values were within the permissible limit according to WHO (2011).

The mean values of cadmium (<0.0008, <0.0008, <0.0008) mg/L were observed during the three seasons respectively. In accordance with the result, the values of cadmium is within the permissible limit according to WHO (2011) during the study period. The mean values of lead (<0.015, <0.015, <0.015) mg/L are within the permissible limit according to WHO (2011).

Lead contamination of the surface waters might be the result of entry from old plumbing, household sewages, agricultural runoff carrying lead containing pesticides and phosphate fertilizers, fall out of lead dust and human and animal excreta. The agriculture drainage water rich in phosphate fertilizers is considered the main source of cadmium (IARC, 1993). Monteiro *et al.*, (2005) recorded aneurysms might be used as a sensitive and reliable biomarker of acute copper exposure. Degenerative changes and necrosis in the fish gill epithelium were reported by Hasan *et al.*, (2014) after heavy metal and pesticide exposure. In the present study aneurysms were found in the rainy and cold seasons while degeneration of lamellaes were found during three seasons. This fact matches with the situation in which the paddy field was cultivated in the hot season and the farmers used some fertilizers and pesticides according to surveys of paddy farmers. Thus, the histological changes in the gill tissues of *Oreochromis* sp. in the present study may be due to the heavy metal mixture that causes similar histopathological changes.

Conclusion

The present study showed that histology is a useful biomarker for environmental contaminations such as heavy metals, parasites and changes of water quality. Histological alterations of gill tissues indicated that the fish responds to polluted water. Therefore, it can be concluded that the histological changes found in gills of the studied fish indicated environmental pollutions in the study area.

Nyaung Kaing In (Lake) is a leasable In and is replenished annually by the water from the Chindwin River. However, during the study period, water entering from Chindwin River was temporarily halted. As a consequence of no input and output of water, the water characteristics in Nyaung Kaing In (Lake) become deteriorated. Therefore, it is needed to replenish the Nyaung Kaing In (Lake) with water from Chindwin River in order to maintain the freshwater ecosystem of the lake.

Acknowledgements

We are deeply grateful to Rector Dr Thuya Oo, Monywa University for his permission to submit this research in MAAS. We are greatly indebted to Dr Khin Soe Win, Professor and Head, and Dr Thet Thet Lwin, Professor, Zology Department, Monywa University, for their supervision.

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GROWTH RATE OF GIFT TILAPIA (*OREOCHROMIS NILOTICUS*) CULTURED IN DIFFERENT AQUAPONIC SYSTEMS

San Htet San[1], Moe Thandar Oo[2], Kay Lwin Tun[3]

Abstract

Aquaponics is the combined culture of fish and plants in recirculating systems (without soil). Aquaponics uses these two in a symbiotic combination in which plants are fed the waste from fish and in return, the vegetables clean the water that goes back to the fish. In the present study, GIFT Tilapia (109 ± 0.7g) was cultured with three different aquaponics systems to investigate the most appropriate aquaponics system for small scale culture. Three sets of aquaponics systems, Media Bed (MB), Nutrient Film Technique (NFT) and Deep Water Culture (DWC) were created to develop the aquaponics systems for tilapia and lettuce. A total of 40 fingerlings of GIFT tilapia (*Oreochromis niloticus*) collected from Hlawga Hatchery Station, Yangon were introduced to each system. Then, 15 seedlings of lettuce were introduced to aquaponic system. Fish were fed floating pellet for 3% of body weight and cultured from January to May, 2020. The highest weight gains were found in DWC (10.3g) followed by MB (10.2g) and NFT (9.5g). The lowest food conversion ratio (FCR) was in DWC, followed by NFT and MB. For plant quality index, 11 plants of grade A were observed in DWC which were followed by 6 plants each in MB and NFT during the first harvest. In second harvest, plant quality index was observed that number of grade A plants was 8, 5 and 3 plants in DWC, NFT and MB, respectively. According to this study, DWC system shows the most favorable outcome for tilapia and lettuce aquaponic than other systems.

Keywords: Systems, GIFT Tilapia, Lettuce, Aquaponics, FCR

Introduction

Aquaponics combines re-circulatory aquaculture system with hydroponics system in an integrated symbiotic farming concept that ensures efficient nutrient recycling. In this system, the excretory products of the fish are broken down by microorganisms and the resultant by products inputted into the hydroponic system for plant growth (Bosma *et. al*., 2017).

There are three main components of an aquaponics system: fish**,** plant and bacteria. At the design of an aquaponic system, several factors have to be considered when selecting fish, especially because they are going to be living in a tank (Somerville *et al*., 2014).

Serious environmental issues are faced by traditional aquaculture, such as high-water consumption, use of extensive land area, and production of nitrogen and phosphorus compounds (Mariscal-Lagarda *et. al*., 2012). New solutions are essential to see the modern standards of high productivity and minimum environmental impact.

Aquaponics may play an important role in water conservation, globally a major concern for food production (Martins *et. al*., 2010; Rijin, 2013).

This integration aims to convert the normally wasted nutrients excreted by fish into valuable plant biomass. This allows for lower water exchange and spillage which should significantly reduce the environmental impact of fish and hydroponic plant production (Delaide *et. al*., 2017).

In aquaponics, the aquaculture effluent is diverted through plants beds and not released to the environment, while at the same time the nutrients for the plants are supplied from a sustainable, cost-effective and non-chemical source (Somerville *et. al*., 2014).

[1] PhD student, Department of Zoology, University of Yangon
[2] Lecturer, Department of Zoology, Pathein University
[3] Professor, Fisheries and Aquaculture, University of Yangon

In aquaponics system, three types of beds are widely used namely: Nutrients film technique (NFT), ebb-and-flow (EAF) and the deep water culture (DWC) also known as the RAFT beds (Delaide *et. al*., 2017). Most EAF beds are composed of heavy substrate such as clay balls, gravels, send, perlite, etc. These serve as support systems for the plants and as bioremediation medias (Rakocy and Hargreaves, 1993). However, the effect beds in growth rate of fish and plants in aquaponics have not understood yet.

Tilapia are extremely popular in aquaponic systems because they have fast growing and resistant to many pathogens, parasites and handling stress (Somerville *et. al*., 2014). GIFT Tilapia has been introduced in 2016 from Malaysia to Hlagaw fish station, Myanmar with the support of World Fish. If GIFT Tilapia has been used in aquponics system, it is expected to produce good growth rate tilapia within limited water body. Moreover, leafy vegetable production such as lettuce receives a good market price which has fast growth with low nutritional condition and excellent adaptation to the aquaponic system. Therefore, this research has been attended to find out the more effective aquaponics system for GIFT Tilapia and lettuce production.

Materials and Methods

Study site

The present study was conducted at the Laboratory of Aquatic Bioscience, Fisheries and Aquaculture, Department of Zoology, University of Yangon.

Study period

The study period (preparing design, building and making fish and plants tanks) was lasted from June 2019 to November 2019. The culture period for fish and lettuce lasted from December 2019 to May 2020.

Experimental setup and operation of aquaponic systems

Three different aquaponics systems including Media Bed Unit (MB), Nutrient Film Technique (NFT) and Deep Water Culture (DWC) were developed (Fig.1). Three systems included the same size of fish tanks, hydroponic tanks and bio-filters. Fiber tanks were used to rare fish. The size of fiber tank was (0.85m×0.61m×0.43m) with a capacity of 225L. The size of hydroponic tanks to cultivate the lettuce is (0.43m×0.6m×0.6m) with the capacity of 250L.

For the design of Media bed Unit (MB) system, the beds hydroponic tanks were prepared by filling the gravel (Diameter = 2 cm) into the height of 24 cm. A total of 15 seedling plants with pots were introduced inside the gravel. Water in the hydroponic tank was filled into 30 cm to submerge the gravel under water. Water pump was placed inside bucket and set up in the tank to protect flowing of the gravel to the pump. Water from the fish tanks was introduced to the hydroponic tank by gravity while water from the hydroponic tank return back to fish tank by the function of electronic water pump.

For NFT system, series of 15 plastic bottles were connected and allocated on the tank with the aid of bamboo support. A small pore was created by knife on the bottle to allow the exceed water to the tank. In each bottle, two whole (D= 5 cm) were prepared with 15 cm distance to introduce the seedling plants. Therefore, there are total of 15 plants pot in the system.

In Deep Water Culture (DWC) as Styrofoam was used floating grow bed on the surface of the plant tank which was filled in the water. The area of floating beds was (0.6×0.4) m^2. The total of 15 plantation pots was allocated in floating bed by drilling the Styrofoam with 5 cm apart each other.

Water pump (75 Watt) were used in all systems. The running water system was set up between plant tank and fish tank with the aid of pump through bio filter.

The biofilter tank is one of the most important components in an aquaponics system as it reduces the toxicity of the nitrogenous waste for fish. In the present study, shells from bivalve were used as substrate in the biofilter to growth the nitrifying bacteria in large surface area. Large plastic bucket (0.39 m in diameter ×0.43 m in height) was filled with shell of bivalves until two-thirds of the bucket. Tapwater was used for all expermental tanks. Each experiment design was set up for triplicate.

Fish stocking

A total of 400 fingerlings GIFT farm Tilapia (*Oreochromis niloticus.*) (2.5 ± 0.7g) were collected from Hlawga Hatchery Station, Yangon. A total of 40 fingerlings GIFT tilapia were put in each tank (0.85 x 0.61 x 0.43 m^2). Floating pellet (3 mm) containing crude protein (33%), crude fat (6%), NFE (47.9%), ash (5.2%), fiber (2.9%), gross energy (18.9%) and digestible energy (15%) were feed with 3% of body weight per day by dividing into two times. Fish were weighed every 30 days to record the growth rate.

Preparation of vegetables

Seeds were put in seedling trays with coconut coir and soil. Seeds were germinating within four days. After two weeks, seedling of lettuce were transplanted in pot (Plate 2). Seedlings of lettuce were cultivated in gravel in MB, in the whole of Coca cola bottles in NFT and in the whole of styrofoam in DWC. The density of plant was 15 plants m $-^2$. Plants were put to the pot together with substrate (coconut coir) which help the plants to stand vertical (Plate 1).

Fish growth parameters analysis

Fish growth performance such as weight gain, specific growth rate and feed conversion ratio were evaluated in accordance with Cerozi and Fitzsimmons (2017).

$$\text{Weight gain (WG, g)} = \frac{Wf-Wi}{\text{number of fish per replicate}}$$

$$\text{Specific growth rate (SGR, \%)} = 100 \times \frac{\ln Wf - \ln Wi}{\text{days of feeding period}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

Plant quality index (PQI)

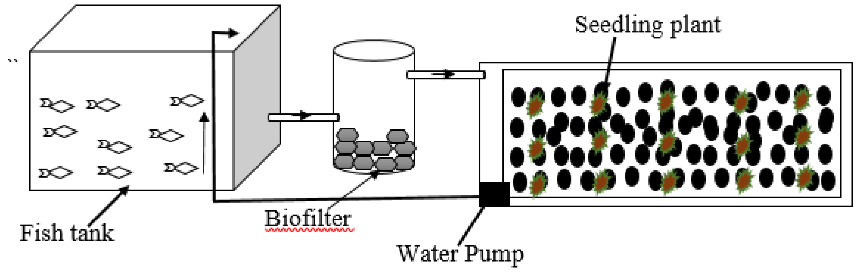
Additionally, a plant quality index (PQI) was evaluated by grades attributed to visual aspect of the leaves. Visual parameters included abnormalities in the leaf surface such as yellowish color and/or imperfections (wrinkles and burns). The grades were from A to D as follows: (Pinho *et. al*., 2017).

A = Excellent, up to 5% of the leaves surface with imperfections
B = Good, 33% imperfections
C = Average, 66% imperfections
D = Poor, 100% imperfections

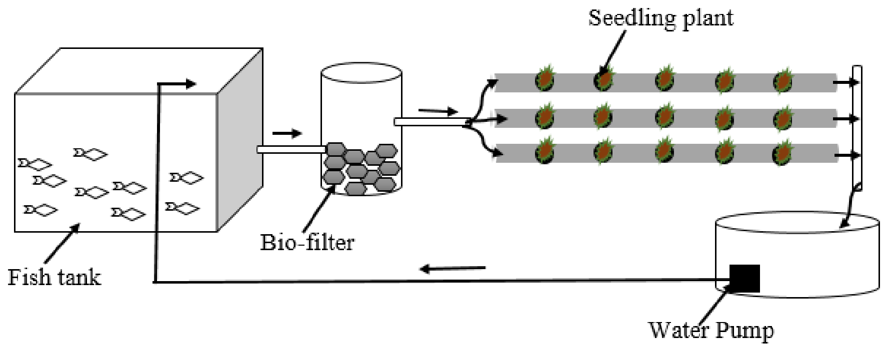
Plants grades assessed using a "blind" approach where three valuators did not know which systems has been used.

Water quality

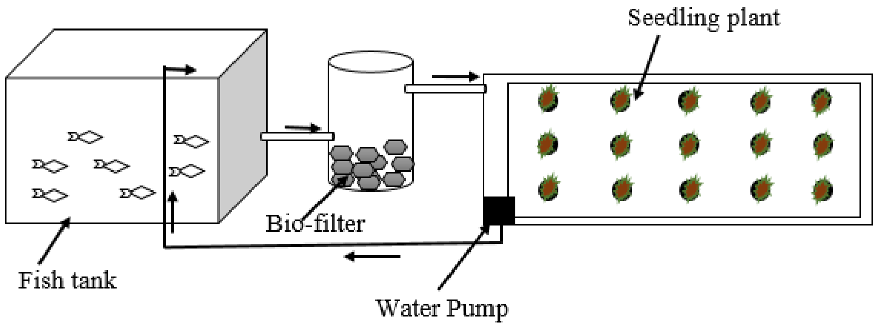
The water quality parameters; pH, dissolved oxygen and temperature were monitored by probes (ID-1100, USA and ID-150, Iijima Electronics Corporation) every day in all tanks. Nitrate and ammonia equipment calibrated from the experimental tanks were measured twice a week using colorimetric test kits.



(A) Media Bed Unit (MB)



(B) Nutrient Film Technique (NFT)



(C) Deep Water Culture (DWC)

Figure 1 Schematic diagram of three aquaponic systems used in the experiment

(A) Media Bed Unit (MB) system

(B) Nutrient Film Technique (NFT) system

(C) Deep Water Culture (DWC) system

Plate 1 Three different units in aquaponics system

Plate 2 Germination of Lettuce before introducing to aquaponics systems

Results

Growth of GIFT Tilapia

Growth of fish was studied within the period of five month cultured in different aquaponics systems. In the beginning of experiment, mean weight of tilapia was 2.5g in all systems. Fish weight gradually increased during study period. At the end of experiment, the highest mean weight was found in DWC (21.8 g), followed by MB (20.6 g) and NFT (19.8 g) respectively (Fig.2).

The highest final weight gains were recorded in DWC (10.3g) while the lowest weight gain in MB (10.2g) followed by NFT (9.5g) in the end of experiment (Fig.3). Final specific growth rate of fish was (2.3) similar specific growth rate in all systems (Fig.4).

Food conversion ratio (FCR) in April, were 1.4, 1.1 and 1 in Media Bed Unit (MB), Nutrient Film Technique (NFT) and Deep Water Culture (DWC) respectively. The highest FCR was found in MB while the lowest FCR was recorded in NFT followed by DWC system. Although the highest FCR was found in MB, in the end of experiment in May, the same FCR was recorded for all systems (Table 1).

The mortality of GIFT tilapia was found 98% in the beginning of experiment. The highest mortality was found in May at the temperature of 32 ºC. The survival rate during the experimental period was 98%, 96%, 93% and 87% in MB, NFT and DWC respectively (Fig. 5).

Lettuce production

During experimental period, the condition of plants was measured in all aquaponics systems. Plants were cultivated and harvested for two production cycles of lettuce during the study period. Plant quality index was assessed as A, B, C and D according to the quality of plant. For plant quality index, 11 plants of grade A were observed stocking density of 15 plants m^{-2} in DWC which were followed by 6 plants each in MB and NFT in the first cycle of harvest. In the first cycle, 1 plant was grade D in MB and NFT. However, none of the lettuce was grade D in DWC. In second cycle of harvest, plant quality index was observed that number of grade A plants was 8, 5 and 3 plants in DWC, NFT and MB respectively (Fig. 6).

Water quality

The water quality was recorded from January to May 31, 2020. In all experiments, average dissolved oxygen ranged 4.9 to 6.0 mg/L while water temperature varied 23.3 to 31 ºC. In all aquaponic systems, pH level ranged from 7.3 to 7.7. The ammonia levels ranged 0 to 0.3 mg/L while Nitrate levels varied 0 to 0.2 in all aquaponics systems (Table 2).

Table 1 Food conversion ratio during experimental period

FCR	February	March	April	May
MB	1.5	2	1.4	1
NFT	1.5	2.1	1.1	1
DWC	1.3	2.1	1	1

Table 2 Water Parameters during experimental period

Parameters	January			February			March			April			May		
	MB	NFT	DWC	MB	NFT	DWC	MB	NFT	DWC	MB	NFT	DWC	MB	NFT	DWC
Dissolved Oxygen	5.7	5.8	5.7	6	6	6	5.8	5.8	5.8	6	6	6	5.1	5.1	5.2
Temperature	24.6	24.6	24.6	25.9	26	26	27	27	27	29	29	29	31	31	31
pH	7.6	7.7	7.6	7.5	7.5	7.6	7.4	7.5	7.4	7.4	7.4	7.4	7.5	7.5	7.6
Ammonia	0	0	0	0	0	0	0	0	0	0.1	0.1	0.1	0.1	0.1	0.2
Nitrate	0	0	0	0	0	0	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2

*MB = Media bed unit
*NFT = Nutrient film technique
*DWC = Deep water culture

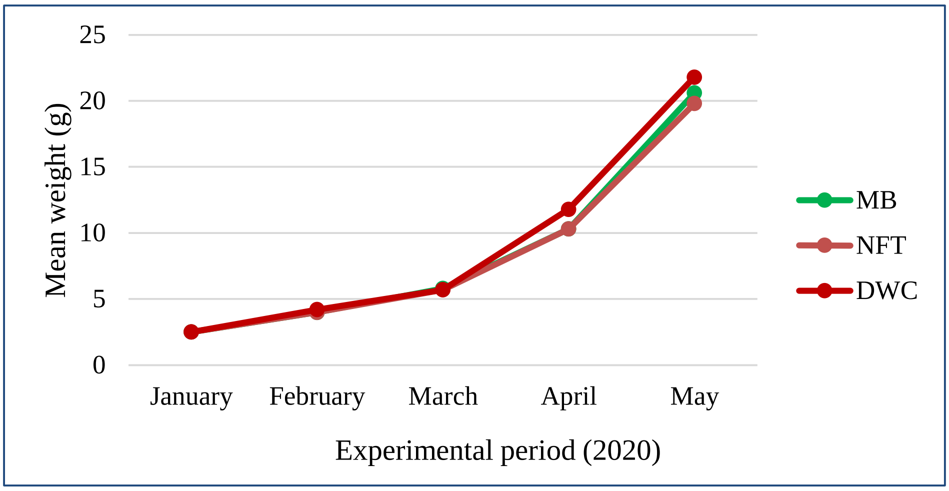


Figure 2 Total weight of GIFT tilapia in three aquaponics systems

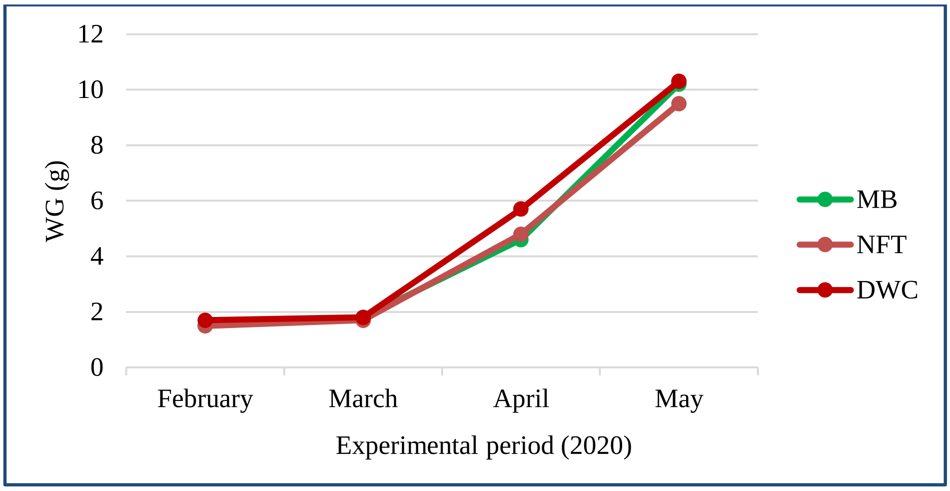


Figure 3 Weight gain of GIFT Tilapia during the experimental period

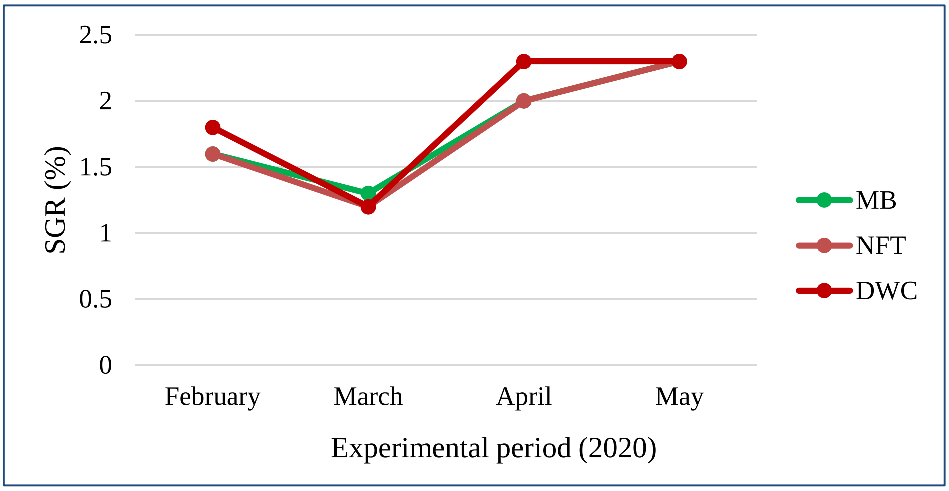


Figure 4 Specific growth rate of GIFT Tilapia in all systems

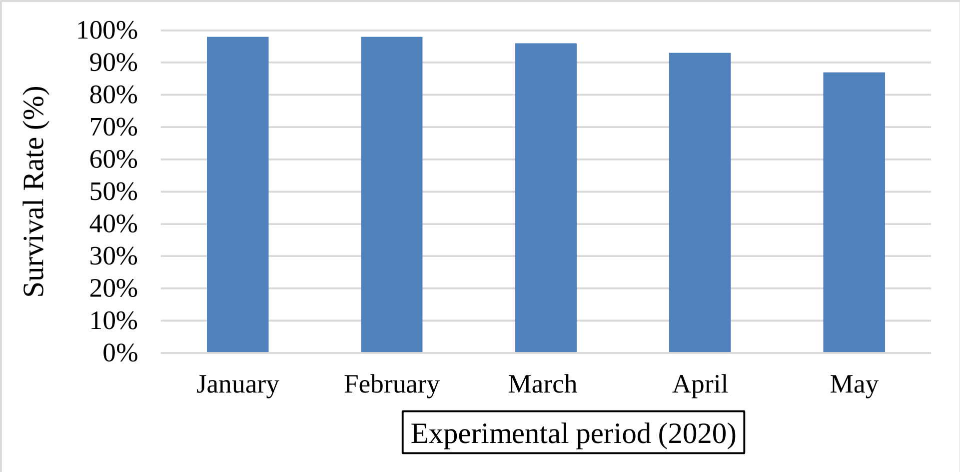


Figure 5 Survival rate of GIFT Tilapia during the experiment

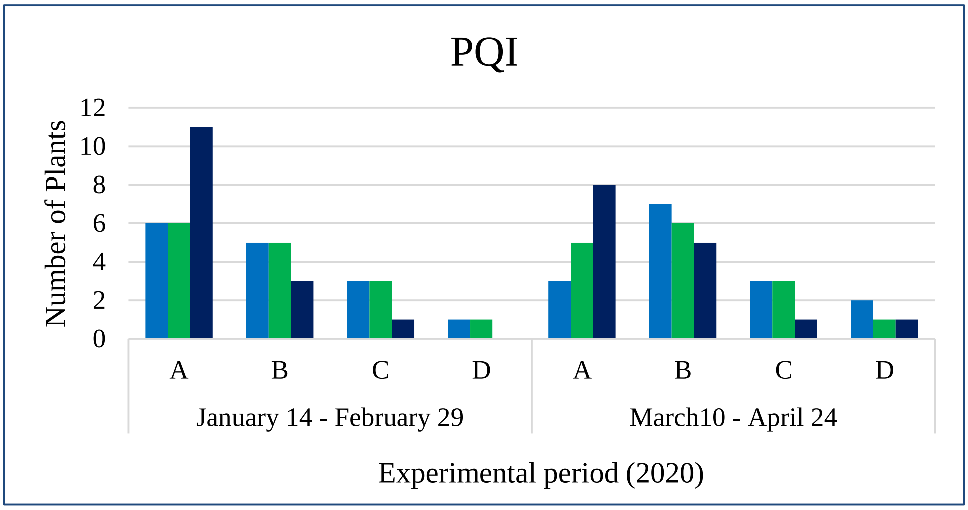


Figure 6 Plant Quality Index of lettuce in all aquaponics systems

Discussion

The growth rate of GIFT tilapia and plant in three different aquaponics systems were studied in the present study. The best growth rate of fish and plant were found in deep water culture (DWC), The weight gains of three aquaponics systems not much differ very yet, however, the highest weight gain was recorded in DWC (10.3g). In April and May, DWC was found in the highest weight gain and SGR. Tilapia grows fast if they are given the right conditions and may achieve approximately 1 kg in 8-9 months (Martins *et. al*., 2009).

The same FCR was found (1.5) in MB and NFT while it was (1.3) DWC in February. However, the same FCR (1) reached in May in all systems. The FCR of present research is better than Rakocy *et. al*., (2006) in which FCR was 1.7 to 1.8. It is assumed that GIFT tilapia is a strain that has been genetically improved for aquaculture purpose.

The best plant quality index was recorded in DWC because it seems that in MB and NFT cannot supply required nutrient for plant and as a result, 30% of plants are PQI grade B. Grade B means 33% of plant is imperfection as the plant is wrinkles and burn (Pinho *et. al*., 2017). The root in DWC system seems freely absorb nutrient from water while roots in MB were restricted in the gravel. On the other hands, roots in NFT was limited in the plastic bottle. Therefore, longer roots in DWC system can absorb nutrient from the water to the leaves of lettuce. Plants in the MB and NFT are nutrition deficiency and as a consequence, grade B plant was more collected (Fig. 5).

The lettuce and basil did not grow efficiently in clay balls contrary to Lennard and Leonard (2006) who compared their growth rate in NFT, DWC and EAF (MB). This may be explained in caused by reduced water flow around the roots and hence reduced nutrients availability (Trang *et. al*., 2010). Plant growth in the present study followed the relationship DWC > Gravel bed (MB)> NFT.

On the aspect of water quality, the average of water temperature ranged 23.3 ·C- 31·C. This was suitable for tilapia but was often warmer than the optimum temperature needs of lettuce (Resh, 2012). Dissolved oxygen were above 5 in all tanks because all tanks were aerated during the study period. The average of pH in all aquaponics systems were (7.3 to 7.7). Rakocy *et. al.*, (2004) suggested that pH should be above 7 in aquaponics to promote nitrification in aquaponic system. Ammonia levels and Nitrate levels in aquaponic system is low because biofilter convert fish waste ammonia into plant food nitrate (Rakocy, 2004). Results indicated that DWC is the most appropriate all aquaponics systems. It can be constructed available facilities, a simple recirculating aquaponics system for combined production of fish and vegetables with a minimum use of water and space.

Conclusion

Three sets of aquaponics systems (MB, NFT and DWC) were created to reveal the appropriate system for Tilapia and lettuce aquaponic system. The highest final weight gain was found in DWC followed by MB and NFT. Lettuce were cultivated and harvested for two production cycles during the study period and the best plant index is also found in DWC. According to the result, DWC system showed the more favorable outcome for good growth rate of tilapia and lettuce than the other aquaponics systems.

Acknowledgements

I would like to express my gratitude to Professor, Dr Aye Mi San, Head of the Department of Zoology, University of Yangon for her permission to do this research. I also thanks to Dr Kevin Fitzsimmons (USAID's Sustainable Industry Infrastructure Project) and Mr. Hendrik Stolz (Myanmar Sustainable Aquaculture Program) for providing the facility for aquaponics setting in the Laboratory of Aquatic Bioscience.

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MYXOSPOREAN INFECTION IN THE INTESTINE OF *CIRRHINUS MRIGALA* (HAMILTON, 1822) AT YEZIN FISHERY STATION, NAY PYI TAW, MYANMAR

Yu Yu Aye[1], Thant Zin[2], Kay Lwin Tun[3]

Abstract

Cirrhinus mrigala fingerlings were sampled monthly from Yezin Fishery Station, over 12 months of study period and examined for intestinal myxosporean parasites. Three myxosporean species, *Myxobolus* sp., *Thelohanellus* sp. and *Henneguya* species under phylum Cnidarian were recorded. Among all these parasites, the most dominant species was *Myxobolus* sp., followed by *Thelohanellus* sp. and *Henneguya* species. Spores of *Myxobolus* sp. elongated and ellipsoid in valvular view. It had 11.6µm±1.1µm in length and 7.6µm±0.8µm in width. The myxospores of *Thellohanellus* sp. were pyriform in shape, blunt at the anterior end measuring 16.0µm±1.3µm x 8.8µm±1.3µm in size. Spores of *Henneguya* species are oval in shape and measurement of its length and width were 27.5µm±1.7µm x 3.8µm±0.7µm in size. The highest prevalence and intensity of *Myxobolus* sp. infection were recorded in December 2018 (84%). The highest prevalence and intensity of *Thelohanellus* sp. infection were found in November (18%). However, *Henneugya* species was found only in December with low prevalence infection (4%). Cyst formation of *Myxobolus* sp. was found on the surface of intestine. To examine the histopathological changes of infected tissues, histopathological slides were prepared and checked under microscope. Histopathological changes such as proliferation of villi, necrosis of serosa, mucosa and submucosa as well as space in villi necrosis and fusion of villi were observed in the intestine of infected fish. Therefore, management practices and pond hygiene should be adopted in nursery operation systems and grow-out ponds for producing quality fish fry and successful harvesting.

Keyword: - myxosporean infection, intestine, histopathology, *Cirrhinus mrigala*

Introduction

In Myanmar, freshwater aquaculture depends mainly on carp culture practices and farming in earthen ponds depicts the major source of aquaculture production. Myanmar's foreign income from fishery exports reached more than US$535 million in 2018. Myanmar's farmed fish exports are dominated by *Labeo rohita*, along with two other species; *Cirrhinus mrigala* (Mrigal carp) and *Catla catla* (Belton *et al*., 2015). Mrigal carp is an important component of polyculture with other native and exotic carp species.

Disease has a serious impact on fish in both captive and natural environments. In cultured fish population, the parasites may involve in the serious outbreak of disease (Kayis *et al*., 2009). It is a major problem that carrying heavy infection of parasites of freshwater fishes in aquaculture. The water quality parameters and stocking density correlate with the development of fish parasites (Bhuiyan and Musa, 2008). In the high stocking density, if the fishes are stressed, the parasites multiply rapidly. Meanwhile the farmers had acquired as a result of research and development as well as their own experiences.

Myxozoan parasites are one of the economically important groups of microscopic parasites as they infect fish harvested for food and most commonly parasitize invertebrates (Kent *et al*., 2001and Lom and Dykova, 2006). They are common in juvenile carps in nursery ponds and high mortality rates caused by their infections in the organs of fish. Due to a site of gaseous exchange and rich blood supply, gills are prone to be more infected (Martins, *et al*., 1997). Myxosporeans infected in the organs of fish, where they may cause serious structural changes depend on the

[1] Lecturer, Department of Aquaculture and Aquatic Diseases, University of Veterinary Science
[2] Professor and Head, Department of Zoology, University of Mandalay
[3] Professor, Fisheries and Aquaculture, University of Yangon

intensity of infections. Myxosporean parasitic infections caused economical losses in the carp nursery ponds (Sanaullah and Ahmed, 1980).

The complex life cycle of myxozoa involves myxospore and actinospore life stages within a fish host and an invertebrate host respectively (Markiw and Wolf, 1983 and Wolf and Markwi, 1984). The life cycle of myxozoa requires a tubificid worm *Tubifex tubifex* as an alternative host, in which the ingested spore further develops as actinospores. When actinospores were released from the tubificid, they enter into the fish and life cycle is completed (Lom and Dykova, 1992). Therefore, transmission of myxozoa among the fish are considered in Myanmar since freshwater fish farms are conducted in the earthen ponds.

In Myanmar, parasitic infections of freshwater fish were studied by Moe Kyi Han (2006), Pa Pa Win (2007), Sein Sein Myint (2007), Myint Myint Win (2012), Hnin Hnin Htay (2014), Su Su Mon (2014) and Yan Naung Tun (2019). However, examination of intestinal Myxoaporea in *Cirrhinus mrigala* is still required to improve the production. In turbot and gilthead sea bream aquaculture, it was reported that the myxozoan parasites invade the intestinal mucosa, causing a cachectic syndrome associated with intestinal barrier alteration. *Myxobolus* sp. cyst infection in the intestine can damage villi and mucosal epithelium causing myolitic (Maftuch *et al*., 2017). Intestine infection leads to myolitic on the intestinal wall of fish (Marjoram and Bagnat, 2015). Moreover, developing and mature spores of plasmodia in the wall of the intestine dispersed throughout the body by the blood stream (Molnar and Kovacs-Gayer 1985).

The intestine is an important organ for digesting and absorbing nutrients to growth fish. The parasite extracts energy and nutrients from the host fish so, which are supported the general health and reproductive effort and also impair mating and gonad maturation (Reddy and Benarjee, 2014). Therefore, investigation on intestinal myxosporeans in *Cirrhinus mrigala* is required from the biosecurity point of view and diseases control strategies. In the present study intestinal myxosporean parasitic infections in *Cirrhinus mrigala* were investigated to understand on fish health status of carp hatchery in Myanmar. Besides, histopathological changes of infected fish were examined to reveal on the impact of parasites on fish physiology.

Materials and Methods

Sample collection

Fish samples, *Cirrhinus mrigala* were collected initially one month old fingerlings from nursery pond at the Yezin Fishery Station, Nay Pyi Taw, Myanmar. *Cirrhinus mrigala* fingerlings were cultured in experimental pond (8.3mx33.3m) at Yezin Fishery Station using extensive culture system. The fifty host fishes, *Cirrhinus mrigala* were collected on regular basis once a month during the study period. Fish were collected for 12 months from September 2018 to August 2019. The fish samples were brought in live condition to the laboratory at Department of Aquaculture and Aquatic Diseases, University of Veterinary Science or laboratory Aquatic Bioscience, University of Yangon with oxygen and water filled plastic bags. The fish samples were kept temporarily in a small aquarium (45.72 × 91.44 × 91.44 cm3) and aeration was given in the laboratory.

Examination of parasites

The fishes were examined immediately after collection. The total length, standard length and body weight of each specimen were immediately measured and recorded. The external symptoms of the whole fish were checked under stereomicroscope before dissecting the fish. The body of the fish then was cut to examine the present of different parasites in the intestine. The whole intestine was transferred on to the petridish with 0.9% saline. Then, intestine was cut into

1cm each, put on glass slide, added with 10 µl of 0.9% saline, covered with coverslip and examined under light microscope (Olympus – CX 31).

Identification of parasites

The identification of myxosporean parasites were conducted according to the guidelines of Lom and Arthur (1989), Lom and Dykova (1992) and Kalavati and Nandi (2007). Identification was made on the basis of various morphological structures of spore such as shape, size, and number of polar capsules, length of polar filaments, number of coils of polar filaments, presence or absence of intercapsular process, presence of any iodinophilous vacuole and number of nuclei in the sporoplasm, length of caudal appendage etc. They were measured and photographed using the light microscope (Olympus CX 31) under 100X magnification.

Data analysis for parasites

Prevalence of parasitic infection was calculated in accordance with the following methods (Bush *et al*., 1997).

$$\text{Prevalence (\%)} = \frac{\text{Number of infected host}}{\text{Total number of host examined}} \times 100$$

Mean intensity of infection was classified four stages according to Bachere *et al*. (1982) and Culloty *et al*. (1999).

Stage (I): 1-20 parasites observed within five minutes of screening under 40 x magnification

Stage (II): 21-40 parasites observed within five minutes of screening under 40 x magnification

Stage (III): 41-60 parasites observed within five minutes of screening under 40 x magnification

Stage (IV): 1-10 parasites in all field of region observed immediately in screening under 40 x magnification

$$\text{Mean Intensity} = \frac{\text{Total Number of parasites recovered}}{\text{Total number of infected fishes}}$$

Preparation of histopathological slides

To understand the histopathogical changes of infected tissues of intestine, the tissue infected with cyst of parasites were cut and fixed in 10% neutral buffered formalin. After fixation for 48 hours, the tissues were cut in order to obtain a size of 1 cm^3. The prepared tissues were dehydrated through a graded series of ethanol, cleared in xylene, and infiltrated in the paraffin. Sections were cut at 5µm in thickness on a microtome (Thermo Scientific Microm hm355s) fitted with a sharpened microtome knife. These sections were then stained with Hematoxylin-Eosin. The permanent mounting of the slides was made by DPX (distyrene, plasticizer and xylene). Histopathological lesions were examined and photographed at different magnifications with the help of binocular light microscope (Olympus – CX 31).

Environmental parameters examination

Environmental parameters such as pH, Dissolved oxygen (DO), Ammonia and Nitrite from fish pond were examined by using portable water Test Kits. Water temperature was measured with a thermometer.

Results

Growth rates of *Cirrhinus mrigala*

A total of 50 fish was sampled monthly from Yezin Fishery Station. The initial weight of fish was 0.8 g (±0.2 g) while it gradually increased during the study period (Fig. 1). In the end of experiment, the mean body weight of fish reached to 14.7 g (±2.1 g).

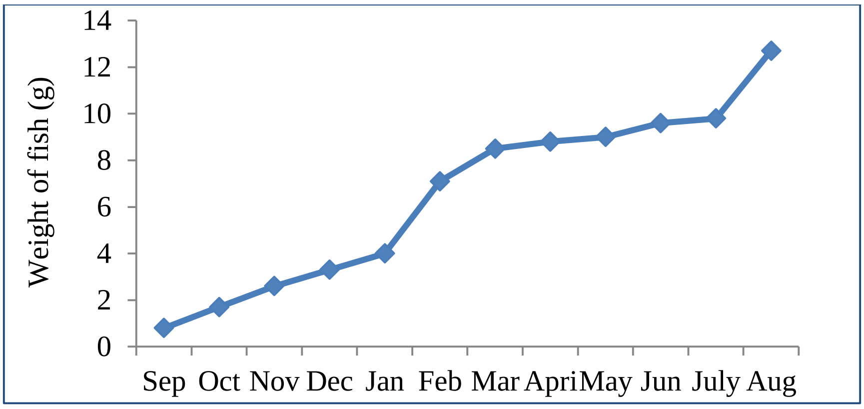

Figure 1 Growth weight of *Cirrhinus mrigala* recorded from Yezin Fishery Station

Morphology and morphometry of myxosporean parasites

Three myxosporean parasites, *Myxobolus* sp., *Thellohanellus* sp. and *Henneguya* sp. were found in the intestine of *Cirhinus mrigala*.

Spores of *Myxobolus* sp., was 11.6µm±1.1µm in length and 7.6µm±0.8µm in width with elongated and ellipsoid in valvular view. Mucus envelope was found around the posterior end. Two polar capsules were slightly pyriform and unequal in shape with 4 to 6 filaments, larger 5.0µm±1.1µm x 3.3µm±0.5µm and smaller 3.5µm±0.8µm x 3.3µm±0.5µm in size (Plate 1, B). Sporoplasm was finely granular and occupied most of the extracapsular cavity of spore.

The myxospores of *Thellohanellus* sp. were pyriform in shape, blunt at the anterior end measuring 16.0µm±1.3µm x 8.8µm±1.3µm in size (Plate 2, B). Polar capsule was elongated pyriform measuring 7.1µm±0.9µm x 4.9µm±0.6µm in size.

Henneguya sp. spores are oval in shape and the measurement of length and width were 27.5µm±1.7µm x 3.8µm±0.7µm in size. It has two equal polar capsules inside, measuring 3.5µm±0.6µm x 1.2µm±0.5µm in size. Length of caudal appendage was 12.5µm±1.3µm long (Plate 3, A).

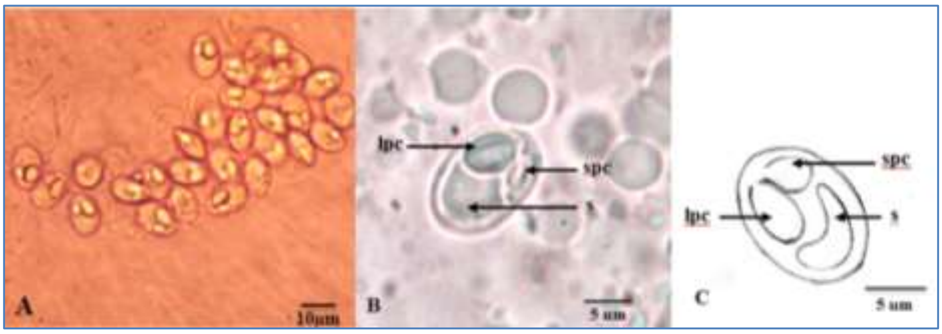

Plate 1 *Myxobolus* sp. recorded in the intestine of *Cirrhinus mrigala* from Yezin Fishery Station (A) Plasmodia of *Myxobolus* sp. (B) Detail morphology of *Myxobolus* sp. (C) Line drawing of *Myxobolus* sp. (lpc = large polar capsule, spc = small polar capsule, s = sporoplasm)

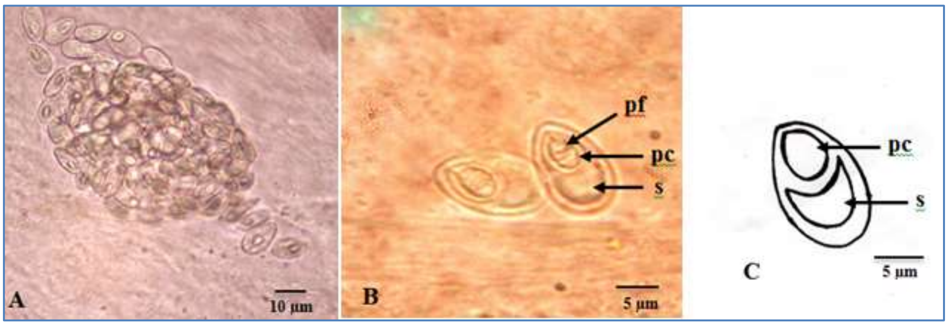

Plate 2 *Thellohanellus* sp. recorded in the intestine of *Cirrhinus mrigala* from Yezin Fishery Station (A) Plasmodia of *Thellohanellus* sp. (B) Detail morphology of *Thellohanellus* sp. (C) Line drawing *Thellohanellus* sp. (pc = polar capsule, s = sporoplasm)

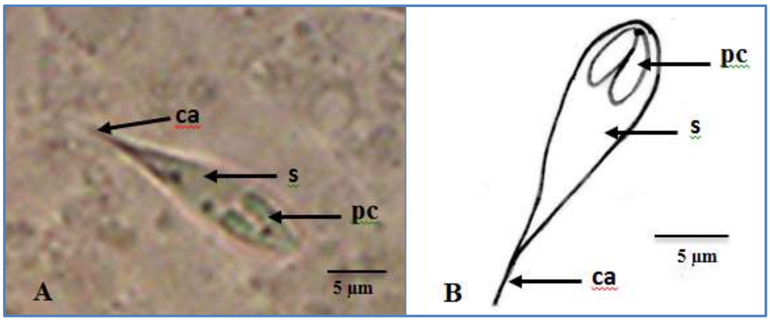

Plate 3 *Henneguya* sp. recorded in the intestine of *Cirrhinus mrigala* from Yezin Fishery Station (A) Detail morphology of *Henneguya* sp. (B) Line drawing of *Henneguya* sp. (pc = polar capsule, s = sporoplasm, ca = caudal appendage)

Prevalence and mean intensity of myxosporean parasites

The prevalence and mean intensity of three myxosporeans fluctuated during the study period (Fig. 2 and 3). It is clear that the prevalence of *Myxobolus* sp. in the intestine of fish was 62% in November 2018 and it noticeably increased to 84% in December 2018. Then, it sharply decreased to 52% in January 2019. The prevalence fluctuated around 40% in April, May, June, July and August 2019. *Myxobolus* sp. was found in the intestine with the highest mean intensity 3.5 in January 2019 and followed by 3.4 in December 2018. However, the lowest mean intensity 1.2 was recorded in May and July 2019 (Fig. 3).

The highest prevalence of *Thellohanellus* sp. was 18% and it was found in November. It gradually decreased to 10% in December 2018, followed by 2% in January and February 2019. After that, it marginally increased to 4%, 6% and 8% in March, April and May respectively and minimally decreased to 6% in June and July 2019. *Thellohanellus* sp. was not recorded in September, October 2018 and August 2019. The mean intensity of *Thellohanellus* sp. was 4 in November, December 2018 and April 2019 but it was only 1.0 in the other months during the study period (Fig. 3).

During the study period *Henneguya* sp. was found only in December 2018 with low prevalence (4%) and mean intensity of infection was (1) (Fig 3).

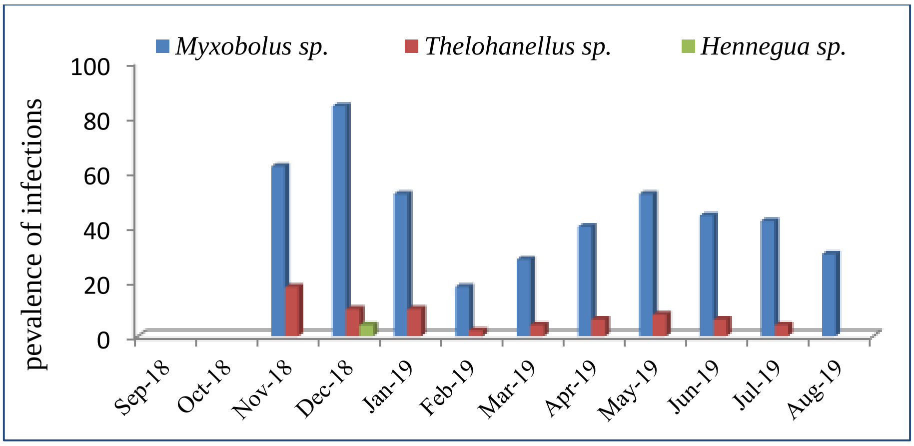

Figure 2 Prevalence of myxosporean infections in the intestine of *Cirrhinus mrigala* during the study period

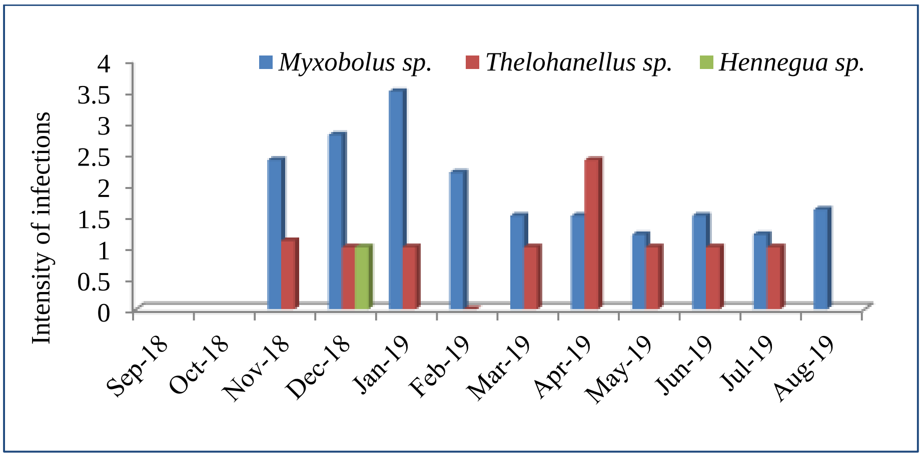

Figure 3 Mean intensity of parasitic infestations in the intestine of *Cirrhinus mrigala* during the study period

Mean body weight of infected and uninfected *Cirrhinus mrigala*

Mean body weight of infected and uninfected *Cirrhinus mrigala* was compared to understand the effect of parasitic infection on growth of fish. The weight of infected fish is slightly decreased than that of uninfected fish from February to August 2019.

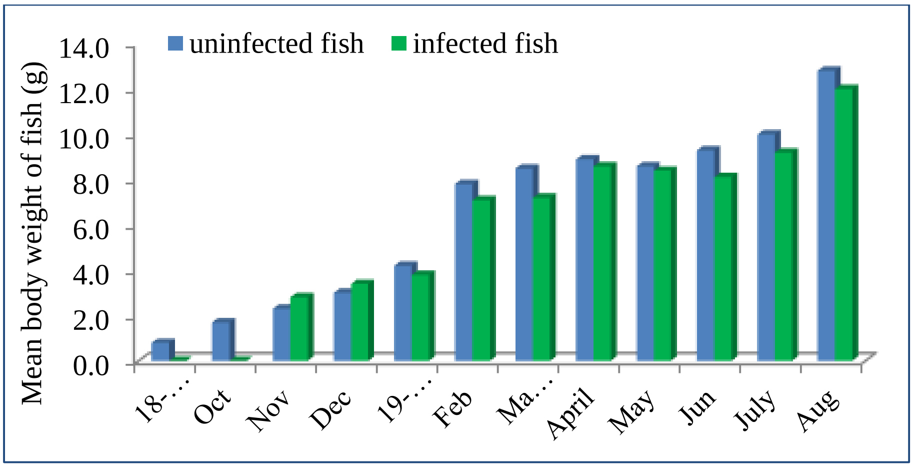

Figure 4 Mean body weight of *Cirrhinus mrigala* during the study period

Histopathological analysis of intestine

Cyst formations in intestines were found only in the fish that was infected with *Myxobolus* species. Pathogenesis in intestine due to the infection of *Myxobolus* sp. was observed. Pathological findings in the intestine of host included severe degenerative and necrotic changes in the intestinal mucosa and submucosa. Proliferation of mucus cells in the intestinal mucosa and intestinal cells damage in the form of necrosis occurred in infected fish.

Cyst of *Myxobolus* sp. had been found in the intestine (Plate 4, A). Hemorrhages and atrophy were found in the epithelial layer of intestine due to the abundance of *Myxobolus* parasites (Plate 4, B). Vacuolization and necrosis were found in the epithelial layers that were followed by hemorrhages (Plate 4, C). In some cases, massive atrophy and hemorrhages were also found in mucosa and circular layer of smooth muscle regions. In the present study, marked histopathological changes in the intestine of *Cirrhinus mrigala* have been observed, proliferation of villi, and necrosis of serosa, mucosa and submucosa as well as space in villi necrosis and fusion of villi.

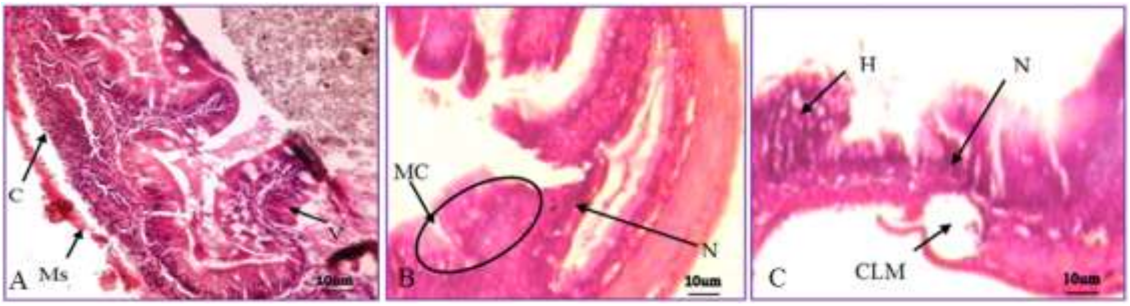

Plate 4 Pathogenesis in intestine of *Cirrhinus mrigala* caused by myxospoean infections (A) *Myxobolus* sp. cyst formation in smooth muscle (C=Cyst of *Myxobolus* sp., Ms=Muscularis, V=Villi) (B) Haemorrhage and aggregations of inflammatory cells in the mucosa and submucosa (MC=Mucus cell, N=Necrosis) (C) Degenerated serosa and muscularis

(H=Haemorrhage, VCLM = Circular layer of smooth muscle, N= Necrosis)

Environmental parameters in fish pond

Water temperature in fish pond varied from 20 Ċ to 28 Ċ during the study period (Fig. 4). The highest water temperature was 28 Ċ (April, 2019) in fish pond. The value of pH in studied pond ranged from 6.5 to 7.6 within twelve months. The highest level of dissolved oxygen (DO) was 7 ppm in September and the other months between 5 to 6 ppm in the fish pond. The concentration of ammonia in water was observed to be highest in March, April, May and June, 2019 (0.4 ppm) and the other months were 0.1 ppm and 0.2 ppm respectively. The value of nitrite in fish pond maintained the level at 0.05 to 0.1 ppm in the study period.

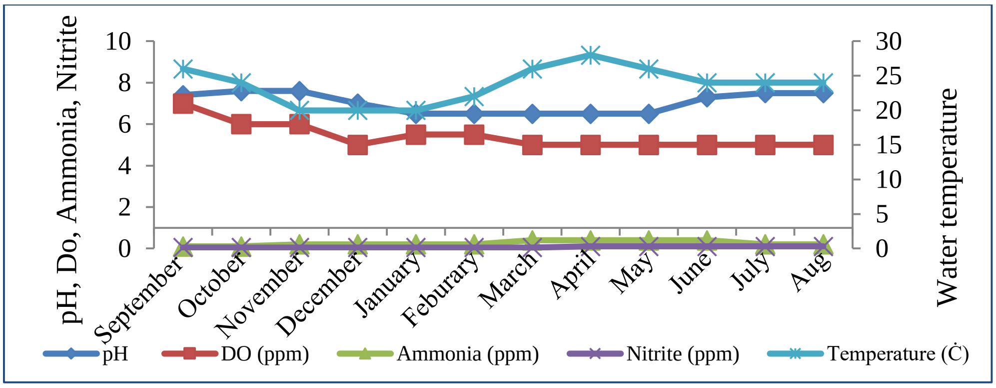

Figure 4 Environmental parameters in fish pond during the study period

Discussion

Three myxosporean parasites, *Myxobolus* sp., *Thellohanellus* sp. and *Henneguya* sp. were recorded in the intestine of *Cirrhinus mrigala* at Yezin Fishery Station. Identification of the parasites cannot be conducted into species level since only morphological study has been done in the present study. *Myxobolus* is the predominant species group within the phylum Cnidarian. Most of the species infect primarily fish, both freshwater and marine species all over the world, and a few numbers of species were found in amphibians. There are 112 nominal species were described for *Myxobolus* (Butschli, 1882).

The shape and dimension of *Myxobolus* sp. recorded in the present study is similar to *Myxobolus eirasi* infected in caudal fin of *Cirrhinus mrigala* and *Myxobolus guangzhouensis* infested in scales of *Cirrhinus mrigala* (Eiras *et al*., 2014). However, length of polarcapsule was slightly different. The shape and size of *Myxobolus* sp. detected in this study is similar to *Myxobolus* sp. 7 infected in gills and kidney of *Cirrhinus mrigala* that was recorded by Pa Pa Win (2007). However, the site of infection is differed from the present study.

Thelohanellus sp. recorded in the present study appeared similar to *Thelohanellus kalavatae* in caudal fin of *Cirrhinus reba* (Zhang *et al*., 2013). The dimension of *Thellohanellus* sp. infected in *Cirrhinus mrigala* of recorded by Pa Pa Win (2007) was nearly similar. However, the width of spore and size of polar capsule were slightly different.

Henneguya species was rear Myxosporadium species. *Henneguya* species was reported only in Mandalay Region by Shwe Sanda *et al*. (2020) from the gills of *Anabas testudineus* in Taungthaman Lake, Mandalay Region. In the present study, only one individual of *Henneguya* species was recorded during the study period. Therefore, it had low prevalence and intensity of infection. The species identification of *Henneguya* species was more difficult than the other two species.

Although the morphology of Myxosporean is the same, the species are assumed to be different if the host fish species and infection sites are varied. Molecular identifications of myxosporean parasites recorded in the present study are needed as further confirmation to identify the species level.

In the present study, parasitic frequency of *Myxobolus* sp. was highest in December 2018 (84%) stated as “stage 4” and lowest in February 2019 (18%) stated as “stage 1”. The result of the present study agreed with the work of Farhaduzzaman *et al*., 2010. They reported that the highest number of parasites was collected in December (94%) and lowest in February (15%). Because of the parasitic infection is greatly influenced by the seasonal especially in winter, which basically interfere with ecology and parasitic condition of the fish. Moreover, environmental parameters fluctuated very quickly during winter and summer seasons, fish becomes affected with diseases in these seasons.

The highest prevalence of infestation was found in *Myxobolus* species. High prevalence of infection was found from November 2018 to January 2019 when the fish was 3 to 4 months old. Tun *et al*. (2014) reported the prevalence of gallbladder myxosporean parasite, *Zschokella honjoi* infection in *Labeo rohita* and they found that the infection decreased when the size of fish increased. They postulated that *Zschokella honjoi* was released from the gallbladder after 6 months of infection. Since the fish in Yesin Fishery station was cultured in earthen pond, it is difficult to estimate the mortality due to infection. The older fish seems to be more resistant than the younger fish. In addition, Brown *et al*. (2016) suggested that adaptive immunity in fish is low during winter. Therefore, parasite was found starting from November when the temperature decreased.

Myxobolus cerebralis infects cartilage tissue and causes a whirling behavior (tail chasing swimming), a black tail, and skeletal deformities of affected fish. Whirling disease was previously known as a hatchery disease, but recently, it has been recognized as one of the causes for the decline of natural rainbow trout population (Hedrik *et al*., 1986). Therefore, *Myxobolus* sp. recorded in the present study might be threatening species for fish hatcheries. In addition, it can have impact on natural population of *Cirrhinus mrigala* in near future.

The mean body weight of infected fish is lower than the uninfected one. Shanchita and Hossain, (2015) reported that the internal parasites can cause physiological damage, cell proliferation and reproductive damage, necrosis in epithelial tissue and mucosal destruction of the intestine due to parasitism of myxosporean parasites in the intestine. Tissue damage such as necrosis and proliferation of mucus cells were recorded in the intestine of fish. The parasite extracts energy and nutrients from the host which are not supported the general health and reproductive effort and also impair mating and gonad maturation (Reddy and Benarjee, 2014). Therefore, the poor absorption of nutrients can cause due to damage of intestinal wall of fish infected by parasites. It can cause malnutrition that led to the reduction of growth of fish. *Cirrhinus mrigala* is important aquaculture species in Myanmar for both local consumption and export market. They have been cultured in earthen ponds which will be one of the factors for disease transmission of Myxosporean since Tubifex in the earthen pond acts as an alternative host in the lifecycle species of Myxozoa. The present finding will support the fishery sector for the management of parasitic infection in earthen pond culture system for *Cirrhinus mrigala.*

Conclusion

The result of the present study indicated that the *Cirrhinus mrigala* is infected with three myxosporean parasites namely *Myxobolus* sp., *Thelohanellus* sp. and *Henneguya* species. High prevalence of infection was recorded when the fish were young ages. Epithelial tissue and mucosal destruction of the intestine caused by parasitism of myxosporean parasites were found. It can cause malnutrition and retards growth of the fish. Therefore, management practices and pond hygiene in nursery operation systems are suggested for producing quality fish fry for successful harvesting.

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I would like to thank Dr. Win Ohnmar Kyaw, Professor and head of Department of Aquaculture and Aquatic Diseases, University of Veterinary Science for her encouragements to carry out this study. Profound indebtedness are due to U Khin Maung Maw, Director General, Department of Fisheries, for allowing me to conduct the study at Yezin Fishery Station; U Soe Paing, Assistant Director, Yezin Fishery Station, Department of Fisheries, for supporting fish specimens and fish pond to conduct this work at Yezin Fishery Station, Nay Pyi Taw.

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DETECTION OF WHITE SPOT SYNDROME VIRUS INFECTION USING NESTED PCR IN *PENAEUS MONODON* CULTURED IN EXTENSIVE FARMS

Nway Ei Khine[1], Cho Cho Thin[2], Kay Lwin Tun[3]

Abstract

White Spot Syndrome Virus (WSSV) is a major shrimp disease in South East Asia. It cause high mortality and losses huge economic to the shrimp farming countries. In the present study, shrimps were collected from natural habitats (mangrove forest) and extensive trap and hold farms located in Pyapon area, Ayeyarwady Region from May to September 2019. The samples were examined for WSSV using PCR and Nested PCR methods. Two out of ten samples from natural habitats showed positive in nest PCR in May 2019. The prevalence of infection ranged 20% to 60% in the shrimps collected form trap and hold systems during sampling from May to August 2019. However, all samples collected in September 2019 showed negative in both PCR and nested PCR. Since the nest PCR detects latent or carrier stage, the result in the present study indicated that shrimps collected from natural habitats and traps and hold farms are latent or carrier stage.

Keyword: WSSV, *Penaeus monodon,* PCR, Myanmar, Trap and hold farm

Introduction

Local shrimp farmers in Ayeyarwady Region and Rakhine state in Myanmar have used traditional trap and hold system for shrimp production. The shrimp farms are constructed near the mangrove forest with inlet and outlet channels to introduce and discharge water to the rivers. The system consists of an earthen pond with three to five sluice gates made up of wood or cement. During high tide, the ponds were filled with brackish water containing wild larvae of different species including shrimps larvae. Shrimp are harvested by releasing the water during the low tide by using fishing nets at the sluice to sieve the outgoing water. Depending on the pond size, harvest time takes 3 to 5 days (interview survey with U Phone Myint Naing, shrimp farmer, Kyonkan Village, Pyapon). Using the trap and hold system, shrimps (*Peneaus* spp., *Metapeneaus* spp.), crab and fishes are produced. Among them, *P. monodon* is the valuable commercial species for trading and local demand.

In Ayeyarwady Region, Pyapon Township is the main areas of producing of *P. monodon* using trap and hold farming system. The farm types are defined based on farm size such as small and medium-scale farms (up to 20 ha) and large scale farms (up to 50 ha) and commercial owned by private investors with an area up to 200 ha (Joffre *et al.*, 2012). The majority of farms in Pyapon Township are larger than 10 ha.

Local farmers rely on *P. monodon* production rather than other aquatic species because of high market demand and price. However, shrimp production in trap and hold farming system has decreased since 2010 without significant reason. Shrimps have been infected with various viruses, bacteria, fungi, parasites, algal toxins, nutritional deficiency or adverse environment. The most lethal for *P. monodon* are White Spot Syndrome Virus (WSSV) and Yellow head virus (YHV) in Asia (Flegel, 2009). The three main pathogens affecting *P. monodon* in Myanmar are WSSV, Taura syndrome virus (TSV) and YHV (Tun *et al.*, 2020). In 2010, Department of Fisheries conducted scanning of disease for 40 individual of *P. monodon* collected from Ayeyarwady Division. Among

[1] Ph.D Student, Department of Zoology, University of Yangon
[2] Lecturer, Fisheries and Aquaculture, University of Yangon, Kamayut Township, Yangon
[3] Professor, Fisheries and Aquaculture, University of Yangon, Kamayut Township, Yangon

them, four samples were found positive for TSV. Yellow Head virus (YHV) was also detected in shrimp which were foreseen to export in 2014 (NACA and FAO, 2015).

There is no information for the distribution of shrimp diseases in trap and hold farming systems in Ayeyarwady Region, Myanmar although shrimp farming is one of the most important productions in aquaculture industry of the country. The present study was undertaken to examine the occurrence of WSSV, one of the lethal pathogens for shrimps, in trap and hold system in Pyapon Environs, Ayeyarwady Region.

Materials and Methods

Study area

Two study areas were chosen to collect the sample. Post larvae shrimp were collected from Mangrove forest (natural habitats) before entering the trap and hold farm. In addition, three trap and hold farms located in Pyapon Township, Ayeyarwady Division, 16º4′48″ N - 95º43′16″ E. (Fig.1. A) were selected to collect the shrimp monthly.

Study period

Study period lasted from May, 2019 to November, 2019.

Collection of specimens

A total of ten post larvae of *Penaeus monodon* were collected from mangrove forest in May 2019 to examine the WSSV infection in natural habitat. Collection of post larvae in mangrove forest was conducted only one time before entering the area of trap and hold farms.

To collect the samples from trap and hold farms, three farms near the mangrove area were selected. The areas of farms were 40 acres (16ha). The farms are two meters apart each other. A total of five sample of *Penaeus monodon* were collected from each farm from May to September 2019. The shrimps brought to Aquatic Animal Diseases Laboratory, Fisheries and Aquaculture, University of Yangon alive. In the laboratory, length and weight of the shrimps were measured. The whole tissue of post larvae and pleopods of adult individuals were collected separately and fixed with 70% ethanol in 1.5ml micro centrifuge tube until DNA was extracted (Plate 1).

DNA extraction

DNA was extracted by PETNAD nucleic acid co-prep kit (GeneReach Biotechnology Corp) (Plate 2). Tissue and pleopods sample (20-25 mg) was ground using disposable plastic grinder with 100 µl PB1 solution. Then PB1 solution (500 µl) was added and vortexed for one minute. A total of 600 µl of PB2 solution, 600 µl of PB3 solution and 600 µl of PB4 solution were added and discharged step by step according to the manual instruction. Finally, 50 µl of PB5 solution was added for elution and stayed at room temperature for one minute and centrifuged. Concentration of extracted DNA were measured NanoDrop 2000 Spectrophotometer and it was stored at -20˚C.

Polymerase chain reaction (PCR) and nested PCR methods for detection of WSSV

Two steps of PCR were used in the present study. First step PCR was to detect the serious infection of WSSV whereas second step PCR was to detect a latent or carrier-state of WSSV (Table1).

Table1 Primers used for PCR analysis of WSSV confirmation (Lo *et. al*., 1996)

PCR	Primers	Sequences 5' to 3'	Product Size
first PCR	146F1	ACT-ACT-AAC-TTC-AGC-CTA-TCTAG	
	146R1	TAA-TGC-GGG-TGT-AAT-GTT-CTT-ACG-A	1447bp
nested PCR	146F2	GTA-ACT-GCC-CCTTCC-ATC-TCC-A	
	146R2	TAC-GGC-AGC-TGC-TGC-ACC-TTG-T	941bp

The volume of the reaction mixture for the first step PCR was 20.0 µl containing 10.0 µl of HS-PCR MasterMix (WizPure™ PCR 2X Master), 0.8 µl of WSSV first PCR Primer Mix, 7.2 µl of ddH_2O 2.0 µl of extracted DNA extract template. In the nested PCR, the amplified PCR products of the first step served as the template DNA for the second step of amplification. After completion of the first step, 20.0 µl of nested PCR containing 10.0 µl of HS-PCR MasterMix ((WizPure™ PCR 2X Master), 0.8 µl of WSSV nested PCR Primer Mix, 7.2 µl of ddH_2O 2.0 µl of first PCR product was prepared. The condition of PCR thermal cycler was described in Plate 3 and Table 2. The positive DNA was provided by the Aquaculture Pathology Laboratory, University of Arizona, United States. The PCR was conducted using thermal cycler (Applied Biosystem 9800) (Plate 3). The condition of PCR thermal cycler was described in Table 2.

Table 2 first and nested PCR amplification for WSSV

Temperature	Time	Cycle	Step
95°C	5 minutes	1	initial denaturing
95°C	30 seconds		denaturing
60°C	30 seconds	25	annealing
72°C	30 seconds		extension
72°C	5 minutes	1	final extension

Gel electrophoresis

PCR products were separated on 1.5% agarose gel containing 30 ml of 1xTAE buffer, 0.15 g of agarose powder and 6 µl of Gel Stain Green (Wizbiosolutions Inc). A 100bp DNA Marker (Wizbiosolutions Inc) was used to compare the base pair PCR results. Gels were visualized under UV Blue light trans-illuminator (Plate 4).

Parameters of soil and water in farms

Water and soil samples from the study sites were collected monthly to analyze water pH, alkalinity, ammonia, temperature, salinity, and soil pH. Water pH, alkalinity, ammonia were checked using the test kits (Advance Pharma Co., Ltd. Bangkok, Thailand). Water temperature was recorded by using a mercury thermometer. The water salinity was measured by using MA 887 Seawater Refractometer. Soil pH was analyzed by using INDEX pH meter ID 1100 USA.

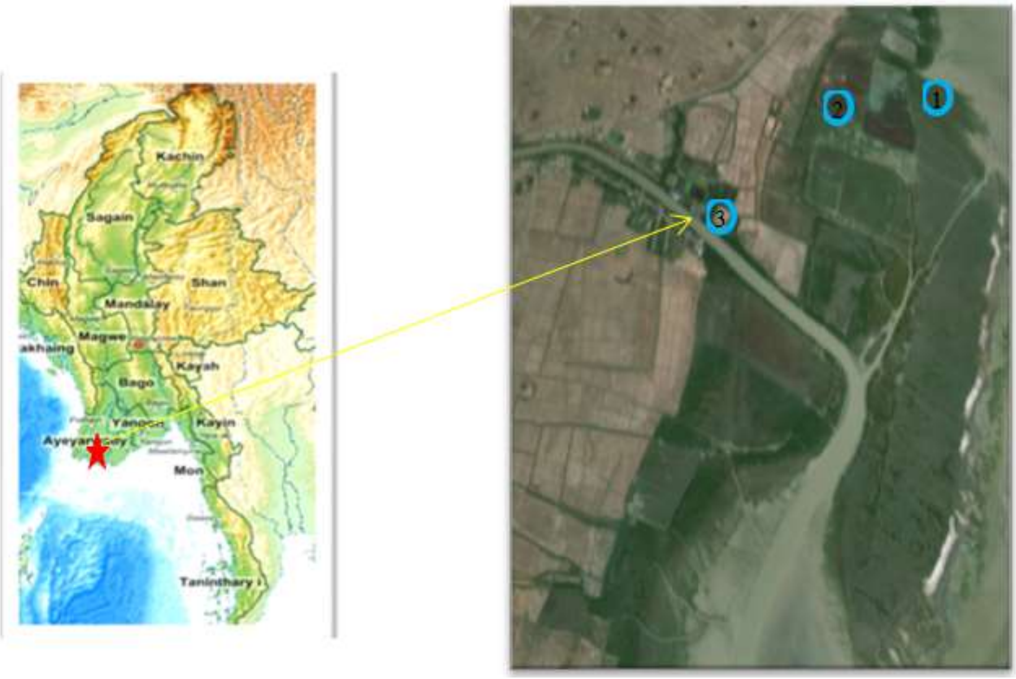


Figure 1 Location of Trap and Hold Shrimp Farms in Pyapon, Ayeyarwady

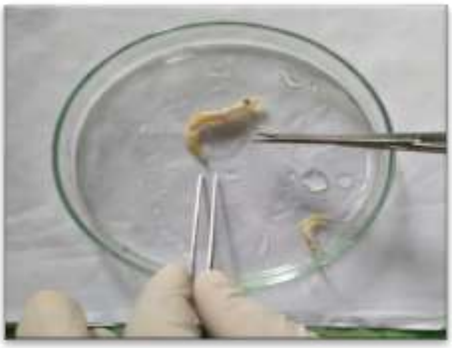


Plate 1 Sampling of *P. monodon*

Plate 2 PETNAD nucleic acid coprep kit (DNA Extraction) (LIR biotech lab-ind resource Co. Ltd, Malaysia)

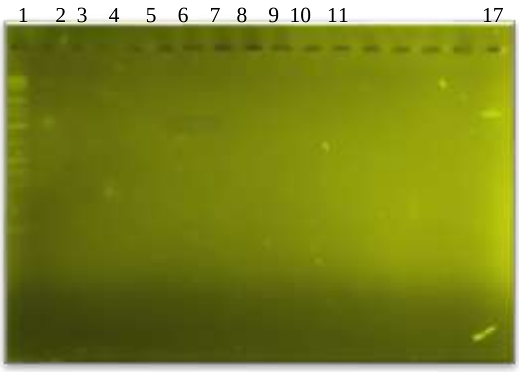
Plate 3 Thermal cycler for PCR assay

Plate 4 Gel electrophoresis

Results

Detection of White Spot Syndrome Virus at Natural Habitats (Magrove forest)

Before entering the cultured farms, 10 individuals of shrimp post-larvae were collected from natural habitats (mangrove forest) in May 2019 to examine the WSSV infection. WSSV infection was negative in the first step PCR in all samples (Plate 5 A). However, two out of ten samples showed positive results in nested PCR (Plate 5 B).



A. First step PCR result of the shrimp sampled shrimp collected in May 2019

B. Nested PCR result of the in May 2019

Plate 5 First step and nested PCR results of WSSV detection in *P. monodon* from natural habitats

A: Well 1 - Ladder (100bp), Well 2 to11 - Samples from natural habitat, Well 17 - Positive control

B: Well 1 - Ladder (100bp), Well 2 to 11 - Samples from natural habitat,

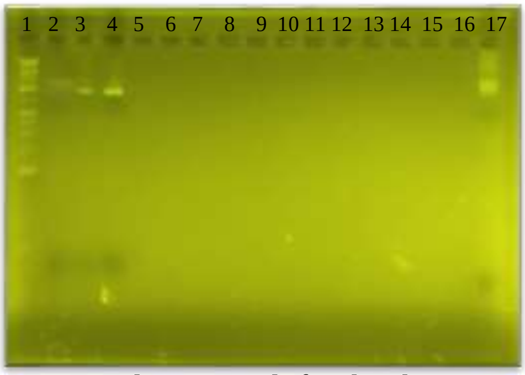
Well 17 - Positive control, Well 2, 3 - WSSV Positive results

Detection of White Spot Syndrome Virus in three trap and hold farms

All samples collected from farm (1), (2) and (3) shows negative results for WSSV infection in first step PCR during the study period.

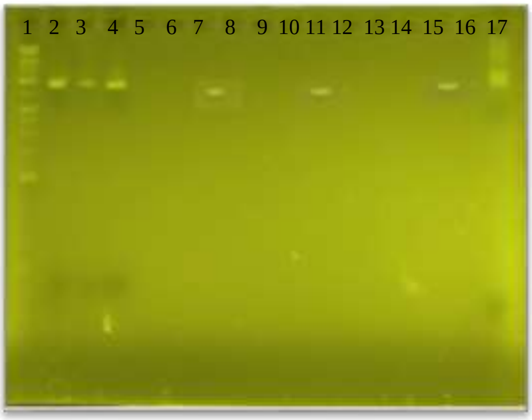
In nested PCR, two samples from the farm (1) were positive in June 2019 (Plate 6 A and Table 3). In July, two samples from the farm (1) and one sample from the farm (2) showed positive in nested PCR (Plate 6 B and Table 3).

All farms showed positive for WSSV in nested PCR in August 2019. The three samples from farm (1), two samples from farm (2), and one sample from farm (3) were positive (Plate 6 C and Table 3). Interestingly, all *P. monodon* samples were negative for WSSV in nested PCR in September 2019 (Plate 6 D and Table 3).

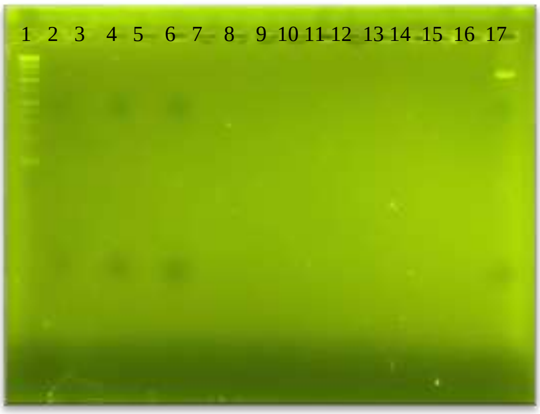


A. Nested PCR result for the shrimp in June 2019

B. Nested PCR result for the shrimp sampled in July 2019



B. Nested PCR result for the shrimp in August 2019



D. Nested PCR result for the shrimp sampled in September 2019

Plate 6 Nested PCR Result for WSSV disease in *P. monodon* (farm 1, 2, and 3) in June – September 2019

Well 1 - Ladder (100bp), Well 2 to 6 - five Samples from farm 1, Well 7 to 11 - five Samples from farm 2, Well 12 to 16 - five Samples from farm 3, Well 17 - Positive control

Table 3 PCR Result for WSSV detection from *P. monodon*

No. of Farm	June				July				August				September			
	+ in 1st PCR	%	+in nested PCR	%	+ in 1st PCR	%	+in nested PCR	%	+ in 1st PR	%	+in nested PCR	%	+ in 1st PCR	%	+in nested PCR	%
Farm (1)	0/5	0	2/5	40	0/5	0	2/5	40	0/5	0	3/5	60	0/5	0	0/5	0
Farm (2)	0/5	0	0/5	0	0/5	0	1/5	20	0/5	0	2/5	40	0/5	0	0/5	0
Farm (3)	0/5	0	0/5	0	0/5	0	0/5	0	0/5	0	1/5	20	0/5	0	0/5	0

Water and Soil Parameters at Extensive Farms

The water temperatures among the three farms were not shown much variation. It was ranged between 27 - 29˚C, 27 - 30˚C and 29 - 31˚C in the farms (1), (2), and (3) respectively during the study period. The range of water pH was 7.2 - 8.3, 7.3 - 8.1, and 7 - 8 in three farms. The range of alkalinity was 100 - 180 ppm in farms (1), (2), and (3). The maximum and minimum concentrations of ammonia were 0.17 ppm and 0 ppm in the farm (1), 0.3 ppm and 0 ppm in the farm (2), and 0.2 ppm and 0 ppm in the farm (3) from June to September 2019. The range of salinity was 1 - 4 ppt throughout the study period in all farms. The range of soil pH varied 7.06 to 7.23 in the farm (1), 6.95 - 7.36 in the farm (2), and 7.1 - 7.3 in the farm (3) (Table 4).

Table 4 Water and soil quality Parameters in shrimp farms

Parameters	June			July			August			September		
	Farm			Farm			Farm			Farm		
	1	2	3	1	2	3	1	2	3	1	2	3
Temperature (ºC)	27	27	29	27	29	29	29	30	31	29	29	31
pH	8	8.1	8	7.2	7.4	7	7.5	7.3	7.5	8.3	7.7	8
Alkalinity (ppm)	160	140	180	120	110	100	120	150	170	110	100	100
Ammonia (ppm)	0.1	0	0	0	0.1	0.12	0.15	0	0.1	0.17	0.3	0.2
Salinity (ppt)	3	3	2	2	2	1	3	4	2	2	2	2
Soil pH	7.06	6.95	7.13	7.14	7	7.1	7.1	7.21	7.3	7.23	7.36	7.19

Discussion

The present study is the first report for the scanning of White Spot Syndrome Virus in shrimp cultured in extensive trap and hold farms in Pyapon area, Ayeyarwady Region. In the present study, PCR and nested PCR methods were conducted for detection of WSSV in post-larvae lived natural habitats and juvenile to adult shrimps cultured in trap and hold farms. In trap and hold system farms, introducing of shrimp post-larvae are totally depended on natural habitats, mangrove forest (Myanmar Shrimp Association, 2015). To understand the disease transmission of post-larvae from natural habitats to extensive cultured farms, shrimp post-larvae were collected from mangrove and examined for WSSV infection. WSSV infection was negative in the first PCR while it was positive in nested PCR. The result is positive in the first step implies a serious infection with WSSV, when the result is positive only in the second step, a latent or carrier-state infection (OIE, 2019). The present work supports collected shrimps at the natural habitats are carrier-state with the light infection which showed positive with nested PCR only.

WSSV had been introduced to Myanmar by imported post-larvae from Thailand and serious outbreaks of shrimp disease were encountered in 1996-1999 (FAO, 2018). After outbreaks of WSSV in Myanmar, penaeid shrimp from the natural population of Myanmar became carrier state for WSSV in outbreak area (Tun *et. al*., 2017). Therefore, it is assumed that post-larvae from mangroves are latent-state for WSSV. Positive WSSVs were found in nested PCR in farm (1) in June 2019 while farms (2) and (3) were negative. All positive samples from extensive farms were also detected in the nested PCR stage in July and August. Tun *et. al.*, (2017) also reported positive for WSSV infection in nested PCR state and they concluded that WSSV in the Rakhine region was only latent or carrier-state. Yu Wai Hlaing (2018) reported the occurrence of WSSV in intensive shrimp culture farms in Myeik, Tanintharyi Region.

The present work supports collected shrimps at the carrier-state with the light infection which is positive with nested PCR only. Lo and Kou (1998) stated that if the shrimps at the nested state PCR are positive only, they may not display any clinical signs of WSSV. The collected post-larvae of *P. monodon* at natural habitats were not found any clinical signs of WSSV. The collected samples of *P. monodon* at extensive farms were not displayed in white spots or patches embedded in the exoskeleton.

Water and solid quality in the shrimp farms were examined and the values were acceptable limit for shrimp culture. According to the guideline of FAO (2017), water and soil parameters in the studied area are suitable for the culture of *P. monodon.* To improve the sustainable shrimp production in Pyapone area, *r*egular examination of WSSV in extensive farms is essential for effective health management. Monitoring of shrimp disease in extensive farms is necessary to reduce the possible risk of an outbreak of WSSV.

Conclusion

This is the first report for scanning of WSSV in extensive shrimp farms in the Ayeyarwady Region. The *P. monodon* collected from mangrove area and trap and hold system farms were negative in first stage PCR while it was positive in nested PCR. The present work supports collected shrimps at the asymptomatic carrier-state with the very light infection which is positive with nested PCR only. WSSV can disperse to shrimp farms where water has been shared among the farms. Therefore, a regular scanning for WSSV in farms should be undertaken.

Acknowledgements

Firstly, I am deeply indebted to Dr. Aye Mi San, Professor and Head, Department of Zoology, University of Yangon, for her permission for my research work. I greatly indebted to MYSAP, Myanmar Aquaculture Sustainable Programme implemented by GIZ for their support. I sincerely thank all farm owners and workers at extensive farms in Pyapon. Finally, my special thanks go to my parents for their moral and financial supports to accomplish this study.

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GONADAL DEVELOPMENT OF *CHANNA STRIATA* (BLOCH, 1793) FROM HMAWBI TOWNSHIP, YANGON ENVIRONS

Khine Min Tun[1], San San Myint[2], Omar Myint[2]

Abstract

Knowledge on reproductive biology of fish species is crucial important for the biodiversity conservation and for the sustainable development of the fishery. The biological information about *Channa striata* has been poorly documented in Myanmar. The present work has been conducted to investigate the gonadal development and fecundity of *C. striata* from their natural environment. Fish were sampled from rice fields of Hmawbi Township in Yangon Region. Gonadal development has been studied by macroscopic and microscopic observation of the different stages of gonad during the study period from November 2017 to October 2019. In the present study, macroscopic characteristics of the female gonads showed four maturation stages and histological observation indicated seven phases of oocyte development. Spawning period has been determined by observing the monthly Gonado-Somatic-Index and Condition factor of the females. Highest GSI value of female *C. striata* was found in June and length at first sexual maturity was estimated at 26.04 cm. The mean absolute fecundity was 33922.08 ± 9708.42 vitellogenic oocytes. The results from the present study clearly showed that the breeding season of *C. striata* was extended from May to July in Myanmar and their peak spawning season was in June.

Keywords: gonadal development, fecundity, length at first sexual maturity

Introduction

Channa striata, commonly known as snakehead, is a freshwater fish and native to Asia, Indonesia and tropical Africa (Herborg *et al*., 2007). It is locally known as Nga-yant in Myanmar. All species of snakeheads are piscivorous and feed on crustaceans and small vertebrates (Dasgupta, 2000). They breed in lakes, river, ponds and shallow water areas such as flooded paddy fields (Jayaram, 1999). Because of its high protein contents, snakeheads are important food fish in Southeast Asia. Snakehead is an economically important species and it is a popular dry fish in Myanmar. The natural population of this species is gradually declining because of over exploitation and habitat degradation.

Knowledge on reproductive biology of fish species provides important information for the rational management of a fishery resource (Greiner and Gregg, 2010; Reuter *et al*., 2010). Animals use various reproductive strategies to maximize their reproductive fitness and/or to ensure the survival of offspring. The reproductive tactics such as size at first sexual maturity, spawning period and fecundity are used by the individual organisms (Potts and Wootton, 1984) to increase their reproductive fitness. Many researchers has been doing research on reproductive biology of fishes in order to provide information for the sustainable development of the fishery and aquaculture.

A thorough understanding on the gonadal development of female *C. striata* provides important information to fish stock management. Due to over exploitation and habitat degradation, the population of *C. striata* is gradually decreasing and there is a growing concern over degradation of population of *C. striata* in their natural habitats. Hence, it is urgently needed to study the reproductive biology of *C. striata* and to monitor systematically in the purpose of fishery management. However, no detail work on reproductive biology of *C. striata* has been performed in Myanmar, thus, the present study was undertaken to study some aspects of reproductive biology of *C. striata*. The aims of the present study was to describe the different stages of gonadal development of female *C. striata* and to estimate their length at first sexual maturity.

[1] Student, Department of Zoology, University of Yangon
[2] Dr, Lecturer, Department of Zoology, University of Yangon

Materials and Methods

A total of 420 female *C. striata* were observed during the study period. The study area was located at Hmawbi township (17˚ 8′ 10″ N and 96˚ 0′ 34″ E) and all fish specimens were collected from the paddy fields with the help of fishermen from November 2017 to October 2019. Fish sample were kept in ice boxes and transported to the laboratory where total length (to the nearest 1mm) and body weight (to the nearest 0.01g) of fish were measured. The sex of fish were identified by observing gonads of the fish. Gonads of female (n = 15) were removed, weighed while wet (nearest 0.01g) and observed the development stages of the macroscopic gonad monthly. The gonadosomatic index (GSI) was calculated by using the formula described by Brook *et al*. (1997): GSI = total gonad weight (g)/ total body weight of fish (g) x 100. Hepatosomatic index (HSI) of females was calculated according to Cek and Yilmaz (2009): HSI = total liver weight (g)/ total body weight of fish (g) x 100. Female reproductive maturity was determined by macroscopic observation of different stages of the ovary. To determine the absolute fecundity of the female, the mean of three sub-samples, anterior, middle and posterior part of the ovary of twenty mature females, were used to calculate absolute fecundity by counting the number of oocytes per sub-samples of ovary to the total ovary weight. Total number of oocytes were calculated by using the formula described by Bagenal (1978): total number of oocyte = total weight of ovaries/subsample weight x number of counted oocytes in the subsample. Oocyte samples, taken from anterior, middle and posterior part of the ovary, were measured by using ocular micrometer and calculated the size of the oocyte by using the formula supported by De Vlaming *et al*. 1982: egg size = (length axis + wide axis)/2.

Histological analysis

The gonad samples of three mature females were preserved in 10 % buffered formalin monthly and they were kept at room temperature. They were dehydrated in graded alcohol series, exposed to Xylene and embedded in paraffin wax. Sections of 0.15cm-0.2cm thickness were prepared and stained with Haematoxylin and Eosin, then mounted with DPX. Finally, photographed with a ZEISS PRIMO STAR TRINOCULAR microscope which was equipped with the Axiocam ERc 5s camera. Gonad developmental stages were estimated observing sections under the microscope as immature, maturing, mature and spent phases (Navarao *et al*. 1989; Morton 1990).

Results

A total of 420 specimens were observed during the study period. The gonadal development of the female *C. striata* were observed through macroscopic and microscopic analysis. The female *C. striata* presented with total length varying from 27.3 cm to 50 cm (35.46 ± 5.61, n = 20) and weight varying from 737.1 to 162.39 g (364.86 ± 151.86, n = 20) (Table.1). The absolute fecundity of females ranged from 18148.68 to 56927.35 eggs (33922.08 ± 9708.42, n = 20). The oocyte diameter is varied from 1.1 to 0.55 mm (0.84 ± 0.13, n = 20) and egg diameter varied from 1.3 to 0.48 mm (0.99 ± 0.15, n = 20) (Table.1). The total length and ovary weight of females showed a positive correlation with a coefficient of determination (r^2) of 0.8756 (Fig. 1). There is a positive correlation between total length and number of eggs (r^2 = 0.8395, Fig. 2) and between ovary weight and number of eggs in the female ovary (r^2 = 0.8105, Fig. 3). The total length at first maturity of *C. striata* was 26.04 cm (Fig.4).

The GSI values of female *C. striata* (n = 360) varied from 0.08 to 2.49 and the highest value was found in May (Fig. 5). The HS

I values varied from 0.60 to 1.10 and the highest value was found in June (Fig. 5).

Through macroscopic observation, the immature stage was found throughout the year, except in July and August (Fig. 6). The developing stage was started to find in September and the mature stage in May to July (Fig. 6). The spent stage was found in June and August. (Fig. 6).

The ovary of *C. striata* is a paired hollow sac-like structures and more or less elongated, lying dorsal to the alimentary canal and ventral to the swim bladder. The two ovaries were united to form an oviduct which opened to the exterior via the oval shaped urogenital papilla. The size of the left ovary was usually larger than the right and the color of the ovary varied from reddish brown to light yellowish.

Macroscopic gonadal maturation stages of female *C. striata*

Macroscopic observation revealed four gonadal maturation stages in female *C. striata* as immature, developing, mature and spent (Plate. 1).

Stage (I) Immature

Ovaries are relatively small, thin, translucent and pale reddish in color.

Stage (II) Developing

In this stage, ovaries are increased in size and reddish in color. Small ova are visible through thick wall of gonad. Blood capillaries become conspicuous.

Stage (III) Mature

The ovaries occupy the whole of the body cavity and it is yellowish in color. The ripe eggs are apparently visible.

Stage (IV) Spent

The ovaries are small in size and dull white in color. The volume and weight of the ovary is decreased in this stge.

Microscopic gonadal maturation stages of female *C. striata*

The microscopic examination of the ovaries of *C. striata* showed seven phases of oocyte development (Plate. 2) constituting four different stages. The first or immature stage included the chromatin nucleolar stage (Phase I) and Perinucleolar stage (Phase II). The second or developing stage included early yolk vesicle stage (Phase III) and late yolk vesicle stage (Phase IV). The third or mature stage included early yolk granule stage (Phase V) and late yolk granule stage (Phase VI). The fourth or spent stage included artretic stage (Phase VII).

Stage (1) Immature

(I) Chromatin nucleolar stage (CN)

In this stage, oocytes were small cells surrounded with a thin peripheral zone. They contained a spherical and large nucleus, which occupies the greater part of the cell with one nucleolus.

(II) Perinucleolar stage (PN)

In perinucleolar stage, the size of the oocyte became larger and the shape vary from polygonal to oval according to the oocyte development. The nucleus became enlarged.

Stage (2) Developing

(III) Early yolk vesicle stage (EYV)

Small yolk vesicles can be seen in the cytoplasm. These yolk vesicles first appeared at the periphery of the oocyte.

(IV) Late yolk vesicle stage (LYV)

In this stage, the yolk vesicles gradually spread towards the central nucleus and the nucleoli were present at the periphery of the nucleus.

Stage (3) Mature

(V) Early yolk granule stage (EYG)

Small yolk protein granules stained in light pink can be seen in the outer cortex. They were gradually increased in size and number and then moved towards the inner cortex.

(VI) Late yolk granule stage (LYG)

The oocytes were greatly increased in diameter and yolk granules and lipid droplets were fused in this stage.

Stage (4) Spent

(VII) Atretic stage (AT)

The nucleus became degenerate followed by the dilution of the yolk and fragmentation of the zona radiata.

Through microscopic observation, the immature stages (chromatin nucleolar stage and perinucleolar stage) were found in October and November and developing stages (early yolk vesicle stage and late yolk vesicle stage) were found between December and April (Plate. 3). The mature stages (early yolk granule stage and late yolk granule stage) were highly found in June. The spent stage (atretic stage) was commonly found in August (Plate. 3).

Table 1 Absolute fecundity, oocyte and egg diameter of mature *C. striata*

	Weight (g) (n=20)	**Length (cm) (n=20)**	**Absolute fecundity (n=20)**	**Oocyte diameter (mm) (n=20)**	**Egg diameter (mm) (n=20)**
Mean±SD	364.86 ± 151.86	35.46 ± 5.61	33922.08 ± 9708.42	0.84 ± 0.13	0.99 ± 0.15
Range	737.1 – 162.39	27.3 – 50.0	18148.68 – 56927.35	1.1 – 0.55	1.3 – 0.48

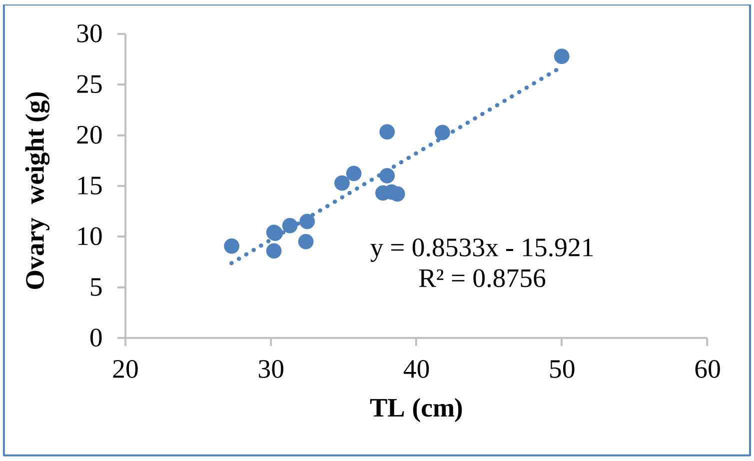


Figure 1 Relationship between total length (TL) and ovary weight of female (n=20)

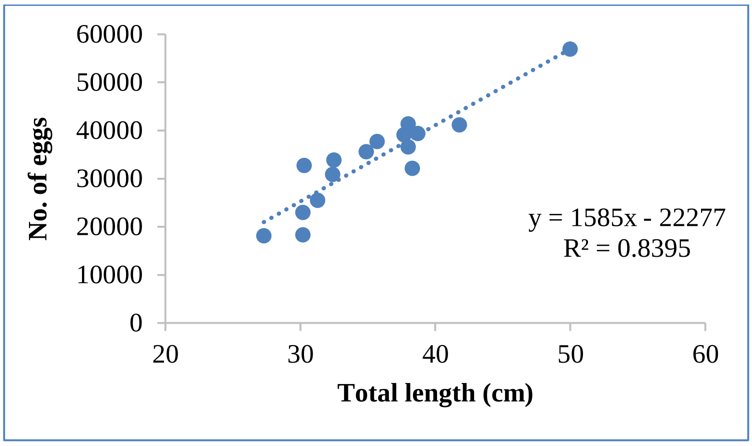


Figure 2 Relationship between total length (TL) and number of eggs of female (n=20)

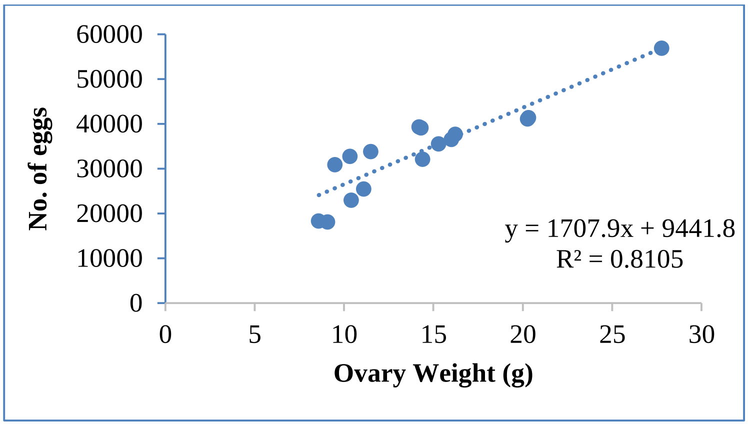


Figure 3 Relationship between ovary weight and number of eggs of female (n=20)

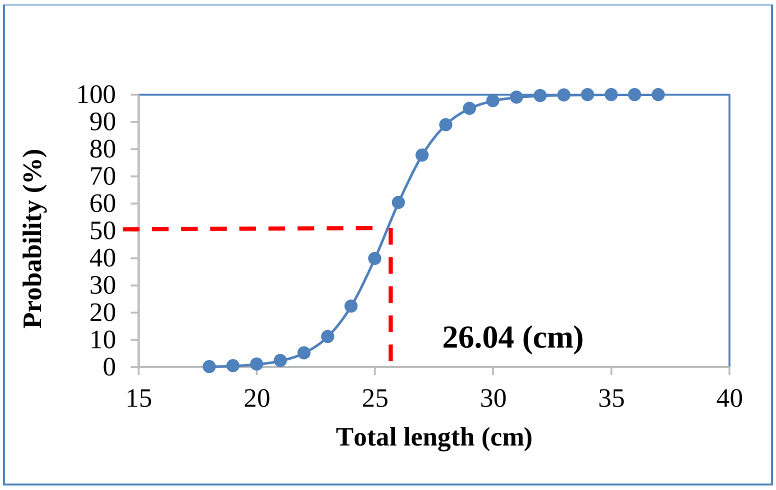


Figure 4 Length at first maturity (L_m50) of female *C. striata* (n=145)

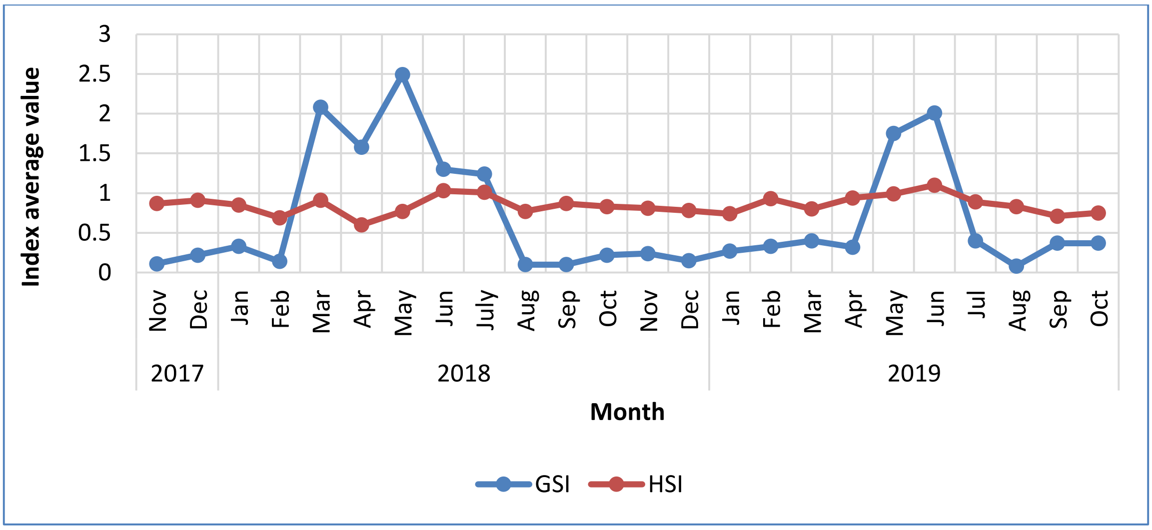


Figure 5 Monthly variation of GSI and HSI of female *C. striata* (n=360)

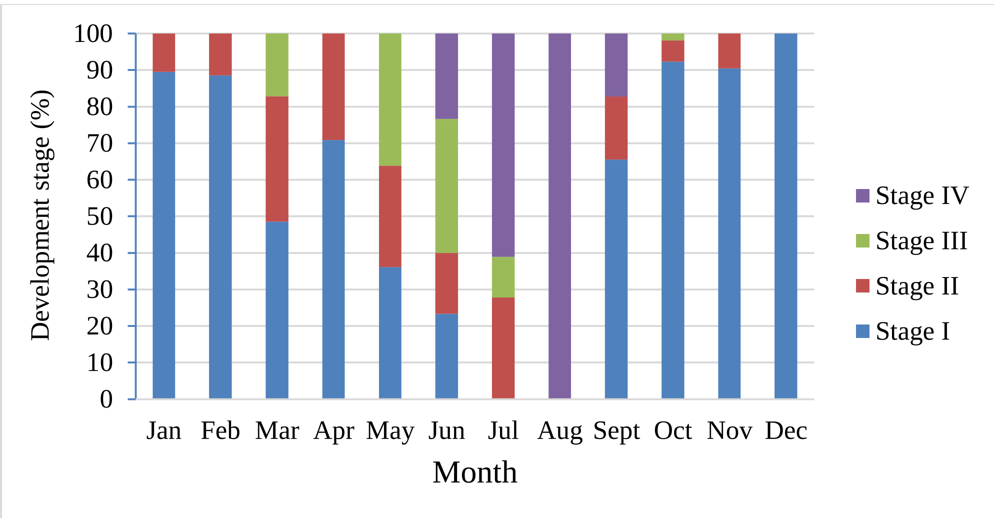
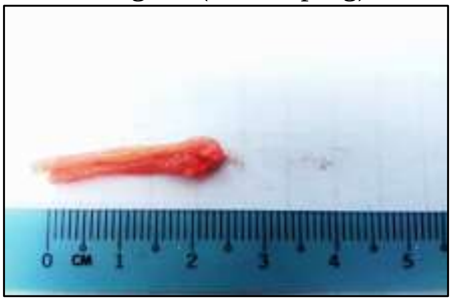


Figure 6 Monthly developmental stages of ovary of *C. striata* (n=180)

Stage I (Immature)

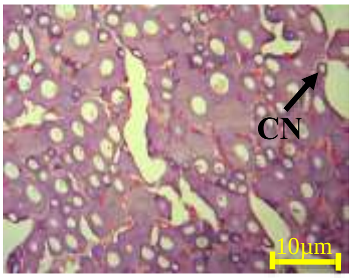

Stage II (Developing)


Stage III (Mature)

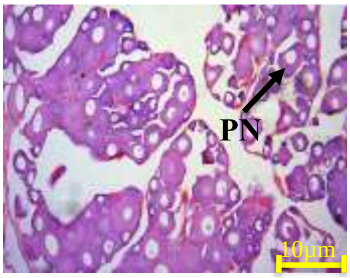


Stage IV (Spent)

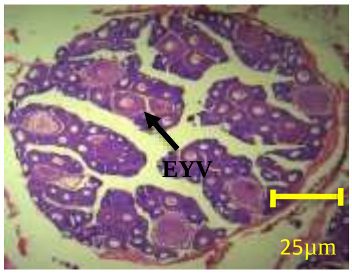
Plate 1 Developmental stages of the female ovary (Macroscopic features)



I. Chromatin nucleolar stage

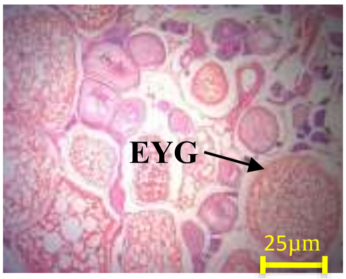


II. Perinucleolar stage



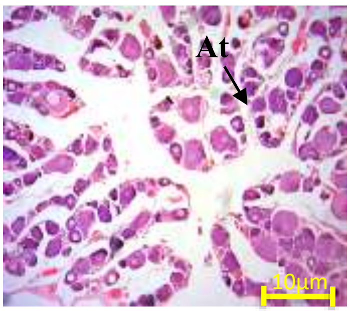
III. Early yolk vesicle stage

IV. Late yolk vesicle stage



V. Early yolk granule stage

VI. Late yolk granule stage



VII. Atretic stage

Plate 2 Developmental stages of ovary of *C. striata* (Microscopic features)

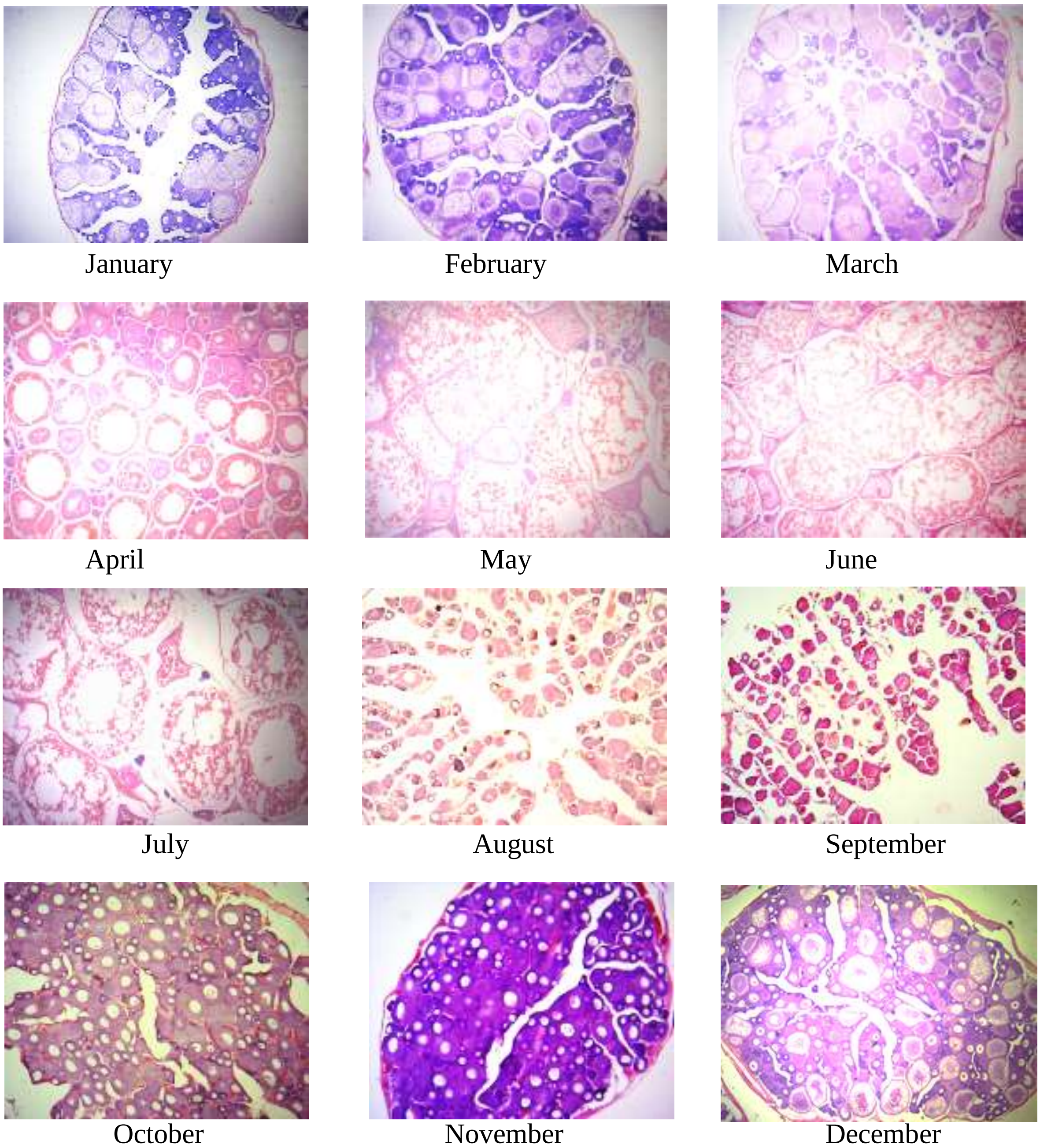


Plate 3 Monthly development of oocyte of *C. striata*

Discussion

In the present study, the macroscopic characteristic of gonads indicated four maturation stages of ovary in *C. striata*. Histological studies of gonads of *C. striata* showed seven phases of oocyte development. The macroscopic features of gonads stated that immature stages was found throughout the year except in July and August. Developing stage was started to find in September and mature stage was found in May, June and July indicating that spawning occurred during the month of May to July. In addition, the occurrence of mature stage of gonads was highest in June. Spent stage was found in June and August. These results indicated that the breeding season of *C. striata* might be the period between May and July in Myanmar.

Meanwhile, the GSI value was highest in May and abruptly decreased in June. The microscopic observation also gave the same result as the mature stage (late yolk granule) was found in May, June and July and Spent stage (atrectic) was found in August. The result from this study clearly indicates that the breeding season of *C. striata* might started in May and proceed until July in Myanmar. The breeding season of *C. striata* was June-July in Malaysia and has only one breeding season (Ghaedi *et al.* 2013). Hence, our result strongly supports to the result of former researcher. The result from this study clearly showed the breeding season of *C. striata* coincided with the onset of rainy season (May to July) in Myanmar.

The absolute fecundity of the female ranged from 18148.68 to 56927.35 with a mean absolute fecundity of (33922.08 ± 9708.42). Jhingran (1984) cited fecundity of *C. striata* in India as 3000 to 30000 per ovary. Ghaedi *et al.* (2013) reported that the absolute fecundity of 33949 ± 3388 in Malaysia. However, Li (2016) recorded an absolute fecundity of *C. striata* in Taiwan ranging from 4484 to 96498. One possible reason for different value of absolute fecundity of *C. striata* is that fecundity of female depends on their life history traits (size at first maturity, life span etc.) and the environmental factors (availability of food etc.). Our result is consistent with the range of estimate value of the absolute fecundity of *C. striata.*

The length at first maturity of *C. striata* in Myanmar was 26.04 cm which is close to the value (25.5cm) recorded in the irrigated rice field of Malaysia (Ali, 1999). Alikunhi (1953) reported that the smallest mature female specimen was 23.4 cm in length with ovaries in the 4th stage of development. In Sri Lanka, the TL for the smallest mature *C. striata* female was 23.2cm (Kilambi 1986). However, Li (2016) reported that the minimum body length of sexually mature females in Taiwan was 28cm. Hence, the result from this study is consistent with the result of former researchers from Southeast Asian countries and was different from Taiwan because Taiwan is located at a higher lattitude and with a greater minimum body length of the mature females. In addition these differences in female length at first maturity are expected and likely to be associated with different environmental conditions and/or availability of the food. This study provides clear and update information on the gonadal development of female *C. striata* and its length at first maturity.

Conclusion

The knowledge on reproductive biology of any fish species is crucial important for fishery management. In this study, gonadal development of female *C. striata* was investigated. In general, it was concluded that the breeding season of *C. striata* coincides with the rainy season (May to July) of Myanmar and ends in August. The length at first sexual maturity of female is 26.04 cm and their absolute fecundity is 33922.08 ± 9708.42. More empirical research on reproductive biology of *C. striata* should be encouraged for the sustainable development of fish species and its culture.

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I would like to acknowledge Professor Dr. Aye Mi San, Head of the Department of Zoology, University of Yangon, for giving me a permission to perform this research and her valuable advice on this research work. My heartfelt thanks are due to my teachers, colleagues and personnel who helped me a lot to accomplish this work within a limited time frame.

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ESTIMATION ON THE LEVELS OF EXPLOITATION AND SUSTAINABILITY OF SOME MARINE AQUATIC FAUNA FROM NYAUNG TAN FISH JETTY, PAZUNDAUNG TOWNSHIP, YANGON REGION

Ei Thazin Thaung[1], Nant Thin Thin Kywe[2]

Abstract

This study is a piece of research work carried out as a sample to analyze the level of marine aquatic fauna exploitation and which species stocks needed to be sustained. The present study was an evidence based study obtained from 47 marine fauna of the daily catch weights and its respective daily prices of each species stock of Nyaung Tan Fish Jetty, Pazundaung Township, Yangon Region. The relationship between yearly average catch weights and yearly average prices were analyzed by Pearson's Correlation Coefficient method. It was observed that there were significant negative correlation between yearly average catch weights and yearly average prices as R^2= 0.7628, r= - 0.8860 for 2018 and R^2= 0.6634, r= - 0.8144 for 2019. To find out the level of exploitation of aquatic species, four groups were classified according to FAO, 2017 as (i) Lesser than 1000 kg, (Over exploited), (ii) 1000 kg - 10000 kg, (Fully exploited), (iii) 10000 kg - 100000 kg (Moderately exploited) and (iv) Above 100000 kg (Under exploited). Among them, levels of exploitation was found to be the highest from (Lesser than 1000 kg), which was regarded as over exploited group. It included 28.7% of aquatic species out from the average records of 2018 and 2019. Therefore, for the 28.7% of the aquatic species that were found to be over exploited, which were high in fishing pressure. The fishery potential has been exceeded and these stocks are in the unsustainable conditions. This will become bad for every thing if the nutritional value and commercially demanded aquatic species are lost in the ecosystem. In order to conserve these aquatic stocks, preventive managements are needed to monitor the decling aquatic species in the catch to control the fishing gears and to exclude the spawners from catching during the breeding seasons of the marine aquatic fauna.

Keywords: marine aquatic fauna, catch weights, prices, exploitation, over exploited, unsustainable

Introduction

In the ancient times, fishing including some aquatic fauna was the major source of food for humanity that provides employment and economic benefit. In recent years,fishery sector has developed drastically for food industry. It is clear that aquatic resources are renewable and should be sustained even if they are in an uncontrolled conditions of exploitation. Proper management is needed to sustain the resources to contribute the nutritional, economic and social well-being of the growing population. Nowadays, fisheries are facing with a range of challenges like anthropogenic disturbance on the ecosystems and on continuous of negative impact on biodiversity and fish stocks throughout the world. (Jessica and Nilsson, 2019).

Marine ecosystems of Southeast Asia has one of the most diverse ecosystem in the world but overfishing and destructive fishing is threatening the fishery natural resources. Across the Asian countries, 64%of the fisheries' resource base is at a medium risk of overfishing. (Kim, *et.al*, 2018). There are many clear signs of over exploitations on the fish stocks. It means that fish stocks are simply the harvested population of fish. It refers to one specific species in one particular place for each fish species where it existed. Therefore, we need to know how much fish can be harvested from the water bodies if we need to maintain the fish stock. To understand the stock assessment there are different kinds of data to estimate the population of fish stock and how much can be fished. Stock assessments can be determined as follows: (Wilcox 2017)

[1] Assistant Lecturer, Department of Zoology, East Yangon University

[2] Dr, Associate Professor, Department of Zoology, Kalay University

- **Abundance of fish –**To estimate how many fish are in the populations. It can be based on samples that are gathered using various methods.
- **Biological data-** recording the age and length of fish to estimate the level of natural mortality and fishing mortality. These data can also determine the reproductive rate of a population which can predict how many fish will be around next year. Environmental data is also essential to be recorded, such as temperature, salinity, dissolved oxygen and other ecological variables.
- **Catch data-** It is a historical records of how many or what weight of fish was caught during a calendar year or a fishing season.

Yearly catch weights and its relevant yearly average prices were collected by creating mathematical models to predict how a population will respond to different level of fishing. There was a global list of fish stocks with different level of fishing that were ranked as follows from 600 marines stocks (FAO, 2017).

They were defined as: under exploited, moderately exploited, fully exploited, over exploited, depleted and recovering.

The recent study will also be ranked as above procedure to find out the level of some aquatic species exploitation. The United Nations Food and Agriculture Organizations (FAO) is focusing on the fisheries resources of how far the fish stocks are “sustainable” or “unsustainable” to the report on conclusions on which fisheries are overexploited and which are not.

Therefore, the aim of the present study was to carry out with the following objectives:

- to find out the relationship between yearly average catch weights and its relevant average prices among trophic levels.
- to classify the level of exploitation of some marine aquatic fauna during 2018 and 2019
- to compare the FAO, 2017 records with the records of recent study on the basis of the levels of exploitation, sustainability, fishery potential and food security during the study period

Materials and Methods

Study site

The species were collected from Nyaung Tan Fish Jetty, Pazundaung Township, Yangon Region. (Fig.1)

Study period

The study period lasted from January 2018 to December 2019.

Data collection

The estimated catching sites were from Ayeyarwady Region and Mon State. Daily catch weights and relevant prices of individual marine species were recorded from Nyaung Tan fish Jetty. Finally they were sum up to yearly average catch weights and yearly average prices respectively. Catching plots for this locations were two places on B11 to B20, C1 to C25 of Ayeyarwady Region and D16, 17, 21, 22 of Mon State. The species were captured by bottom trawl net which were attached to the fishing vessels in fishing grounds throughout the study period. The length of trawl net fully stretch ranges from 60-76 m. The net is with 68 m in bottom rope and 55 m in head rope. The mesh size is 64 mm, 50 mm, 38 mm and 30 mm respectively. The size of fishing vessel is 27-29 m long, 7-8 m width. In a vessel, the total fishermen are about 22-25.

Identification of species

Species identification and each Trophic Level (TL) were divided as follow: www. Fishbase.org (Froese & Pauly 2012)

Herbivores + Detritivorous group TL 2 into 2.0 – 2.9

Carnivores + Detritivorous group TL 3 into 3.0 – 3.9

Top predator (Omnivores) group TL 4 into 4.0 – 4.9

Data analysis

The relationship between the yearly average price and yearly average weights were analysed by Pearson's Correation Coefficient method and shown in histogram and graphs for the year of 2018 and 2019.

Pearson's Correation Coefficient (Galton, 1877)

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n(\sum x^2) - (\sum x)^2]\,[n(\sum y^2) - (\sum y)^2]}}$$

x = yearly average weight
y = yearly average price
n = number of exploited groups

Depending on the average catch weights, species were classified into the following groups according to FAO's report 2017 "Review of the State of World Fisheries Resources" which are regarded as the level of exploitation.

(i) Lesser than 1000 kg, (ii) 1000 kg-10000 kg, (iii) 10000 kg-100000 k

(iv) Above 100000 kg, (v) Depleted, (vi) Recovering

At each level of above average catch weight the number of species included were analyzed in ratios according to its trophic groups. Based on the level of exploitation the results were designated into four groups according to FAO's report 2017.

To estimate the type of sustainability, fishery potential and food security of fish species, it was referred to Bridgette Wilcox, 2017 "The type of sustainability and food security" as if a fishery is:

Fully–exploited or Fully-fished (Moderately fished). This group estimates as 60% of world fisheries

- Sustainable
- Fishery potential being reached
- Best for food security

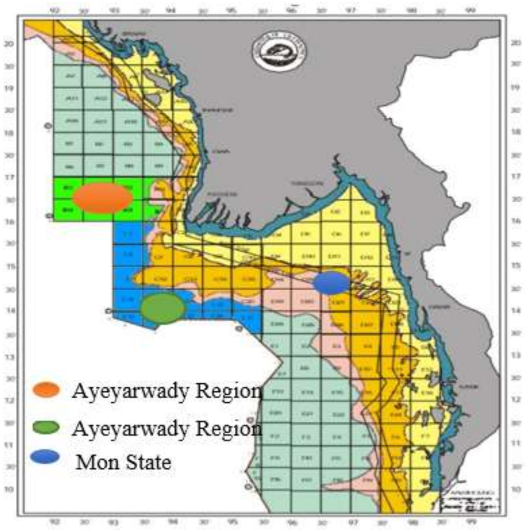
Over-exploited or Overfished. This group estimates as 33% of world fisheries

- Unsustainable
- Fishery potential exceeded
- Bad for everything

Under- exploited. This group estimates as 7% of world fisheries

- Sustainable
- Fishery potential not being reached
- Not good for food security

The outcome of the results will be compared according to the above definitions. Comparisons will be shown in Tables and graphs.



Source: BOBLME (2015)

Source: Google (2018)

Figure1 Fishing grounds of Myanmar and Map of Study site

Results

A total of 47 marine aquatic fauna were recorded from Nyaung Tan fish Jetty. Relationship between yearly average catch weights and yearly average prices were analyzed by Pearson's Correation Coefficient which were recorded as R^2= 0.7628, r = - 0.8860 for 2018 and R^2= 0.6634, r =-0.8144 for 2019 respectively Fig 10 and 11. It showed that they were significant negative correlations between yearly average catch weights and prices.

Among the catch weight groups, the least catch group was lesser than 1000 kg. Generally, this group showed that the average prices of these species were too high, but the average catch weights were extremely low. This group was regarded as **over exploited group**, 12 species existed in 2018 and 15 species existed in 2019.There were only 5 species, *Panulirus polyphagus* and *Oratosquilla nepa* from TL2, *Pampus argenteus*, *Pristolepis pentacantha* and *P.chinensis* from TL3 which cost more than 10000 kyats during these periods. (Fig 2, 3, 12)

In the average catch weight group between 1000-10000 kg, average catch weights were increased compared to (Fig 2 and 3). This group was regarded as **fully exploited group.** There were altogether 17 species existed in 2018 and 13 species included in 2019. But two species from TL 3 *Pampus chinensis* and *Dasyatis kuhlii* TL3 which appeared in 2018 were absent in 2019. Among them, 2 species from TL4, *Katsuwonus pelamis and Scomberomorus commerson* were absent in 2019, but *Argyrosomus amoyenenis* TL4 appeared instead. *Tenulosa ilisha* TL2*, P. chinensis* TL3 and *Leptomelanosoma indicum* TL3 touched the highest price of 10000 kyats. In TL4 all appeared to be in moderate prices, less than 10000 kyats. (Fig 4, 5, 12)

In the catch weight group between 10000-100000 kg they were regarded as **moderately exploited group.** In this group the averge catch weights increased while the prices were appeared to decrease**.** There were 15 species existed in 2018 and 14 species included under 2019. Both groups were absent of TL2. *Arius thalassinus* from TL3 appeared in 2018 was absent in 2019 and *Dasyatis kuhlii* TL3 appeared instead in 2019. Regarding to the averge prices, there were no high priced species contained in this catch range 10000-100000 kg. (Fig 6, 7, 12).

In the catch weight group above 100000 kg only a few species were obtained. Although the catch weights were high their prices were too low. They were regarded as under exploited group.There were merely three species existed in 2018 and five species included in 2019. There were no species of TL2 in both 2018 and 2019. In the year 2019 two species increased one species was *Katsuwonus pelamis* from (TL4) and another species was *Arius thalassinus* from (TL3). Catch weighst were too high and those that were caught have poor value ranging below 5000 kyats (Fig. 8, 9, 12).

Results from Fig 2, 3, 4, 5, 6, 7, 8 and 9 indicated that there were negative correlation between yearly average catch weights and yearly average prices. Total percentage of aquatic species recorded during 2018 were 25.5% from overexploited group lesser than 1000 kg catch weight, 36.2% from fully exploited group 1000 kg-10000 kg catch weight, 31.9% from morderately exploited group 10000 kg-100000 kg catch weight and finally 6.4% from under exploited group greater than 100000 kg catch weight (Table 3).

In 2019 31.9% from overexploited group lesser than1000 kg catch weight 27.7 % from fully exploited group 1000 kg-10000 kg catch weight, 29.7% from morderately exploited group 10000 kg-100000 kg catch weight and finally 10.7% were from under exploited group greater than 100000 kg catch weight (Table 3).

Among the exploited groups, over exploited group is the group that world fisheries recorded as more than a sustainable amount of species exploited. The fishing pressure has already been exceeded that proved to be in the state of unsustainable conditions. In addition to this, these species are highly demanded by the consumers because they provide high content of nutrition to low poverty population. If the havesting pressure continued than the limited level these species can be wiped out from the water bodies affecting the dietary requirements, welfare of the people and national ecomomic growth. (Table 3)

The recent findings of over exploited group appered to be 28.7%which agreed with FAO, 2017 records of world fisheries 33%. (Fig. 13, Table 2)

Over exploited aquatic fauna included are *Oratosquilla nepa* and *Panulirus polyphagus* were included under TL2, *Pristolepis pentacantha*, *Pampus argenteus*, *Pomadasys kaakan* and *Aluterus monoceras* were recorded from TL3 and *Lactarius lactarius*, *Lobotes surinamensis*, *Lutjanu*s *johnii*, *Rachycentron canadum*, *Psettodes erumei*, *Lutjanus sanguineus* and were observed under TL4 (Table 1). For fully exploited and moderately exploited groups, the havesting process has been carrying on and the catch rates are still in the stationary condition proving as sustainable species. They also served as high-protein sources which are best for food security. The recent combined data recorded as 62.6% which also agreed with FAO, 2017 world fisheries records as 60%. The lowest number of species obtained in under exploited group. They are non targeted species because the have low content of nutritional value. Mosty they were caught by by-catch, they are still abundant in the water bodies and not being reached to the maximun catching level .Still sustainable and not good for food security. The recent data recorded as 8.4% which is also compatible with FAO, 2017 world fisheries records as 7% (Fig. 13, Table 2)

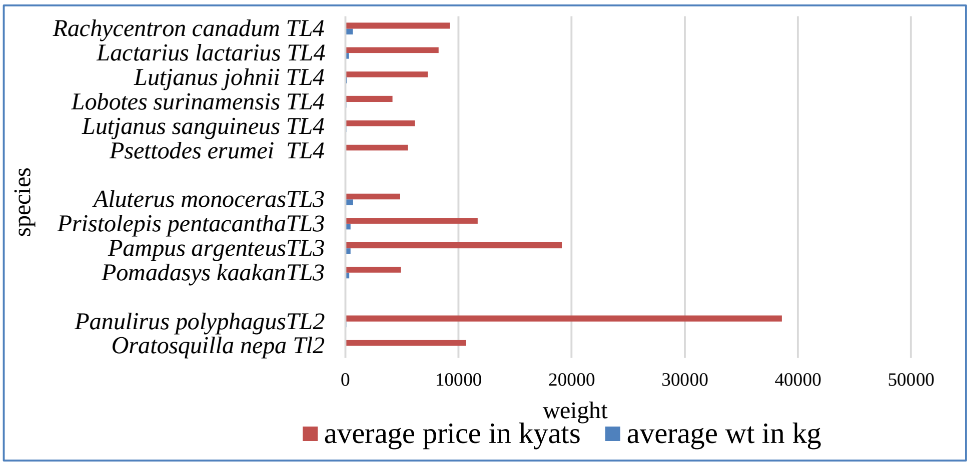


Figure 2 Relationship between average catch weight (lesser than 1000kg) and average price among TL2, TL3 and TL4 during 2018

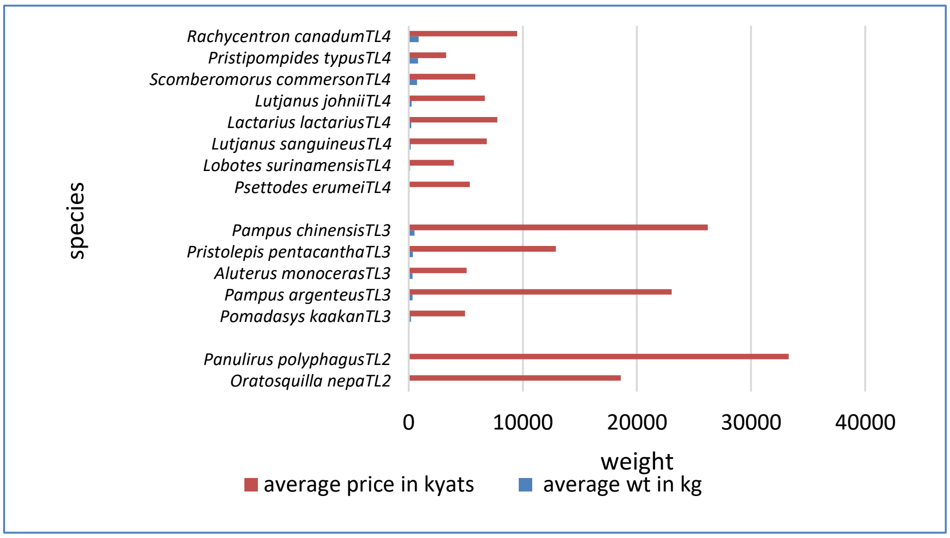


Figure 3 Relationship between average catch weight (lesser than 1000kg) and average price among TL2, TL3 and TL4 during 2019

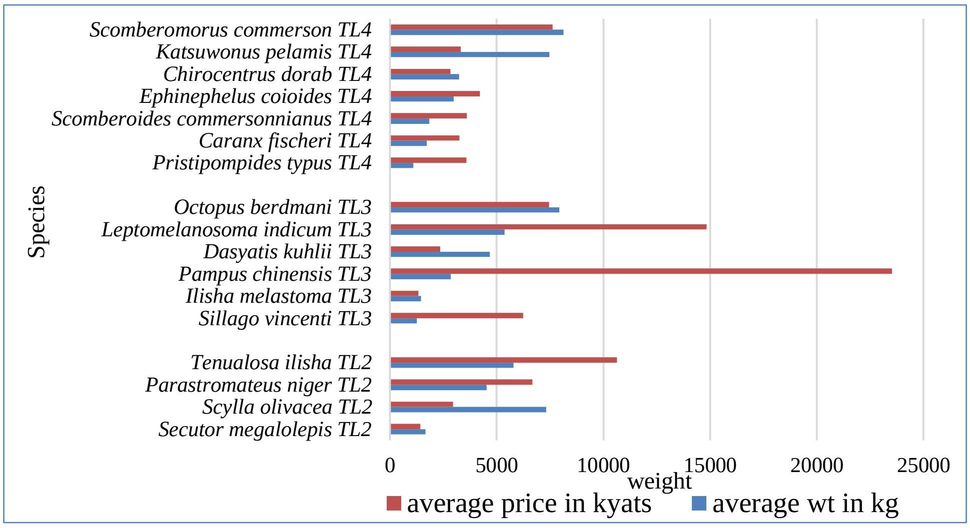


Figure 4 Relationship between average catch weight (1000-10000 kg) and average price among TL2, TL3 and TL4 during 2018

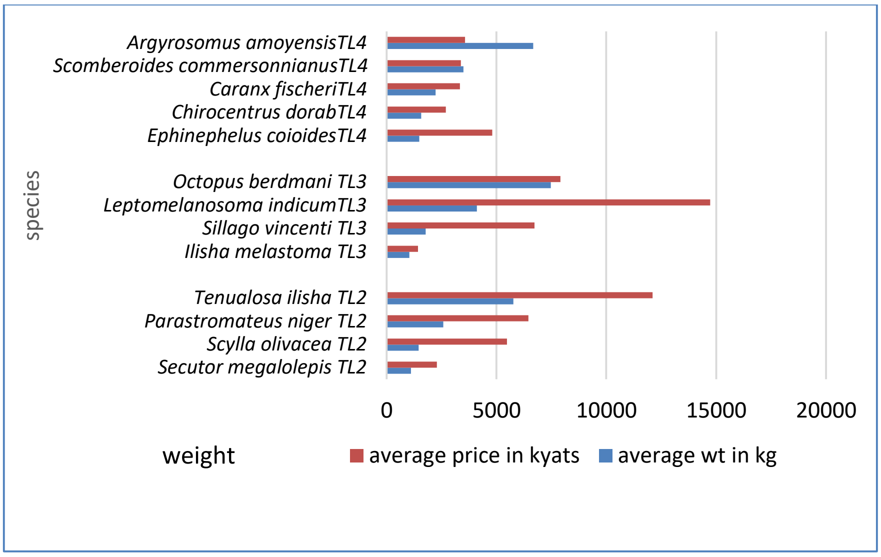


Figure 5 Relationship between average catch weight (1000-10000 kg) and average price among TL2, TL3 and TL4 during 2019

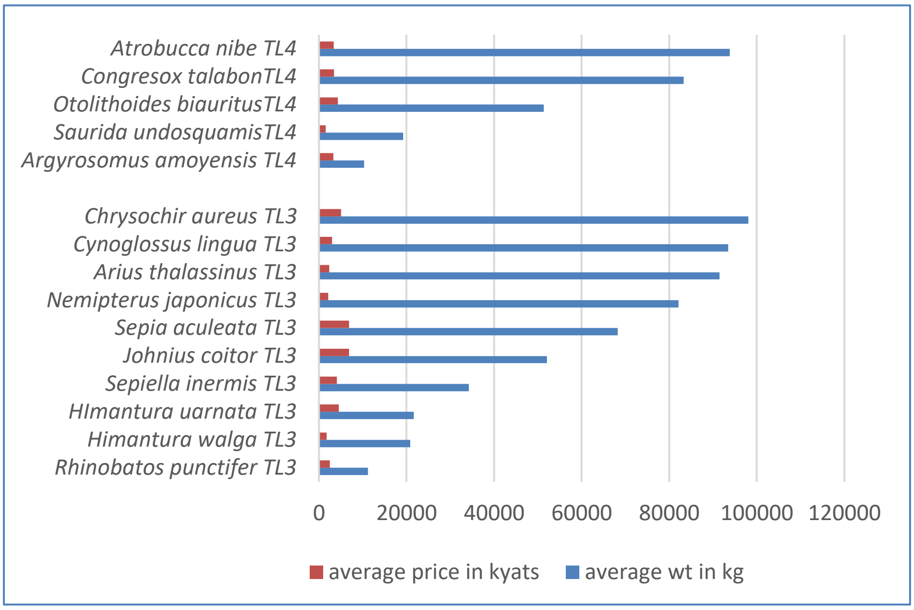


Figure 6 Relationship between average catch weight (10000kg-100000kg) and average price among TL3 and TL4 during 2018

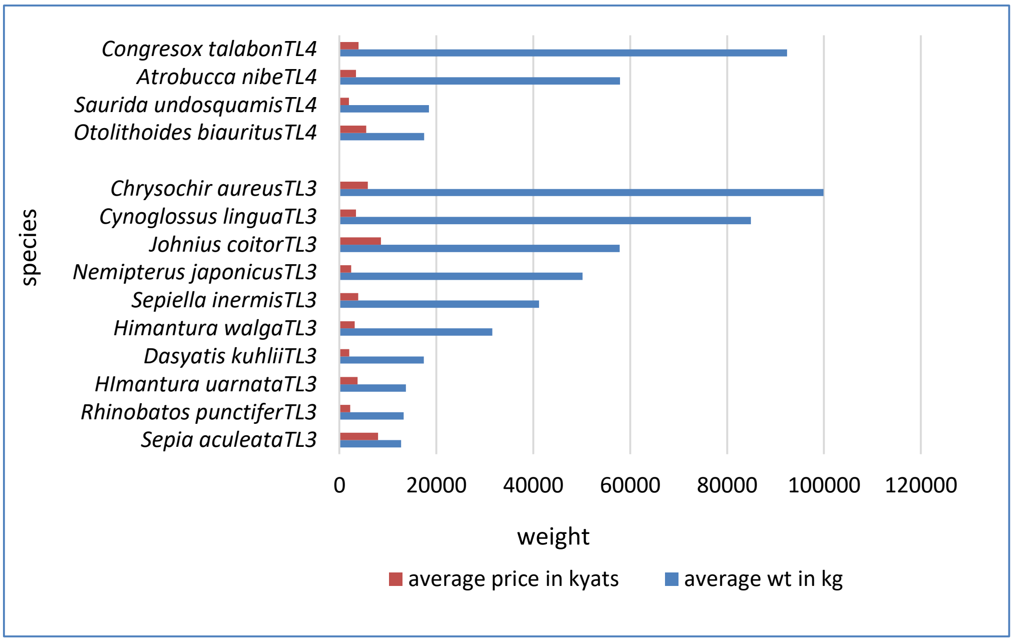


Figure 7 Relationship between average catch weight (10000kg-100000kg) and average price among TL3 and TL4 during 2019

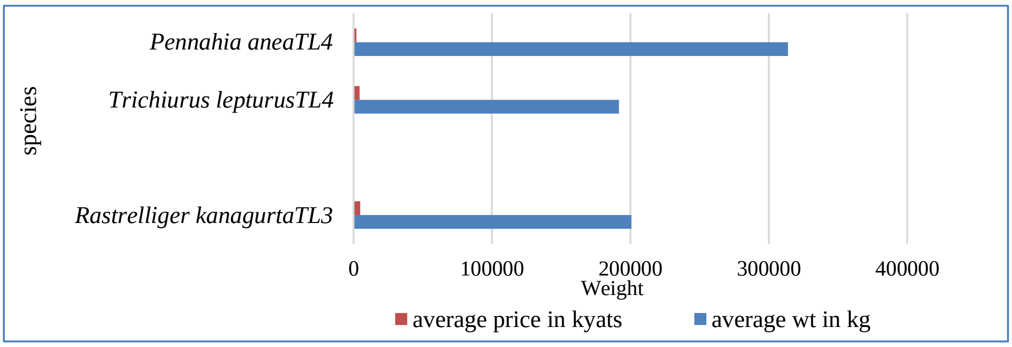


Figure 8 Relationship between average catch weight (>100000kg) and average price among TL3 and TL4 during 2018

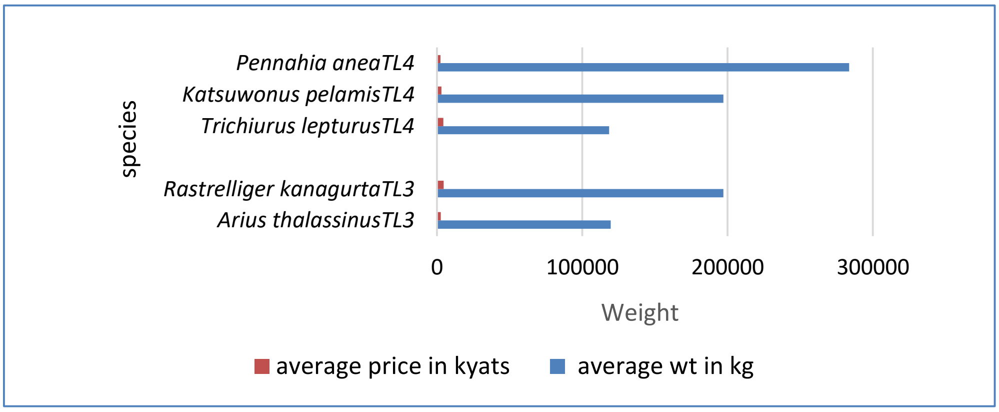


Figure 9 Relationship between average catch weight (>100000kg) and average price among TL3 and TL4 during 2019

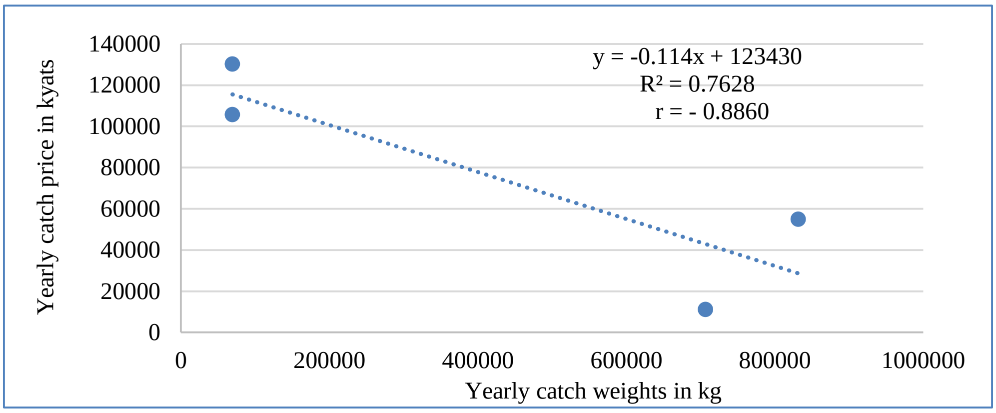


Figure 10 Relationship between yearly averge catch weights and yearly average price in kyats 2018 among four groups

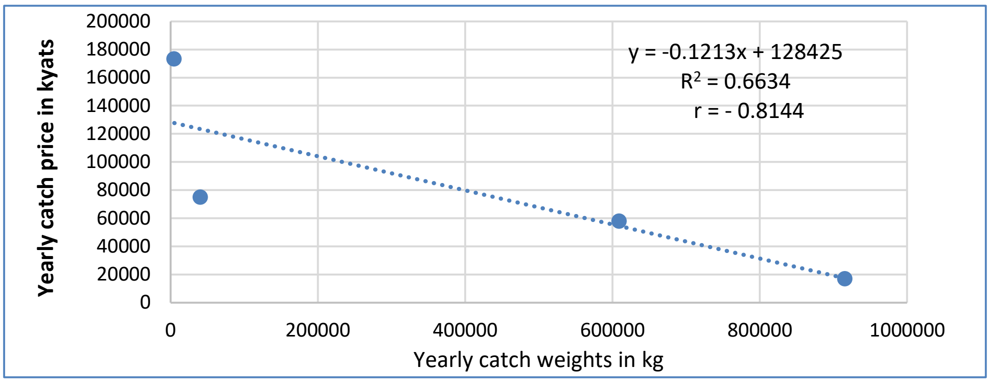


Figure 11 Relationship between yearly average catch weights and yearly average price in kyats 2019 among four groups

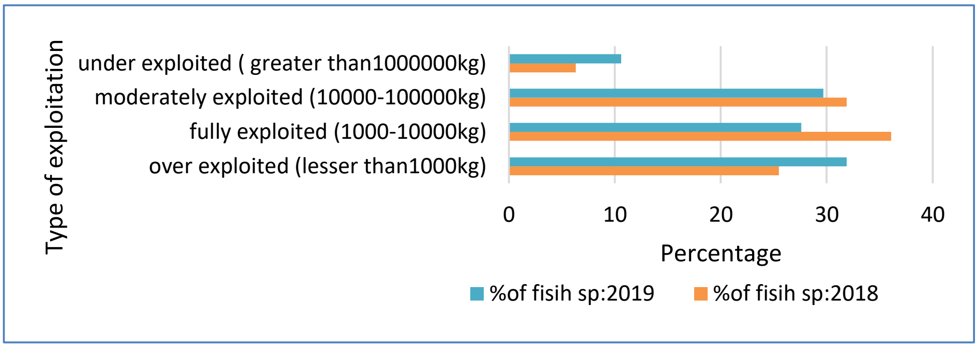


Figure 12 Level of exploitation showing the percentage of marine aquatic species during 2018 and 2019

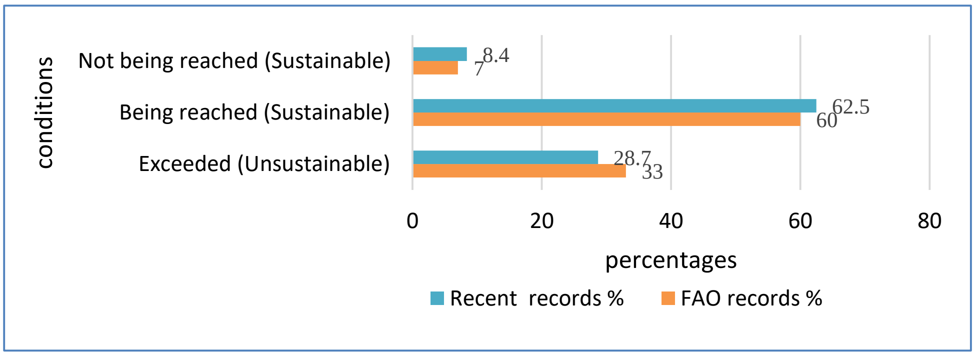


Figure 13 Comparison of FAO and recent records based on fishery potential and sustainability of some marine aquatic species

Table 1 Trophic levels, exploitation and conditions of sustainability of studied species

Sr No	Marine aquatic Spesies	Levels of exploitation				Conditions of sustainability	
		Over exploited	Fully exploited	Moderately exploited	Under exploited	Sustainable	Unsustainable
1	*Congresox talbon* TL 4.0			√		√	
2	*Ephinephelus coioides* TL 4.0		√			√	
3	*Rachycentron canadum* TL 4.0	√					√
4	*Caranx fischeri* TL 4.0		√			√	
5	*Lobotes surinamensis* TL 4.0	√					√
6	*Argyrosomus amoyensis* TL4.0			√		√	
7	*Atrobucca nibe* TL 4.0			√		√	
8	*Pennahia anea* TL 4.0				√	√	
9	*Otolithiodes biauritus* TL 4.1			√		√	
10	*Lactarius lactarius* TL 4.2	√					√
11	*Lutjanus johnii* TL 4.2	√					√
12	*Pristipomoides typus* TL 4.2		√			√	
13	*Scomberoides commersonnianus* TL 4.3		√			√	
14	*Chirocentrus dorab* TL 4.4		√			√	
15	*Trichiurus lepturus* TL 4.4				√	√	
16	*Katsuwonus pelamis* TL 4.4				√	√	
17	*Psettodes erumei* TL 4.4	√					√
18	*Lutjanus sanguineus* TL 4.5	√					√
19	*Scomberomorus commerson* TL 4.5		√			√	
20	*Saurida undosquamis* TL 4.6			√		√	
21	*Rastrelliger kanagurta* TL 3.2				√	√	
22	*Sillago vincenti* TL 3.3		√			√	
23	*Pristolepis pentacantha* TL 3.3	√					√
24	*Pampus argenteus* TL 3.3	√					√
25	*Ilisha melastoma* TL 3.4		√			√	
26	*Johnius coitor* TL 3.4			√		√	
27	*Dasyatis kuhlii* TL 3.5		√			√	
28	*Himantura walga* TL 3.5			√		√	
29	*Arius thalassinus* TL 3.5				√	√	
30	*Pomadasys kaakan* TL 3.5	√					√
31	*Chrysochir aureus* TL 3.5			√		√	
32	*Cynoglossus lingua* TL 3.5			√		√	
33	*Himantura uarnata* TL 3.6			√		√	
34	*Pampus chinensis* TL 3.6		√			√	
35	*Sepia aculeate* TL 3.6			√		√	
36	*Sepiella inermis* TL 3.6			√		√	
37	*Rhinibatos punctifer* TL 3.7			√		√	
38	*Nemipterus japonicus* TL 3.7			√		√	
39	*Aluterus monoceras* TL 3.8	√					√
40	*Octopus berdmani* TL 3.8		√			√	
41	*Leptomelanosoma indicum* TL3.9		√			√	
42	*Oratosquilla nepa* TL 2.6	√					√
43	*Panulirus polyphagus* TL 2.6	√					√
44	*Secutor melagalolepis* TL 2.8		√			√	
45	*Scylla olivacea* TL 2.8		√			√	
46	*Tenualosa ilisha* TL 2.9		√			√	
47	*Parastromateus niger* TL 2.9		√			√	

Table 2 Comparison of FAO 2017 records and recent results on sustainbility, fishery potential and food security

Average catch weight	Level of exploitation	Conditions of Sustainbility	Fishery potential	Food security	FAO records Of world fisheries	Recent findings from 2018 and 2019 average records
Lesser than 1000kg	Highly exploited	Unsustainable	Exceeded	Bad for every thing	33%	28.7%
1000kg-10000kg	Fully exploited	Sustainable	Being reached	Best for food security	60%	62.6%
10000kg-100000kg	Moderately exploited	Sustainable	Being reached	Best for food security		
Above 100000kg	Under exploited	Sustainable	Not being reached	Not good for food security	7%	8.4%

Table 3 Levels of exploitation sustainability, fishery potential and food security of some study marine aquatic species based on FAO records of sustainable fisheries 2017

Average catch weight	Total Percentage of fish species 2018	Total Percentage of fish species 2019	Level of explotation	Conditions of sustainability	Conditions of sustainability among Trophic groups	Fishery potentials	Food Security
Lesser than 1000kg	25.5%	31.9%	Highly exploited	Unsustainable	TL4>TL3>TL2	Exceeded	Bad for everything
1000kg-10000kg	36.2%	27.7%	Fully exploited	Sustainable	TL4>TL3>TL2	Being reached	Best for food security
10000kg-100000 kg	31.9%	29.7%	Moderately exploited	Sustainable	TL3>TL4 TL2 absent	Being reached	Best for food security
Higher than 100000 kg	6.4%	10.7%	Under exploited	Sustainable	TL4>TL3 TL2 absent	Not being reached	Not good for food security

Discussions

In the present study a total of 47 marine aquatic species were recorded from Nyaung Tan fish Jetty. They were arranged into trophic groups based on the feeding types.Six species of herbivorous type included under TL2. Twentyone species of carnivorous type belonged to TL3, while the rest 20 obtained under TL4. Based on the yearly catch weights the previous species were classified into four groups for the year 2018 and 2019, accordingly FAO, 2017:

(i) Lesser than 1000 kg were regarded as over exploited,

(ii) 1000 kg-10000 kg fully exploited,

(iii) 10000 kg-100000 kg moderately exploited and

(iv) Above 100000 kg under exploited

There were no records of depleted fish species from the earlier days of Myanmar. Recovering stage is far more impossible to conduct. Because it needs an expertise to reintroduce the depleted species back into the natural ecosystem. Thus, conditions of (v) Depleted and

(vi) Recovering stages could not be discussed in this study. Therefore, four groups will be classified.

To begin with the highest catch weight groups it will start from (iv) Above 100000 kg. They were regarded as under exploited group, these group included five species *Pennahia anea*, *Trichiurus lepturus, Katsuwonus pelamis* in TL 4, *Rastrelliger kanagurta, Arius thalassinus in TL 3.* They are abundantly caught from the study sites and poorly demanded by the consumers.They are still sustainable in the water bodies with poor nutritious value regarding as not good for food security. Thus this population results indicated as 8.4%, which are nearer to the records of FAO, 2017 showing as 7%.

For the two groups that ranged between (ii) 1000 kg-10000 kg which were fully exploited group, these group included 16 species *Scomberomorus commerson, Chirocentrus dorab, Ephinephelus coioides, Scomberoides commersonnianus, Caranx fischeri, Pristipompides typus,* in TL 4, *Octopus berdmani, Leptomelanosoma indicum, Dasyatis kuhlii, Pampus chinensis, Ilisha melastoma, Sillago vincenti* in TL 3, *Tenualosia ilisha, Parastromateus niger, Scylla olivacea, Secutor megalolepis* in TL 2, another group ranged between (iii) 10000 kg-100000 kg were moderately exploited, these group included 14 species *Atrobucca nibe, Congresox talabon, Otolithoides biauritus, Saurida undosquamis, Argyrosomus amoyensis* in TL 4, *Chrysochir aureus, Cynoglossus lingua, Nemipterus japonicus, Sepia aculeata, Johnius coitor, Sepiella inermis, Himantura uarnata, Himantura walga, Rhinobatos punctifer* in TL 3. In both of the groups fishing potential was still in stationary conditions and proved to be sustainable in the study area. Most of the species that included in this group have commercial value, highly demanded by the consumers. They have high-protein content known as best for food security. Therefore, the combinations of this two groups comprises as 62.6%, which proved to be nearer to the world fisheries records of FAO, 2017 which showed 60%.

Finally, the catch weights (i) Lesser than 1000 kg were designated as over exploited group, these groups included 12 species *Rachycentron canadum, Lutjanus johnii, Lobotes surinamensis, Lutjanus sanguineus, Psettodes erumei, Lactarius lactarius* in TL 4, *Aluterus monoceras, Pristolepis pentacantha, Pampus argenteus, Pomadasys kaakan* in TL 3, *Panulirus polyphagus, Oratosquilla nepa* in TL 2. This group existed as 28.7% which was nearer to the records of FAO, 2017 as 33%. The fishing pressure in this group passed over the maximum sustainable limited. The amount of marine stocks were heavily havested every year, they are unsustainable because the population of these species became declining. From the perspective of food security they are ideal as they produced nutritious high content of protein rich food in the ocean. At one time, most of the marine stocks from this group served as commercial species exporting to the adjacent countries and supporting our country for economic growth. They were highly demanded by the consumers. The number of marine stocks obtained from TL4, showed that they are omnivores with larger body sizes and these stocks became the targeted species for catching.Thus, proper management and guidelines are urgently needed to prevent the loss of valuable marine stocks from their envirnment. It shoud be primarily addressed to the decision-makers, policy-makers in the marine fisheries sector and non-government organizations with the interest in sustainable development of fisheries resources. (FAO, 1999)

Conclusion

It could be concluded that they were significant negative correlation between yearly average catch weight and yearly average price. In this study it showed as when the average catch weights increased the average price rates decreased in this study. Among the classification of four groups by yearly catch weights, level of exploitation was found to be from the overexploited group (Lasser than 1000 Kg, it included 28.7% of fish species out of the total collected records. The recent results indicated as 28.7% for over exploited, 62.6% for the combination of fully exploited and moderately exploited group and finally 8.4% for under exploited group. These findings the nearer to the records of FAO, 2017 reports, showing as 33% for over exploited, 60% for the combination of fully exploited and moderately exploited group and finally 7% for under exploited group. Therefore, the conclusion was drawn as these species are unsustainable because of over exploitation. Due to that consequences, there will be a loss of nutritionally valued marine aquatic species in the environment affecting the health and economic benefits of human population. The comments for the food security for this group will be "bad for everything". Therefore, there is a need in fishery management to monitor the marine species especially fish that are decling in catch to control the type of fishing gears and to excluded the spawners from catching during the breeding seasons.

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SCANNING OF *MACROBRACHIUM ROSENBERGII NODAVIRUS* (*Mr*NV) IN *MACROBRACHIUM ROSENBERGII* HATCHERIES

Tin Mar Cho[1], Saw Malar Than[2], Nway Ei Khaing[3], Yu Wai Hlaing[4], Kay Lwin Tun[5]

Abstracts

Macrobrachium rosenbergii nodavirus (*Mr*NV) is a pathogen of giant freshwater prawns that causes white tail disease (WTD). It leads to high economic losses in hatcheries and farms of freshwater shrimp culture area across the world. In the present study, two prawns hatcheries: Twantay and Htantabin hatcheries located at Yangon Division were selected as a study sides and to examine the distribution *Macrobrachium rosenbergii* nodavirus *(Mr*NV) in different life stages of *Macrobrachium rosenbergii* using RT-PCR and RT-nested PCR (nRT-PCR). Negative results were record in all life stages of *Macrobrachium rosenbergii* from Htantabin hatchery. However, different life stages broodstocks, post-larvae and juveniles of *M. rosenbergii showed positive* in nRT-PCR at Twantay hatchery. The result in the present study indicated that *Mr*NV has been introduced to Twantay hatchery through the broodstocks.

Keyword: *Macrobrachium rosenbergii* nodavirus *(Mr*NV), RT-PCR, *Macrobrachium rosenbergii*, hatchery, Myanmar

Introduction

Farming of the giant freshwater prawn, *Macrobrachium rosenbergii* de Man 1879 is a rapidly growing industry, due to its high commercial value and inland based production. The global aquaculture production of the giant freshwater prawn has been showing 130,689 t in 2000 to 203,211 t by 2011 (FAO, 2013). However, production of the prawns has gradually declined due to various disease infections in aquaculture (Cheng and Chen, 1998).

The production success depends on disease causing agents environmental factors and sustainable management practices influence in the success of the hatchery operations (Shailender, 2012). The important disease causative agents of infectious diseases in prawns are viruses, bacteria, fungi, protozoans and other parasites. Viral diseases in shrimp aquaculture have resulted in large economic losses and it has been estimated that over the past 15 years global losses due to diseases reached more than US $15 billion (Flegel, 2012; Flegel *et al*., 2008). The pathogen can enter the hatchery system by various pathways, most important through feed, broodstocks, instruments, and water as well as unhygienic handless of workers (Sahul Hameed *et al*., 2004).

Macrobrachium rosenbergii is moderately disease-resistant in comparison to penaeid shrimp. The diseases of cultured prawn include syndromes with infectious and noninfectious. Viruses are responsible for main considerable economic losses worth prawn industries (Ganjoor, 2015). Among the pathogens, prawn viruses are very important and responsible for huge economic losses, particularly in the hatchery and nursery ponds. Reported prawn viruses include *Macrobrachium* hepatopancreatic parvo-like virus (MHPV), *Macrobrachium* muscle virus (MMV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), white spot syndrome virus (WSSV), *Macrobrachium rosenbergii* nodavirus (*Mr*NV), and extra small virus like particle (XSV).

Nowadays, the decline in the production of *M. rosenbergii* was mainly attributed to the emergence of a new disease namely, white tail disease (WTD) or white muscle disease (WMD)

[1] Assistance Lecturer, Fisheries and Aquaculture, University of Yangon, Kamayut Township,
[2] Dr, Lecturer, Fisheries and Aquaculture, University of Yangon, Kamayut Township, Yangon
[3] PhD Candidate, Department of Zoology, University of Yangon
[4] PhD Candidate, Department of Zoology, University of Yangon
[5] Dr,Professor, Fisheries and Aquaculture, University of Yangon, Kamayut Township, Yangon

(Sahul Hameed *et al*., 2004). This disease pathogen is *Macrobrachium rosenbergii* nodavirus (*Mr*NV). This virus is a small, non-enveloped virus and containing the genome of two pieces of RNA. MrNV was first reported in the French West Indies in 1999 (Arcier *et al*., 1999). Very few post-larvae with *Mr*NV survive beyond 15 days in an outbreak, and post-larvae that survive may grow to market size like any other normal post-larvae. Adults are resistant to WTD, but act as carriers.

The disease infects hatchery-reared larvae and post-larvae in addition to nursery-raised early juveniles. *Mr*NV is responsible for large-scale mortalities in hatchery and nursery phases of prawn culture system (Vijayan *et al.*, 2005). This virus might be responsible for high economic losses particularly in hatchery in Myanmar. However, there was no scanning on *Mr*NV infection in prawn hatcheries in Myanmar. Hence, the present study was focused to detect *Mr*NV infection in Twantay and Htantabin hatcheries using RT- PCR and nRT-PCR methods.

Materials and Methods

Study site

Samples were collected from two Prawn Hatcheries in Twantay Township (16˚ 71' 23" N - 95˚90' 59" E) and Htantabin Township (17˚1' 4" N - 95˚58' 54" E). Two hatcheries are located in Yangon Division (Plate. 1).

Study period

The prawn sample was collected from both hatcheries from November 2018 to October, 2019.

Specimen transportation and tissue sampling

Live Specimens were transported from each hatchery to the Aquatic Animal Diseases Laboratory, Fisheries and Aquaculture, University of Yangon. Samples were extracted RNA immediately after arriving at the Laboratory. From each hatchery, the pleopods of five broodstocks, eggs from the abdomen of broodstocks, thirty nine post-larvae and five juvenile stages were collected. The samples were collected in 1.5 ml tube separately.

For eggs, a total of twenty eggs were pooled into one sample tubes and altogether five sample tubes were prepared. Similarly, for post-larvae, three post-larvae were pooled into one single 1.5 ml tube and extracted for RNA. Therefore, there were a total of thirteen post-larvae tubes for RNA extraction for post-larvae. For juveniles, five juveniles were collected in the tube separately. The number of samples is described in Table 1.

Table 1 Number of samples collected from Twantay and Htantabin hatcheries

Tissue	Collected samples from each hatchery	Number of samples for RNA extraction
Pleopods of broodstocks	5	5
Eggs of broodstocs	100	5 (20 eggs in each sample tube)
Pleopods of juvenile	5	5
Post-larvae	39	13 (3 individuals in each sample tube)

RNA extraction

Total RNA was extracted from tissue using the PETNAD nucleic acid co-prep kit (GeneReach Biotechnology Corp) (Plate 2). Tissue (20-25 mg) was ground using disposable plastic grinder with 100 µl PB1 solution. Then PB1 solution (500 µl) was added and vortexed for one minute. A total of 600 µl PB2 solution, 600 µl of PB3 solution and 600 µl of PB4 solution were added step by step according to the manual instruction. The dry RNA pellet was eluted with 50 µl of PB 5 solution. Extracted RNA concentrations were measured using NanoDrop 2000 Spectrophotometer and stored at -20˚C.

RT-PCR and nRT-PCR analysis for *Mr*NV infection

Two set of primers were used to detect *Mr*NV using RT-PCR and nRT-PCR**.** First step RT-PCR was carried out for specific detection of *Mr*NV in serious infected prawns. Nested RT-PCR was detected low viral load of *Mr*NV in initial infection stage (Table 2).

Table 2 Primers used for RT-PCR and nRT-PCR analysis in *Mr*NV (Sahul Hameed et al., 2004 and Sudhakaran *et al*., 2007)

RT-PCR	Primers	Sequences 5' to 3'	Product Size
RT-PCR	Forward	GCG-TTA-TAG-ATG-GCA-CAA-GG	425bp
	Reversed	AGC-TGT-GAA-ACT-TCC-ACT-GG	
Nested PCR	Forward	GAT-GAC-CCC-AAC-GTT-ATC-CT	205bp
	Reversed	GTG-TAG-TCA-CTT-GCA-AGA-GG	

The volume of the reaction mixture for the first step RT-PCR was 20 µl containing 10.0 µl of RT-PCR MasterMix (WizPure™ RT-PCR 2X Master), 1.2 µl of MrNV RT-PCR Primer Mix, 6.8 µl of ddH2O, 2.0 µl of RNA. The total volume is 20.0 µl using the following cycles for RT-PCR: at 42°C for 30 minutes; initial denaturation at 95°C for 10 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and elongation at 72°C for 20 seconds. The cycle was ended with an additional elongation step for 5 minutes at 72°C. The condition of PCR thermal cycler was described in Table 3.

In the nRT-PCR, the amplified products of the first step RT-PCR served as the template DNA for the nested PCR of amplification. After completion of the first step RT-PCR, nRT-PCR reaction was made with 10.0 µl of HS-PCR master Mix ((WizPure™ PCR 2X Master), 1.2 µl of *Mr*NV nRT-PCR Primer Mix, 7.8 µl of ddH2O, 1.0 µl of RT-PCR product. The total volume is 20.0 µl using the following cycles: 95°C for 5 minutes, followed by 25 cycles of 30 seconds at 95°C, 30 seconds at 60°C and 10 seconds at 72°C with a final extension at 72°C for 5 minutes. The condition of PCR thermal cycler was described in Table 4. RT-PCR and nRT-PCR were conducted using miniPCR™ mini8 thermal cycler (minipcr^{TM} bio) (Plate 2).

Table 3 Thermal cycling condition for RT-PCR to detect *Mr*NV

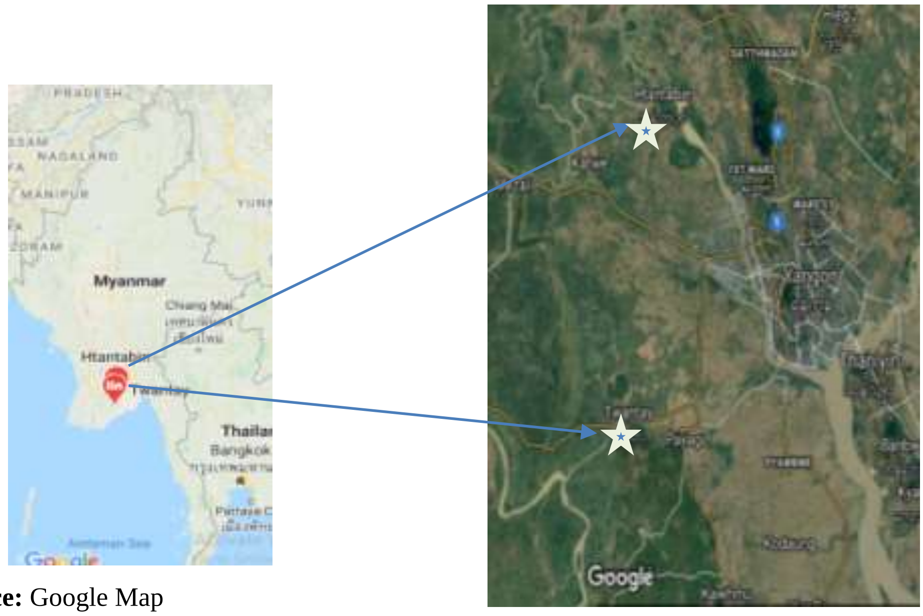
Temperature ℃	Time	cycles	Step
42	30 minutes	1	Reverse transcription
95	10 minutes	1	Initial denaturing
95	30 seconds		denaturing
60	30 seconds	25	annealing
72	30 seconds		extension
72	5 minutes	1	Final extension

Table 4 Thermal cycling condition for nRT-PCR to detect *Mr*NV

Temperature ℃	Time	cycles	Step
95	10 minutes	1	initial denaturing
95	30 seconds		denaturing
60	30 seconds	25	annealing
72	30 seconds		extension
72	5 minutes	1	final extension

Gel electrophoresis

Both RT-PCR and nRT-PCR products were separated and visualized by agarose gel electrophoresis using 1.5 % agarose gel containing 30ml of 1XTAE buffer. Gel were prepared with 0.15g of agarose powder diluted in 40ml of 1x TAE buffer together with 6 µl of Gel stain green (Wizbiosolutions Inc). A 100bp of DNA ladder marker (Wizbiosolutions Inc) was used as an indicator to compare the specific base pair of *Mr*NV. The gel was check under UV Blue light transilluminator (minipcrTM bio) for the result (Plate 4).



Source: Google Map

Plate 1 Location of Twantay and Htantabin Hatcheries

Plate 2 PETNAD nucleic acid co-prep kit (GeneReach Biotechnology Corp)

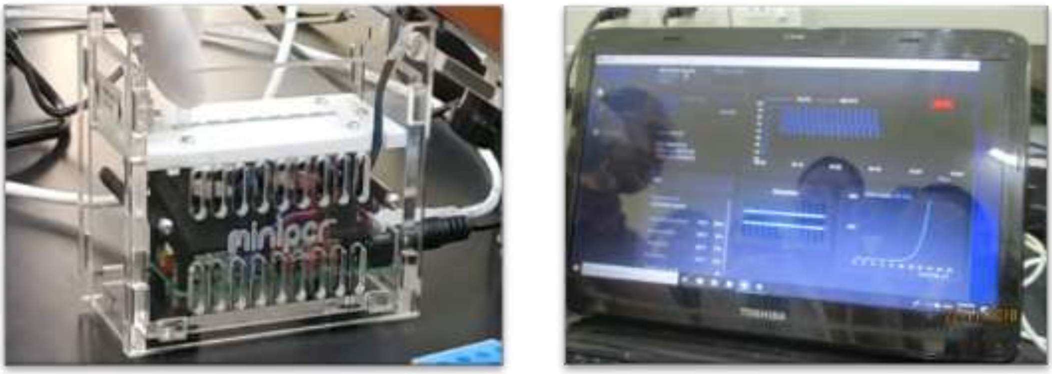


Plate 3 MiniPCR™ mini8 thermal cycler (minipcrTM bio)

Plate 4 Gel electrophoresis (minipcrTM bio)

Results

Concentration of extracted RNA

Concentration of RNA in all samples ranged 105 to 297 ng/µl and it has over 2.1 in 260/280 purity ratio.

Detection of *Macrobrachium rosenbergii* nodavirus (*Mr*NV)

Broodstocks, juveniles and post-larvae of *M. rosenbergii* were obtained from Twantay and Htantabin hatcheries to detect the *Mr*NV. In Twantay hatchery, all samples were showed negative in RT-PCR. However, three out of five broodstocks samples showed positive in nRT-PCR (Plate5, Table 4). However, eggs collected from the broodstocks were all negative (Plate 6 and Table 4).In juvaniles stage, two out of five samples were positive in nRT-PCR (Plate 6 and Table 4). Nine out of thirteen post-larvae were also showed positive in the samples collecd from Twantay hatchery (Plate 7 and Table 4). All positive samples indicated the presence of *Mr*NV at 205 bp.

All samples collected from Htantabin hatchery were negative in both RT-PCR and nRT-PCR (Plate 8,9,10 and Table 5).

Table 4 The RT-PCR and nRT-PCR results for *Mr*NV detection in prawn collected from Twantay Hatchery

Tissue	Examine samples	Positive in RT-PCR	Positive in nested PCR
Pleopods of broodstocks	5	0/5	3/5
Eggs	5 pooled sampes	0/5	0/5
Pleopods of juvenile	5	0/5	2/5
Post-larvae	13 pooled samples	0/13	9/13

Table 5 The RT-PCR and nRT-PCR results for *Mr*NV detection in prawn collected from Htantabin Hatchery

Tissue	Examine samples	Positive in RT-PCR	Positive in nested PCR
Pleopods of broodstocs	5	0/5	0/5
Eggs of broodstocs	5 pooled sampes	0/5	0/5
Pleopods of juvenile	5	0/5	0/5
Post-larvae	13 pooled samples	0/13	0/13

M P 1 2 3 4 5

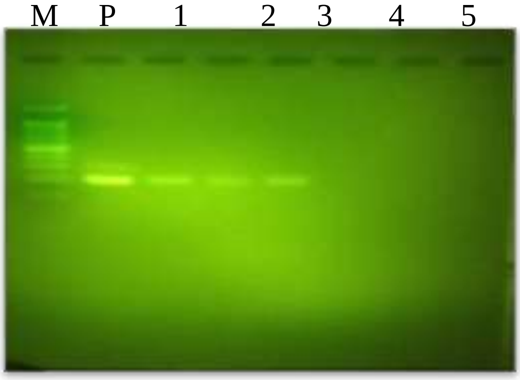


Plate 5 The result of nRT-PCR for the broodstocks collected from Twantay hatchery

M: 100bp DNA marker, P: positive control, N: negative control, Well 1 to 5: Broodstock samples

Note: Well 1 to 3 show positive for *Mr*NV.

M P 1 2 3 4 5 N

Plate 6 The result of nRT-PCR for the eggs (upper gel) and juveniles (lower gel) collected from Twantay Hatchery

M: 100bp DNA marker, P: positive control, N: negative control, Well 1 to 5 (upper gel): eggs pooled samples, Well 1 to 5 (Lower gel): Juveniles samples

Note: Well 1 and 2 (lower gel) show positive for *Mr*NV.

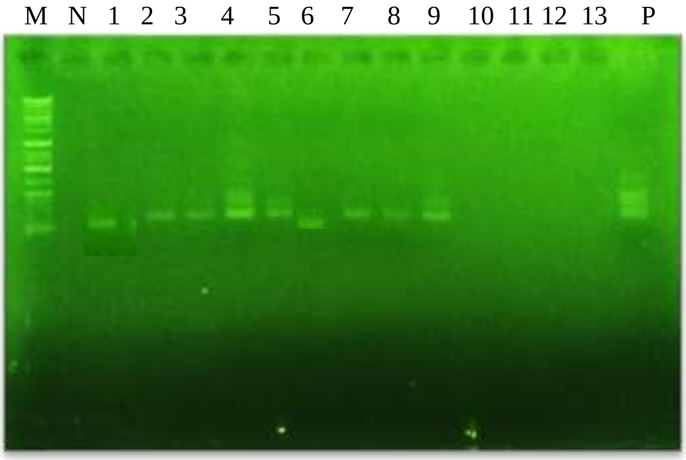


Plate 7 The result of nRT-PCR for post-larvae collected from Twantay Hatchery

M: 100bp DNA marker, P: positive control, N: negative control, Well 1 to 13 post-larvae pooled samples

Note: Well 1 and 9 show positive for *Mr*NV

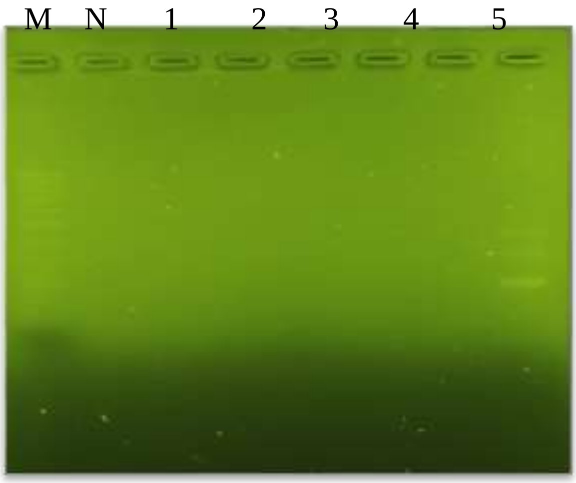
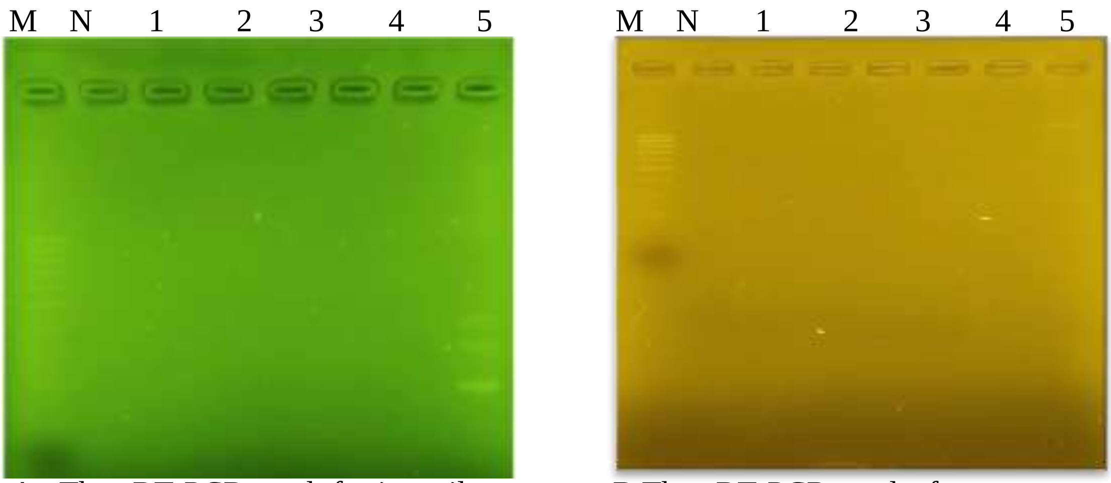


Plate 8 The result of nRT-PCR for the broodstocks collected from Htantabin Hatchery

M: 100bp DNA marker, P: positive control, N: negative control, Well 1 to 5: broodstock samples



A. The nRT-PCR result for juveniles B.The nRT-PCR results for eggs

Plate 9 The result of nRT-PCR for the juveniles and eggs collected from Htantabin Hatchery

M: 100bp DNA marker, P: positive control, N: negative control, Well 1 to 5: collected samples

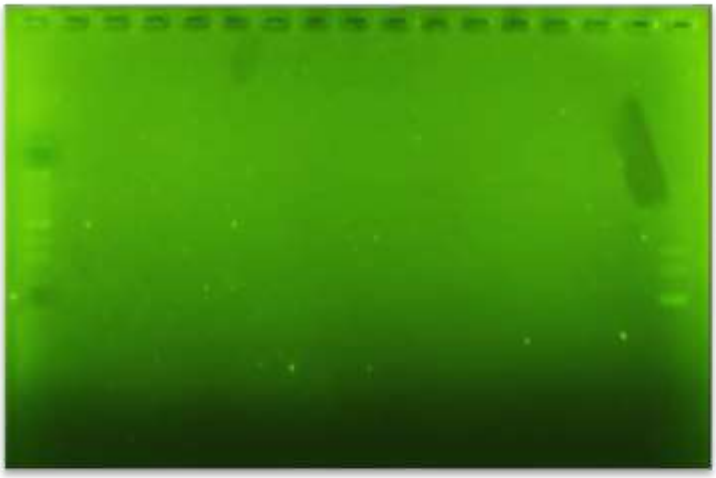


Plate 10 The result of nRT-PCR for the post larvae collected from Htantabin Hatchery

M: 100bp DNA marker, P: positive control, N: negative control, Well 1 to 13: collected samples

Discussion

M. rosenbergii nodavirus (*Mr*NV) has been identified the causative agent for white tail disease in the freshwater prawn and the diseases was reported in West Indies in 1994 (Arcier *et al.,* 1999), Taiwan and China in 1999 (Tung *et al*., 1999; Sri Widada *et al*., 2003), Thailand in 2007 (Sudthongkong *et. al.,* 2007) and Malaysia in 2012 (Tayebeh Azam Saedi *et al*. (2012). This report is first report of the scanning of *Macrobrachium rosenbergii* nodavirus *(Mr*NV) in giant freshwater prawns, *M. rosenbergii,* hatcheries in Myanmar. Different stages of freshwater prawns were obtained from Twantay and Htantabin hatcheries and diagnosed for *(Mr*NV) using RT-PCR and nRT-PCR methods.

We used both RT-PCR and nRT-PCR since nRT-PCR is more sensitive and useful for screening of *Mr*NV even in low viral load. Different diagnostic methods are available to detect MrNV/XSV in prawn and among these methods, RT-PCR is the most sensitive (Romestand and Bonami 2003; Sri Widada *et al.* 2003; Sahul Hameed et al. 2004; Yoganandhan *et. al*., 2005; Sudthongkong *et. al*., 2007). In the present study, *Mr*NV infection was negative in RT-PCR while it was positive result in nRT-PCR except from eggs at Twantay hatchery. The positive of 1st RT-PCR indicates a serious infection of *Mr*NV, when the nested PCR positive result shows low viral load. According to the results, it was identified that *Mr*NV infection has been introduced to Twantay hatchery through the broodstocks.

Yoganandhan *et. al.,* (2006) have been reported that *Mr*NV could be detected in broodstock of prawns but not showed gross sing of disease. Similarly the previous authors were reported that adults are tolerance to *Mr*NV infection, but act as carriers (Qian *et al*., 2003 and Sahul Hameed *et al*., 2004). In Twantay hatchery, although the broodstocks,post-larvae and juveniles were positive of *Mr*NV in the nested PCR, were not show any prominent signs of disease. The present study supports the collected prawns at Twantay hatchery are carrier state with light infection because positive results are only appeared in nRT-PCR. *Mr*NV was not detected in egg collect from Twantay hatchery. Egg has height concentration of lipid and it may take time for the virus to penetrate the eggs.

*Mr*NV have been reported to infect larvae and post-larval of *M. rosenbergii* but not adult prawn (Qian *et a*l., 2003, Sri Widada *et al.,* 2003).Larvae, post-larvae and early juveniles are susceptible, whereas adults are resistant (Qian *et al*., 2003; Sahul Hameed *et al*., 2004). The viral transmission routes are vertical and horizontal (Sahul Hameed *et al*., 2004). In the present study, *Mr*NV was found in the broodstocks, post-larvae and juveniles by nRT-PCR methods in Twantay Hatchery.Therefore, it is assume that vertical transmission of *Mr*NV was occurred in the hatcheries. However, there was negative result in eggs samples. Further study are needed to confirm the reason of negative result in eggs.

All stage of prawns were negative in both 1stRT-PCR and nRT-PCR at Htantabin hatchery. It is assume that broodstocks introduced to Htantabin hatchery was free from the infection of *Mr*NV and as a consequent all life stages of prawns were free from the virus. Therefore, scanning on *Mr*NV infection before introducing to the hatchery is essential to increase the biosecurity in the area.The important role of biosecurity highted according to the results of the present study.

Conclusion

*Mr*Nv iinfection was scanning in two prawns hatcheries in Myanmar using RT-PCR and nRT-PCR methods. Broodstocks, post-larvae and juveniles were negative in 1st RT-PCR but they were positive in nested PCR except from eggs in Twantay hatchery. The present work revealed that collected prawns from Twantay hatchery were infection with light infection of *Mr*NVwhich are positive with nRT-PCR only. The virus can be transmitted horizontally from broodstocks to post-larve and juvinile. In the other hands, all life stages of prawn showed negative result in both first RT-PCR and nRT-PCR in Htantabin hatchery. Therefore, screen for *Mr*NV in broodstock should be undertaken before introducing to the hatcheries to control the *Mr*NV infection in farms.

Acknowledgements

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SOME ASPECTS ON REPRODUCTIVE BIOLOGY OF THREE CRAB SPECIES IN RAKHINE COASTAL REGION

Aye Aye Khine[1], Khin Win Thein[2], Kay Lwin Tun[3]

Abstract

Reproductive biology of three crab species, *Portunus sanguinolentus, P. pelagicus* and *Scylla serrata* collected from Sittway area were studied. A total of 30 individuals of each species were monthly sampled from September to December 2015. Morphometric measurement of crabs, sex ratio and their reproductive development stages were examined. In *Portunus sanguinolentus* and *P. pelagicus*, female individual were abundantly occurred throughout the study period. In contract, high male ratio was recorded in *Scylla serrata* in which more than 25 individual per month are male crab. The fecundity of mud crabs is five times higher than the other two species. *Poutunus sanguinolentus* and *P. pelagicus* carried eggs throughout the study period but *S. serrata* carried egg only in the September 2015. Due to the migration behaviour of *S. serrata,* it is assumed that they migrate from brackish water to deep sea of high saline water at the time of spawning.

Keywords: Reproductive biology, Morphometric measurement, Sex ratio, fecundity

Introduction

Decapods crustacean is a major component of commercial fisheries in Myanmar. The production of crab in fisheries sector is dominated by a few members of a single family, the Portunidae. They include four species of mud crab, *Scylla serrata*, the three-spotted crab, *Portunus sanguinolentus,* the blue swimming crab, *Portunus pelagicus* and the gazami crab, *P. trituberculatus* (Lai *et al.*, 2010).

In the coastal region in Rakhine state, crab production plays an important role in fisheries and aquaculture industry. Mud crab, *Scylla serrata*, farming has been developed in the region since 2003. Crab farming based on collection of crablets or crabs from the wild for fattening or grow-out has probably taken place for hundreds of years. However, there is an unmet demand for mud crabs and this has led to over-exploitation in many areas. Difficulties with obtaining wild caught juveniles for farming operations, plus concerns of further over-exploitation, have led to major investment in research into hatchery techniques. Of the four species of mud crabs, hatchery technology is only being developed for *S. serrata* and S. *paramamosain* (FAO, 2011).

However, production of crablets in Myanmar has not been developed yet and they are collected from natural resources. Myanmar Sustainable Aquaculture Project (MYSAP) supported crab hatchery in Labutta Township, Ayeyarwady Region for artificial production of crablets (Global New Light of Myanmar, 2019). However, they are still in trial stage. According to the conversation of crab trader in Rakhine environs said that production of *Portunus* spp. And *Scylla* spp. is decreasing day by day.

On the other hand, commercial fishery of marine crab species in Rakhine State have increased greatly during the last ten years due to market demand (DOF, 2013). Myanmar crab association located in Myanmar Fishery Federation (IWO are trying to develop *Portunus* spp. and *Scylla* spp. hatchery in Myanmar (Soe Tun, personal communication). To develop the crab industry in Myanmar, understanding on their reproductive biology, especially for egg carrying period and differentiation of male and female crab are fundamentally needed. The fulfill the requirement of

[1] Assistant Lecturer, Fisheries and Aquaculture, Department of Zoology, University of Yangon
[2] Demonstrator, Department of Zoology, Taunggok University
[3] Professor, Fisheries and Aquaculture, University of Yangon

aquaculture industry in Myanmar, the present study were conducted to investigate the male and female ratio of crab species in natural population and estimate the egg carrying period and fecundity in female crabs in Rakhine area.

Materials and Methods

Study site

Sittway Coastal region (20°08'N and 92 °53'E), Rakhine State is selected as study site (Fig.1). *Portunus sanguinolentus* and *P. pelagicus were* collected from fishing boats which are catching crab and fish in intertidal shallow areas. *Scylla serrata* was collected from muddy bottom of Kisapanadi River near Mangrove forest.

Study period

Study lasted from June to December 2015.

Sampling Methods

Three crab species *Portunus sanguinolentus*, three spotted crab, *P. pelagicus* blue swimming crab and *Scylla serrata,* mud crab were selected to be studied (Plate.1). A total of 30 individuals from each species were collected from September to December 2015. After collecting from the sample sites, the collected specimens were placed in ice box and sent to the Laboratory at Department of Zoology, Sittway University. Crabs were weighted when they were arrived to the laboratory. To record the morphometric measurements of male and female crab, carapace length, carapace width, short carapace width, chela length, carpus propodus, abdominal width, chela height and length of arm were measured.

Gonado-Somatic Index (GSI) and Fecundity

Gonado-Somatic Index (GSI) and Fecundity were calculated by following formula. Fecundity was calculated for female crabs that were carrying eggs in their abdomen.

Gonado-Somatic Index (GSI) (Rabia *et al.,* 2008)

GSI = (Gonad weight/total body weight-gonad weight) x 100

Fecundity (Rabia *et al.,* 2008)

Fecundity = F = nV/v

where n = number of eggs in the subsample, V = volume to which the total number of eggs is made up and v = volume of the subsample (1g)

In addition, diameter of 50 eggs from each species were measured monthly under the stereomicroscope to compare the egg sizes of different species.

Reproductive development of crab species

Reproductive developments of crab species are cartelized into three stages, stage 1, stage 2 and stage 3 according to egg color and shape followed by Paulo (2002).

State 1- Freshly extruded egg mass sponge with an orange color due to a large quantity of yolk.

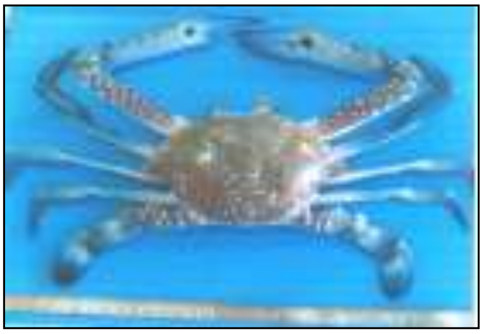
Stage 2- incubation at its halfway period, the egg mass sponge has a light brown col our tending to grey.

Stage 3- the egg mass sponge is dark brown tending to black and little yolk is left.

Source: googlemap.com

Figure 1 Map of showing the location of Sittway Seashore, sampling area

(A) *Portunus sanguinolentus*



(B) *Portunus pelagicus*

(C) *Scylla serrata*

Plate 1 Three studied crab species

Results

Average body weight and length of three crab species

Body weight and length of each species were measured and sizes between male and female were compared. Body weight of *Portunus sanguinolentus* ranged from 52.4 g to 154.6g in male and 51.6 g to 150.3 g in female while *P. pelagicus* ranged from 187.5 to 243 g in male and 190.4 g to 243.2 g in female. In *Scylla serrata* lower body weight ranged from 99g to 142.5 g in male and 99.3 g to 141.3 g in female (Fig.2). In October 2015, body weight of *Portunus sanguinolentus* and *P. pelagicus* were higher than that of female. Apart from that month, the body weight of male and female was not differing much in all species.

Body length of *Portunus sanguinolentus* ranged from 21.8 to 28.9 cm in male and 20.5 to 28.4 cm in female while *P. pelagicus* ranged from 33.8 cm to 49.3 cm in male and 33.3 cm to 48.9 cm in female. In *Scylla serrata,* body weight of male crab ranged from 19.5 cm to 34 cm while it was 20 cm to 30 cm in female (Fig.3). In all species, the body length of male and female was not differing much.

Sex ratio of studied crab species

The sex ratio of crab species was described in (Fig.4). In *Portunus sanguinolentus,* female individual was abundantly occurred in September and October while high male ratio was found in November 2015. In *Portunus pelagicus*, high ratio of female was found thought the study period especially from September to November 2015. In contract, high male ratio was recorded in *Scylla serrata* in which more than 25 individual per month are male crab.

Gonado somatic index of three crab species

Gonado somatic indexes of studied crab species were described in (Fig 5). In *Portunus sanguinolentus,* high female GSI 6.7 was found in October 2015 and it decreased to 2.9 in November 2015. Similarly, in *P. pelagicus,* high GSI, 9.2, was found in September 2015 and it sharply decreased to 2.8 in October 2015. In *Scylla serrata,* GSI of female crab is as high as 3.8, 1.7, 8.2 and 4.5 in September, October, November and December, respectively.

Fecundity of studied crab species

To estimate the fecundity of each crab species, number of egg per gram of female crabs were counted under stereomicroscope and calculated according to the formula. In *P. sanguinolentus,* number of egg is 39,975 average in September, 10,195 average in October and no egg was found in November 2015. In *P. pelagicus,* number of egg was much higher than *P. sanguinolentus.* Female collected in October and December 2015 carried egg and number of egg are 252,308 average and 234,613 average respectively.

Interestingly, an extremely high fecundity was found in *S. serrata* in which number of egg are exceed 198,387 average although female crab was found in only September 2015. (Fig.6)

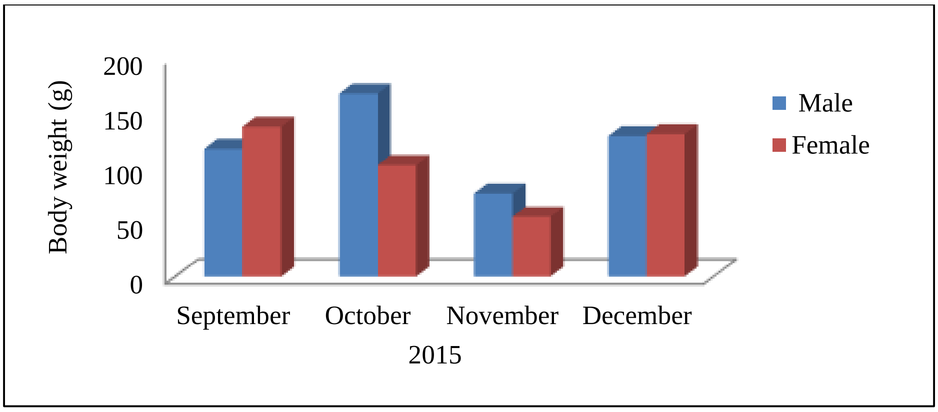
Mean diameter of eggs of studied crab species

Mean diameter of egg of each crab species was measured under the stereo microscope. Mean diameter of *Portunus sanguinolentus* is 398.35±7.9 um (n=800) *Portunus pelagicus* is 386.86 ±17.9um (n= 600) and *Scylla serrata* is 423.55±12.2 (n=500).

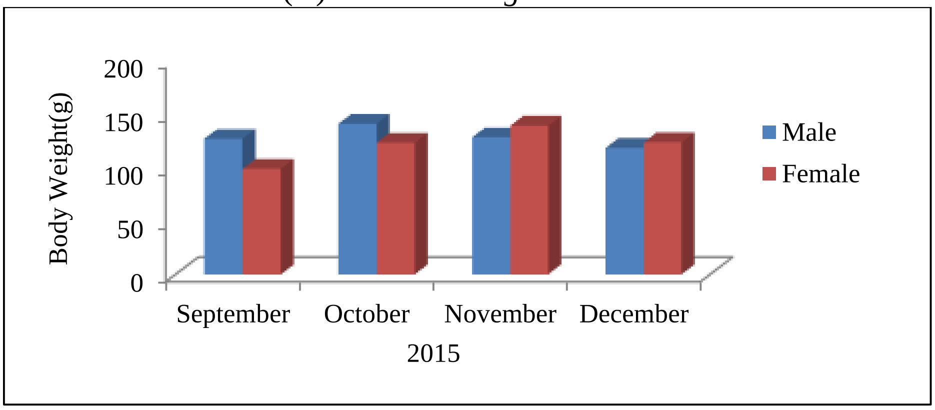
Reproductive development of crab species based on egg morphology of female crab species

Among the collected crab species, only female crab carrying eggs were classify for stages of reproductive development. In stage 1 the egg color was orange, in stage 2 the egg mass sponge was a light brown or grey and in stage 3 the egg was black (Fig.7 and 8).

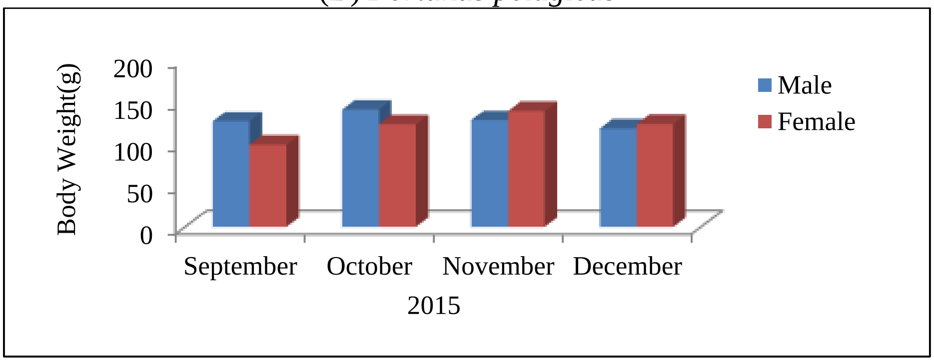
In *Portunus sanguinolentus,* more than 10 females crabs carried eggs throughout the study period, while one to six egg carrying female individuals were found in other two species. Three stages of reproductive development were found in all studied months from September to December 2015 in *Portunus sanguinolentus* and *P. pelagicus*. However, in *Scylla serrata*, two stages, stage 1 and 3 were found in September while only one stage either stag 1 or stage 3 was found in October and November (Fig. 9).



(A) *Portunus sanguinolentus*

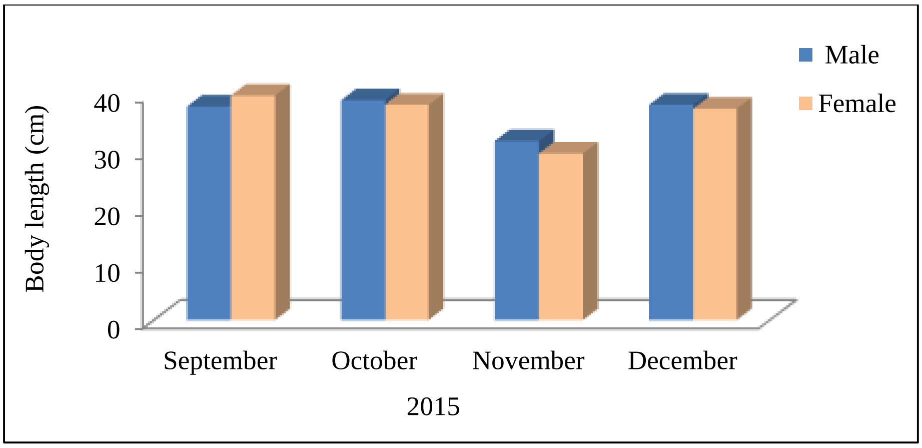


(B) *Portunus pelagicus*

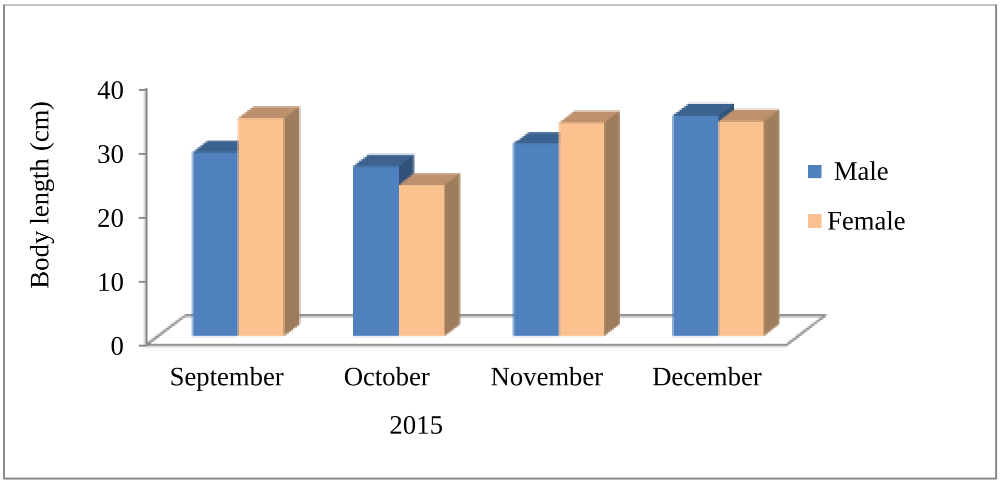


(C) *Scylla serrata*

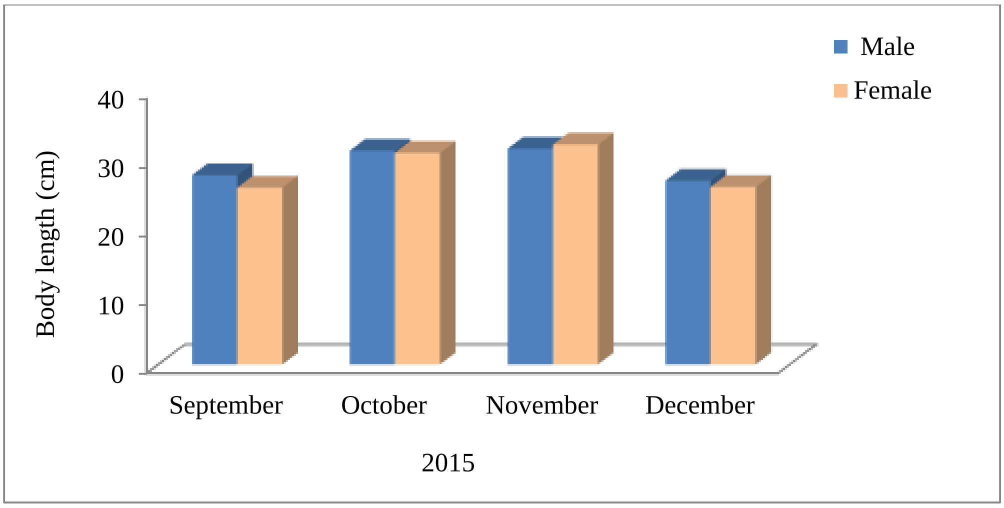
Figure 2 Average body weight (g) of three crab species



(A) *Portunus sanguinolentus*

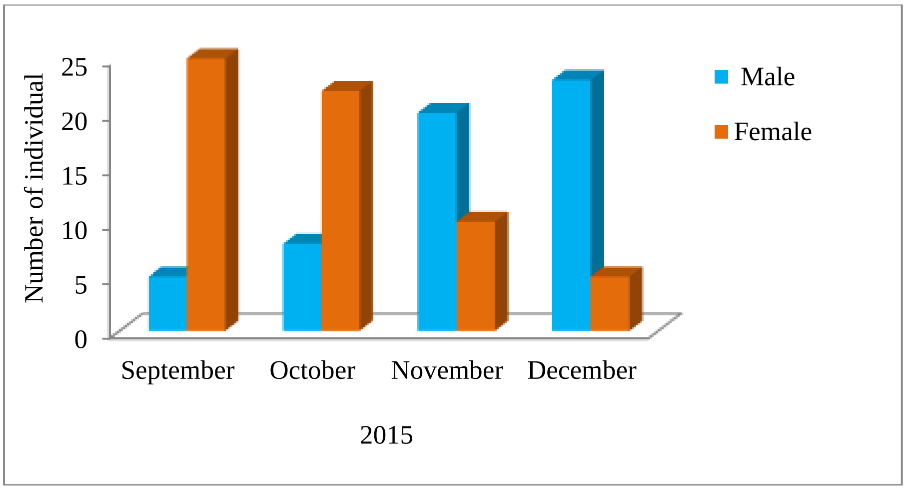


(B) *Portunus pelagicus*

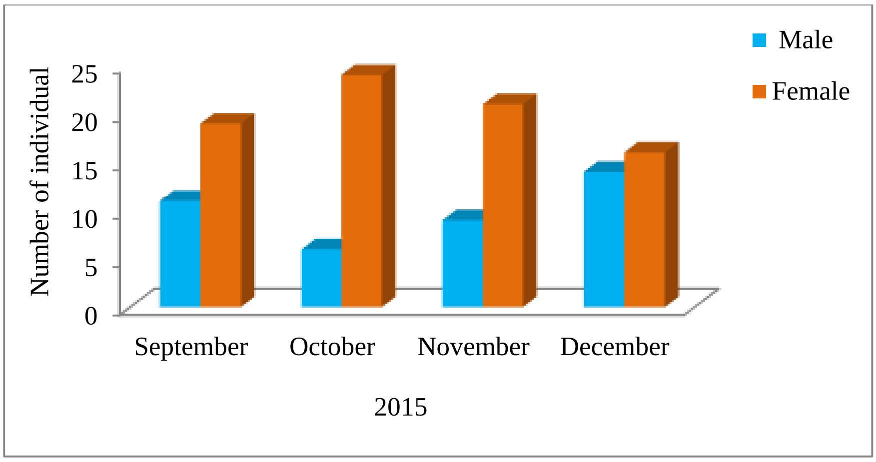


(C) *Scylla serrata*

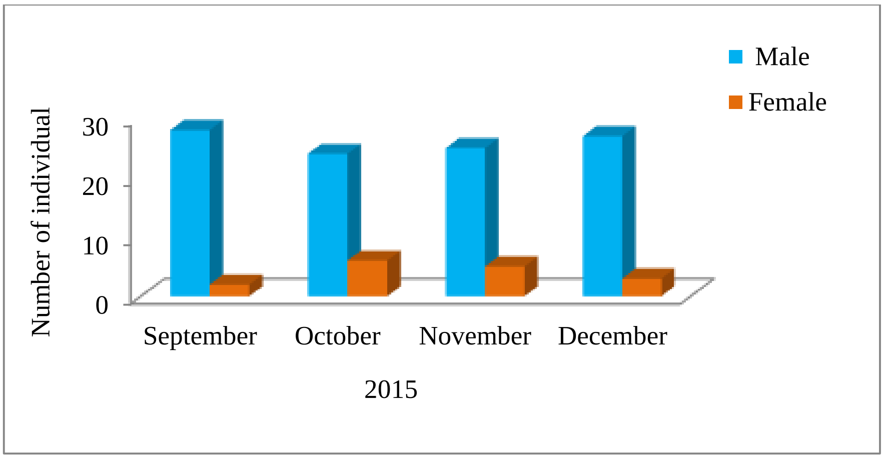
Figure 3 Average body length (cm) of three crab species



(A) *Portunus sanguinolentus*

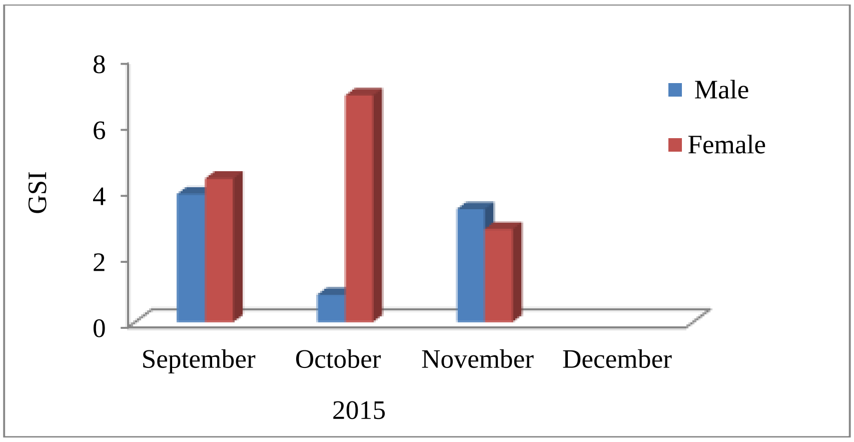


(B) *Portunus pelagicus*

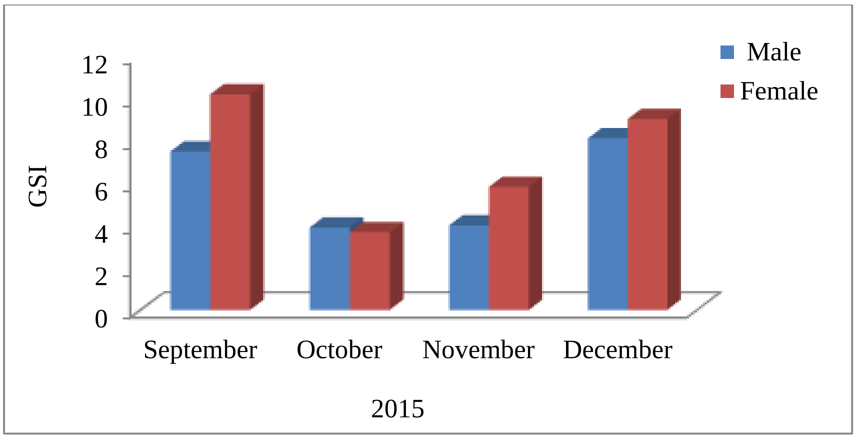


(C) *Scylla serrata*

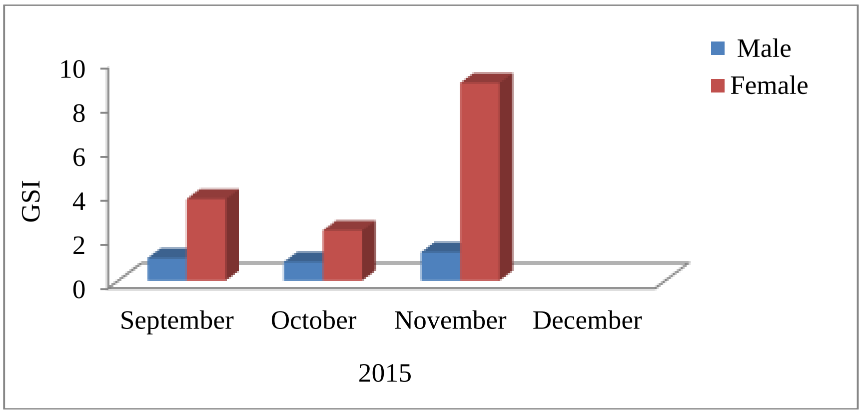
Figure 4 Sex ratio of three crab species



(A) *Portunus sanguinolentus*

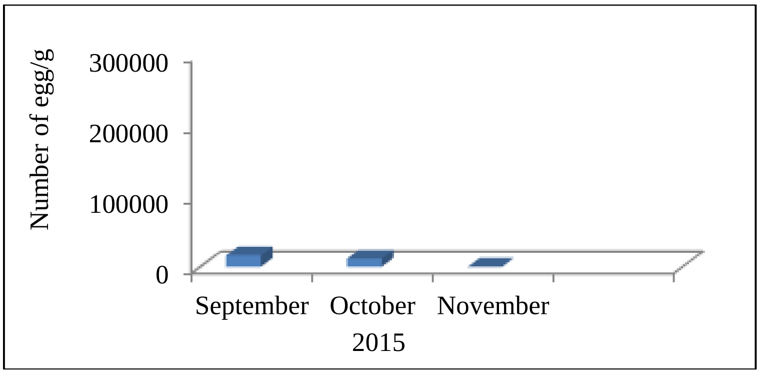


(B) *Portunus pelagicus*

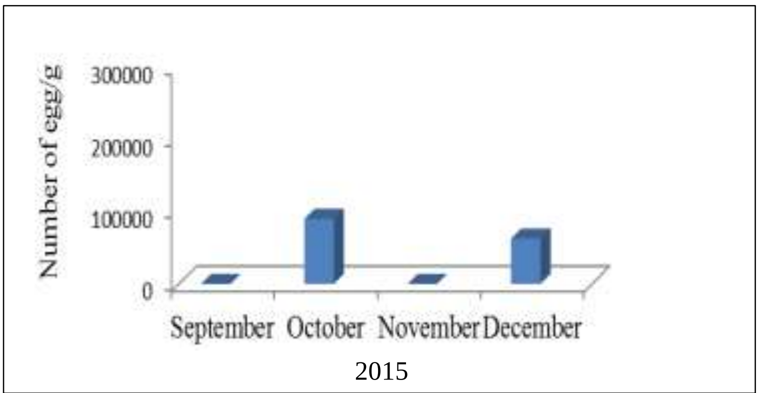


(C) *Scylla serrata*

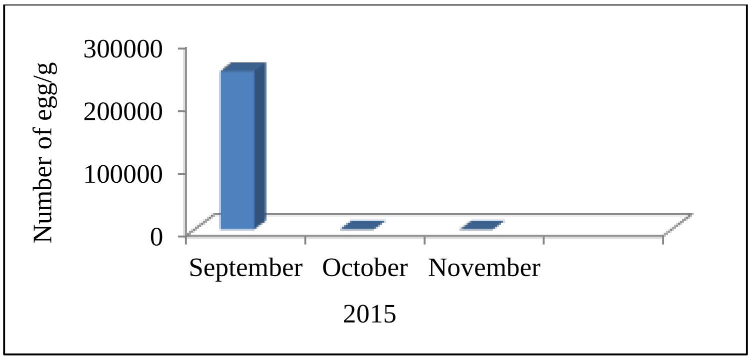
Figure 5 Gonodo somatic index of three crab species



(A) *Portunus sanguinolentus*

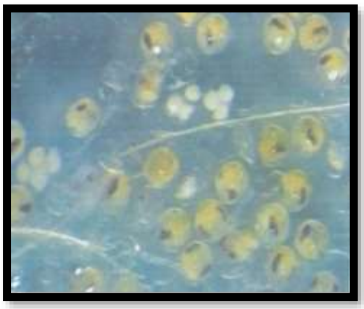


(B) *Portunus pelagicus*



(C) *Scylla serrata*

Figure 6 Mean fecundity of studied crab species



(A) *Portunus sanguinolentus*

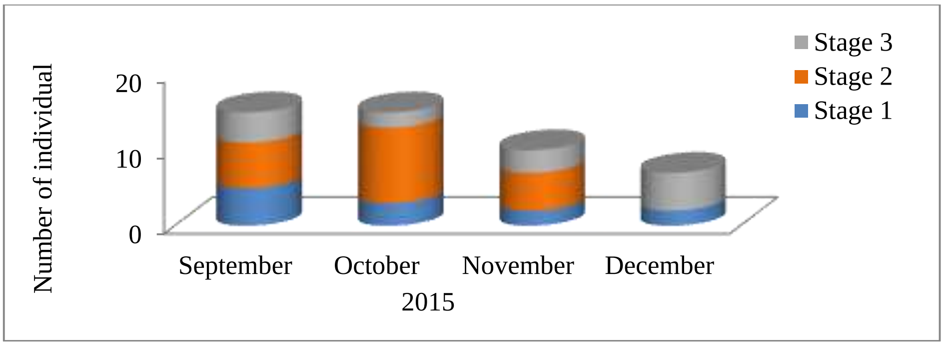
(B) *Portunus pelagicus*

(C) *Scylla serrata*

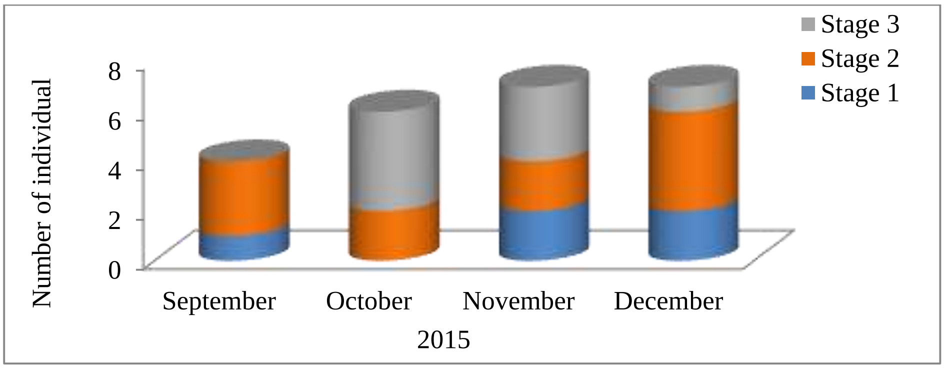
Figure 7 Egg morphology of three crab species

Stage (1) Stage (2) Stage (3)

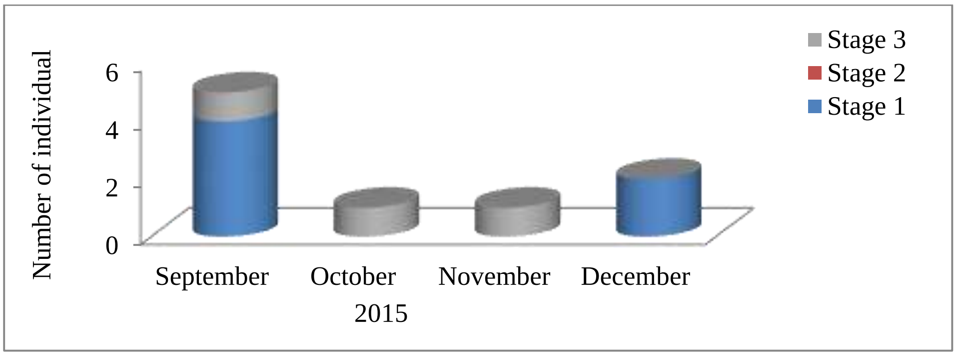
Figure 8 Reproductive stage of crab based on egg morphology (A) Stage 1 of *Scylla serrata* **(B)** Stage 2 of *Portunus sanguinolentus* (C) Stage 3 of *Portunus pelagicus*



(A) *Portunus sanguinolentus*



(B) *Portunus pelagicus*



(C) *Scylla serrata*

Figure 9 Stages of gonad of studied crab species

Discussion

In the present study, three crab species *Portunus sanguinolentus, P. pelagicus* and *Scylla serrata* were collected from September to December in 2015 and their gonadal development and sex ratio were studied. Except *Portunus sanguinolentus* collected in September 2015, body weight of male and female crab did not differ in all species. However, Normant *et al*., (2004) reported that the weight of male mud crab *Rhithropanopeus harrisii*, was heavier than that of female. They concluded that the difference is caused by the much larger, and heavier, male claws: the claw weight accounts for up to 64% of the body weight in males and for 11.1-28.0% in females. In the present study, although body weight of male and female crab are not much differ in each species, it was notice that chelae size of male is lager than the female. Hardnoll (1989) has shown that chela are secondary sexual character of the male. He proved that the size of chela in male significantly increase after the first molt of their life as sexual character.

Male and female population of *Scylla serrata* was a tread towards a contently higher percentage of male. The pattern likely emerged because the studied was carried out in monsoon to winter season. The migration of female *S. serrata* to offshore has been reported during their spawning season and it was only few female left in the mangrove forest (Sara, 2006; Jirapunpipat *et al*., 2008). We collected mud crab in the mangrove forest only although the other two species were obtained from offshore. Long term study is needed to estimate the actual sex ratio of the mud crab.

Portunus sanguinolentus and *P. pelagicus carried* egg throughout the study period while *Scylla serrata* mostly carried in September and did not carried at all in October and November. The migration behaviour of mud crabs suggests that at the time of spawning they migrate from brackish water to deep sea of high saline water (Ali *et al*., 2020). Due to this reason, mud crabs with stage 2 and 3 were rare in the mangrove area.

Several authors have recently reviewed the reproductive pattern of commercial crabs (Emmerson, 1994; Yamaguchi, 2001a; Costa and Negreiros-Fransozo, 2003; Litulo, 2004a), and most of them are based on the frequency of egg-bearing females. The number of eggs produced by mud crabs is five times higher than the other two species. The fecundity of crabs can vary even in different individual of same crab species within the same area and among females of the same species in different areas within the same region and is influenced by several intrinsic and extrinsic factors. According to Ramirez-Llodra (2002), the major intrinsic factors contributing to differences in fecundity among females in the same population include variation in individual female size or maternal size, nutritional history related to food availability and quality, age and the age at sexual maturity or first reproduction. According to the results of the present study, it is concluded that the larval production of mud crab is higher than the other two species.

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