REQUENCY DISTRIBUTION OF MITOTIC CELL DIVISION IN DECCAN CARP, *LABEO POTAIL* (SYKES, 1839) AND STRIPED CATFISH, *PANGASIANODON HYPOPHTHALMUS* (SAUVAGE, 1878)

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Abstract

The deccan carp *Labeo potail* and striped catfish *Pangasianodon hypophthalmus* were obtained from Arrthit Private Fish Farm, Patheingyi Township to investigate the chromosomal configurations of mitotic cells division by treating with mitotic inhibitor during January to August 2022. The colchicine concentration 0.30 % and 0.50 % were injected into the musculature of both fishes depend on the fish weight ml/g. The various organs such as oral cells, gill filaments, liver, kidney and blood cells of these fishes were treated for durations 3 hrs, 4 hrs 30 mins, 5 hrs and 6 hrs in *L. potail*. However, 5 hrs, and 5 hrs 30 mins were applied in *P. hypophthalmus*. The hypotonic solution 0.56 % KCL was used to cell explosion. The highest frequency of interphase stage was observed in blood cells 93.97 % (n=187), kidney 94.21 % (n=114), 91.46 % (n=439), and prophase stage was also found in kidney 38.10 % (n=16) and blood cells 56.19 % (n=168) in both fishes. The most frequent distribution of metaphase stages was observed in the colchicine concentration 0.50 % for duration 4 hrs 30 mins with 0.56 % KCL for duration 1 hr in *L. potail* whereas 5 hrs, 5 hrs 30 mins in *P. hypophthalmus* with 0.56 % KCL for duration 1 hr. The most accelerated mitotic stage of cells such as interphase, prophase, metaphase, anaphase and telophase were observed. These results will provide not only the basic information of mitotic technique of cell division but also resolve the mitotic check point of the chromosomal configuration for other freshwater fishes.

Keywords: fishes, organs, colchicine, KCL, mitotic stages

Introduction

Labeo is a large genus having several species which are of considerable importance as an article of food. Some of the species of the *Labeo* genus are reared for ornamental purpose, some as food species, some for extracting oil and some are considered to be of medicinal value also. Among them, *Labeo potail* has good market value and high consumer preference, important fisheries and in aquaculture activities (Sarma *et al*., 2017). *Pangasianodon hypophthalmus* (Nga-tan) is one of the largest and most important inland fisheries, the Mekong River Fishery, in the world. Striped catfish is also riverine freshwater species that can be found in Ayeyarwady Basin of Myanmar (Griffiths *et al*., 2020).

Every organism generated the vital activities through the checkpoint of cellular process in different stages of cell cycle: mitosis and meiosis. Mitosis maintains the chromosome number and generates new cells for the growth and maintenance of an organism (Iwasa and Marshall, 2018). The natural resources of various kinds of the indigenous species as well as the exotic species must be characterized their phenotypic expression as well as their genotypic attributes before utilizing the species for various purposes.

The objective of this study was to investigate the chromosomal characteristics of mitotic checkpoint of cells in *Labeo potail* and *Pangasianodon hypophthalmus* (locally known as Nga-tan) from Arrthit Private Fish Farm treated with mitotic inhibitors.

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Materials and Methods

The present study was conducted at the Laboratory, Department of Zoology ,University of Mandalay.

The study period was from January to August 2022.

Forty fishes samples were collected from Arrthit Private Fish Farm which is located at 21° 59′ 50.90" N and 96° 07′ 50.23"E , Patheingyi township Mandalay, the collected fishes were reared at Laboratory, Department of Zoology, University of Mandalay (Plate 1 and 2). The fishes were fed twice a day with formulated commercial feeds. The water was changed twice a week and kept in well-aerated aquarium (Plate 2. A).

The fishes were the mean length of *Labeo potail* (n = 10) was 16.80 ± 1.09 cm, standard length 13.90 ± 3.70 cm and the body weight 10.32 ± 3.40 g. In *Pangasianodon hypophthalmus*, the mean total length of striped catfish (n =4) was 22.80 ± 1.37 cm, standard length 21.00 ± 1.19 cm and the body weight 37.90 ± 0.97 g**.**

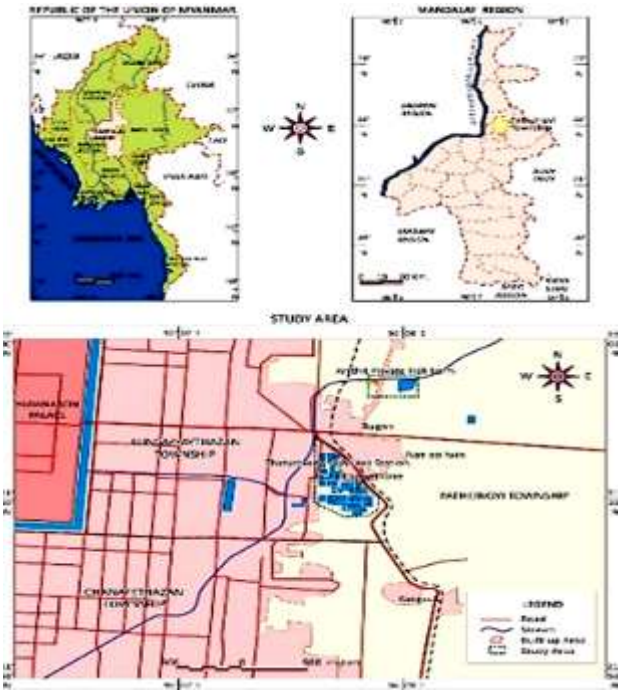


Figure.1.Study map of Arrthit Private Fish farm, Patheingyi Township, Mandalay Region (Source: UTM)

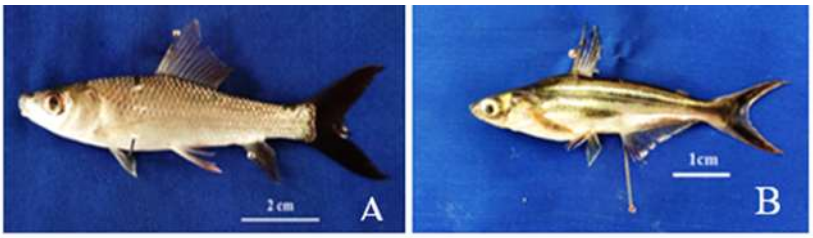


Plate 1 Lateral view of (A) *Labeo potail* and (B) *Pangasianodon hypophthalmus*

Identification of species

The identification of fish species was followed by Talwar and Jhingran (1991) and Khamees *et al*. (2013).

Injection technique

The body weight of each fish was recorded with digital kitchen scale to the nearest 0.01g. The standard length and total length were recorded to the nearest 0.1 cm by using a ruler. The concentrations of colchicine solutions 0.30 % and 0.50 % (AVI CHEM, India) were prepared and injected into the intramuscularly to fishes depending upon their weight 1ml/100 g (Plate 2.B and C).

Collection of tissues

Blood was extracted from the caudal peduncle of fish by using a syringe. The oral cells, gill,heart, liver and kidney were harvested immediately from anesthetized fish (Plate 2. D and E).

Extraction of cells

The sample tissues were incubated in 0.56 % KCL (MERCK Ltd, Munbal) for 15 mins, 20 mins, 1 hr and 1 hr 30 mins and minced with a glass rod. These samples were mixed with vortex mixer (NANOVA) by adding 3 methanol: 1 acetic acid and centrifuged (Firlabo) for

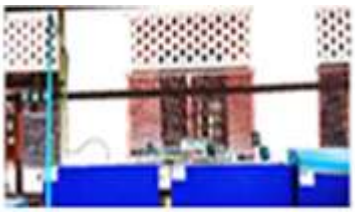
10 mins at 2000 rpm. The supernatant was discarded by using a pipette. This procedure was repeated again (Plate 2. F, G and H).

Preparation of slides

The slides were heated in the oven (Gallenhamp). One or two drops of pellets were placed onto the pre-warmed slides in a far distance and stained with undiluted Giemsa stain (AVI CHEM, India) for 10 mins and 15 mins and diluted Giemsa stain for 20 mins. The stained slides were washed under running tap water and dried at room temperature. Then, these stained slides were covered with pre-cleaned coverslips and finally coated with Canada balsam (Kanto Chemical Co., Inc, Tokyo, Japan) (Plate 2. I, J and K).

Identification of mitotic cell division

The microphotographs were recorded with a biological microscope with an attached camera (G-303P, Taiwan) (x1000). Good quality of chromosomal spreads was recorded for each slide (n = 10) and micro photographed for analysing various stages of cells in mitotic division. All recorded data was performed by Microsoft Excel 2010 (Plate 2. L).

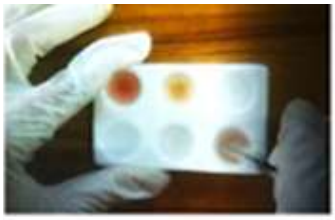


A. Fish reared in glass tank

B. Weighing the fish

C. Injection of fish

D. Extraction of blood

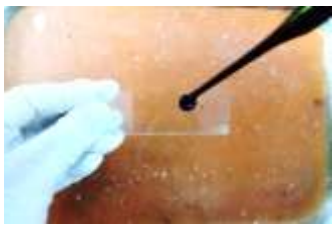


E. Mechanical dissociation of tissues

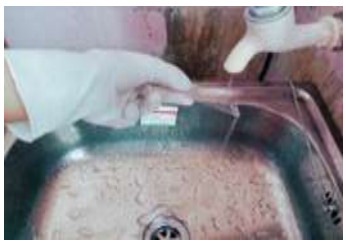
F. Homogenization of tissues

G. Centrifugation of pellets

H. Sucking the pellet from tube to drop on the slide



I. Dropping the Giemsa stain to the slide



J. Washing the slide under tap water

K. Coating with coverslip

L. Examination of prepared slides

Plate 2 Preparation of cytological process from fish tissues

Results

The concentrations of 0.30 % and 0.50 % colchicine solution were exposed to different organs such as gill filaments, oral cells, kidney, blood and liver of deccan carp and striped catfish. The optimum colchicine concentration and duration of *Labeo potail* was 0.50 % for 4 hrs 30 mins with1 hr hypotonic solution (0.56 % KCL) in gill filaments whereas 0.50 % for duration 5 hrs 30 mins with 1 hr 30 mins hypotonic solution in kidney tissues of *Pangasianodon hypophthalmus*. The good pictures of different chromosomal patterns in mitotic cell division were observed by staining the undiluted Giemsa stain for 10 mins.

Percent and frequency distribution

The different mitotic stages of cells were observed at 3 hrs treatment of colchicine concentration 0.30 % with 0.56 % KCL for duration 25 mins in blood cells, oral cells and gill filaments (Table 1). The highest percentage of interphase stage 94.22 % (n=114) were observed in the kidney cell and followed by 93.97 % (n=187) in blood cells and 81.48 % (n= 22) in oral cells for duration of 3 hrs with 25 mins in deccan carp. The most frequent mitotic stages, prophase 11.11% (n=3) and metaphase 7.41 % (n=2) were observed in oral cells. The lowest frequency of prophase stage 1.65 % (n= 2) was recorded in oral cells (Fig.2).

Colchicine concentration 0.50 % for duration 4 hrs 30 mins with 0.56 % KCL for duration 1 hr generated the interphase stage 3.33 % (n=1) in liver, 9.09 % (n=2) in kidney cells and 23.33 % (n=7) in gill filaments. In addition, the same chromosome spreads (n=2) were generated with various frequencies of prophase stages 8.33 % in oral cells, 9.09 % in kidney and 6.67 % in gill filaments expect the blood cells and liver. The highest distribution of metaphase stages was observed with different frequencies in 96.67 % (n=18) in liver, followed by 91.67 % (n= 22) in oral cells, 81.82 % (n= 18) in kidney and the lowest was found in gill filaments 70.00 % (n= 21) expect blood cells (Fig. 3).

The same frequency distribution 2.78 % (n=1) of interphase and prophase stage were observed in liver cells by rising colchicine solution for 5 hrs with the same incubation time of 0.56 % KCL. The highest percentage of metaphase stage 100.00 % (n=25) was found in gill filaments and prophase stage 11.11 % (n=2) in kidney cells. The metaphase stage was found in liver cells 94.44 % (n=34) followed by 88.89 % (n=16) in kidney cells (Fig. 4).

The highest percentage of interphase and prophase stage 61.90 % (n= 26) and 38.10 % (n= 16) in kidney cells were observed in colchicine concentration 0.50 % for duration 6 hrs with 0.56 % KCL for 1 hr. The lowest frequency of metaphase stage of chromosomes 13.33 % (n=4) was also recorded in liver cells of decan carp. Unfortunately, the distribution of metaphase stage was not found in kidney cells (Table 1 and Fig. 5). However, kidney cells operated the other mitotic stages such as anaphase and telophase in the same treatment of colchicine solution and 0.56 % KCL (Plate 3. E and F).

In *Pangasianodon hypophthalmus*, the frequency distribution of interphase stage was not observed in colchicine solution 0.50 % for duration 5 hrs in hypotonic solution 1 hr (Table 2). Prophase stage 10.34 % (n=3) was the most frequent in oral cells. Blood and kidney cells generated the prophase stage 9.09 % (n=2) and the lowest in blood cells with 4.69 % (n=3). Especially, all tissues generated the metaphase stage. The same percentage of metaphase stage was operated with 100.00 % (n=25) in liver and n= 16 in gill filaments followed by 95.31 % (n=6) in blood cells, 90.91 % (n=20) in kidney cells, 89.66 % (n=26) in oral cells (Fig.6).

When the incubation time of colchicine solution was raised up to 5 hrs 30 mins with KCL solution for 1 hr showed the distribution of interphase stage 4.17 % (n=1) and prophase stage 25.00 % (n=6) in blood cells; and 3.30 % (n=1) in gill filaments. The metaphase stage of

100.00 % (n=4) in liver, kidney (n=23) and oral cells (n=2) and 70.83 % (n=17) in blood cells were observed (Fig.7).

The incubation time of hypotonic solution 0.56 % KCL with the same treatment of colchicine duration was raised for 1 hr 30 mins resolved the highest frequency of three mitotic stages found in blood, oral and kidney cells. The highest percentage of interphase stage 91.46 % (n=439) was recorded in kidney cells and the lowest 42.27 % (n=127) in blood cells. The maximum distribution of prophase stage 56.19 % (n=168) was appeared in blood cells and the minimum 1.88 % (n=9) was found in kidney cells. The metaphase stage was recorded in four tissues expect liver cells with frequencies of 21.43 % (n=3) in oral cells, 6.66 % (n=32) in kidney cells, 2.65 % (n=4) in gill filaments and the lowest was 1.34 %(n=4) in blood cells (Fig.8).

Table 1. Percent and frequency distribution of mitotic division in *L. potail*

Duration	0.30 % Colchicine solution (3 hrs)			0.50 % Colchicine solution (4hrs 30 mins)			0.50 % Colchicine solution (5 hrs)			0.50 % Colchicine solution (6 hrs)		
	0.56 % Hypotonic solution (25 mins)			0.56 % Hypotonic solution (1 hr)			0.56 % Hypotonic solution (1 hr)			0.56% Hypotonic solution (1hr)		
Tissues/ Mitotic stages	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase
Blood cells	93.97 % (n=187)	3.52% (n=7)	2.51 % (n=5)	0	0	0	0	0	0	0	0	0
Oral cells	81.48 % (n=22)	11.11 % (n=3)	7.41 % (n=2)	0	8.33 % (n=2)	91.67 % (n=22)	0	0	0	44.44 % (n=16)	5.56 % (n=16)	50.00 % (n=18)
Kidney	94.22 % (n=114)	1.65 % (n=2)	4.13 % (n=5)	9.09 % (n=2)	9.09 % (n=2)	81.82 % (n=18)	0	11.11 % (n=2)	88.89 % (n=16)	61.90 % (n=26)	38.10 % (n=16)	0
Liver	0	0	0	3.33 % (n=1)	0	96.67 % (n=18)	2.78 % (n=1)	2.78 % (n=1)	94.44 % (n=34)	53.34 % (n=16)	33.33 % (n=10)	13.33% (n=4)
Gill filaments	0	0	0	23.33 % (n=7)	6.67 % (n=2)	70.00 % (n=21)	0	0	100.00 % (n=25)	39.47 % (n=30)	36.84 % (n=28)	23.69 % (n=18)

Table 2. Percent and frequency distribution of mitotic division in *P. hypophthalmus*

Duration	Colchicine solution – 5 hrs						Colchicine solution – 5 hrs 30 mins					
	Hypotonic solution – 1 hr						Hypotonic solution – 1 hr 30 mins					
Tissues/ Mitotic stages	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase	Interphase	Prophase	Metaphase
Blood cells	0	4.69 % (n=3)	95.31 % (n=6)	4.17 % (n= 1)	25.00 % (n=6)	70.83 % (n=17)	42.47 % (n=127)	56.19 % (n=168)	1.34 % (n=4)			
Oral cells	0	10.34 % (n=3)	89.66 % (n=26)	0	0	100.00 % (n=2)	64.28 % (n=9)	14.29 % (n=2)	21.43 % (n= 3)			
Kidney	0	9.09 % (n=2)	90.91% (n=20)	0	0	100.00 % (n=23)	91.46 % (n=439)	1.88 % (n=9)	6.66 % (n=32)			
Liver	0	0	100.00 % (n=25)	0	0	100.00 % (n=4)	0	0	0			
Gill filaments	0	0	100.00 % (n=16)	3.03 % (n=1)	3.03 % (n=1)	93.94 % (n=3)	89.40 % (n=135)	7.95 % (n=12)	2.65 % (n=4)			

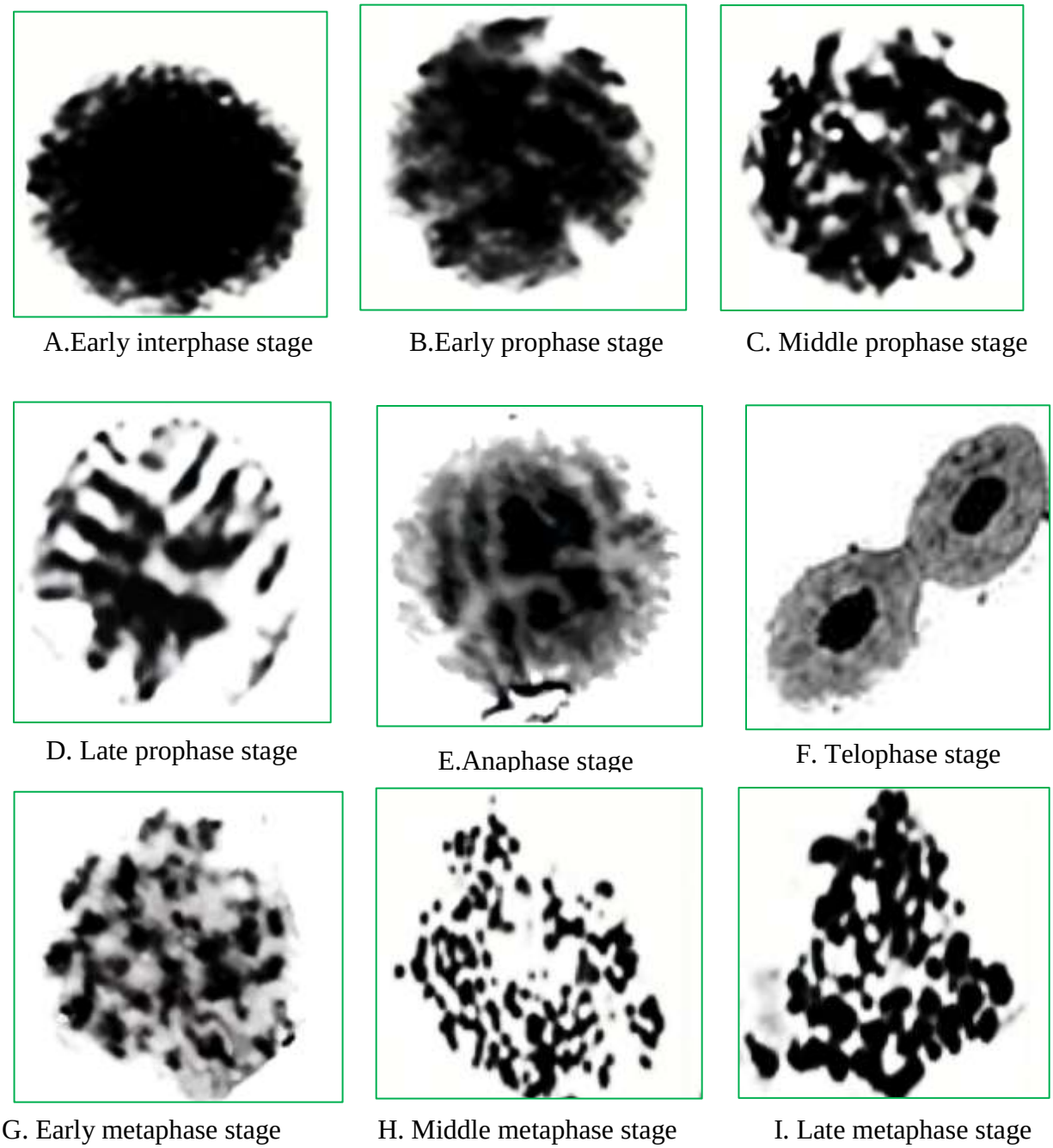


Plate 3. The arrested stages of chromosomal configurations in mitotic division (x1000)

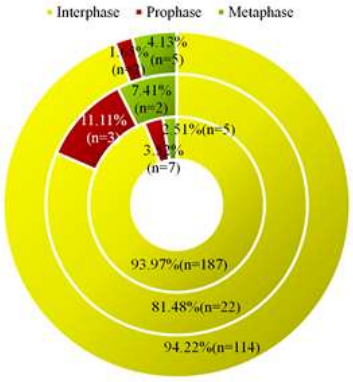


Figure.2. Effect of colchicine concentration 0.30% for duration 3 hrs with 0.56 % KCL for 25mins on the mitotic division of different tissues *Labeo potail (*1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney)

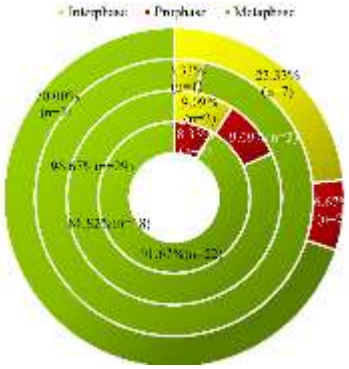


Figure.3. Effect of colchicine concentration 0.50 % for duration 4 hrs 30 mins with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Labeo potail (*1st ring- oral cells; 2nd ring– kidney; 3rd ring- liver; 4th ring- gill filaments)

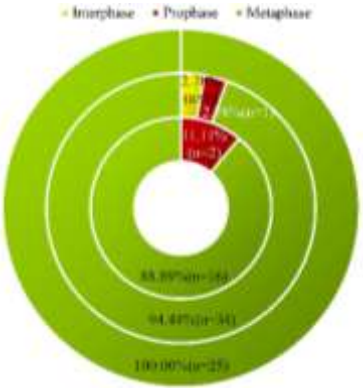


Figure.4. Effect of colchicine concentration 0.50 % for duration 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Labeo potail (*1st ring- kidney; 2nd ring- liver; 3rd ring- gill filaments)

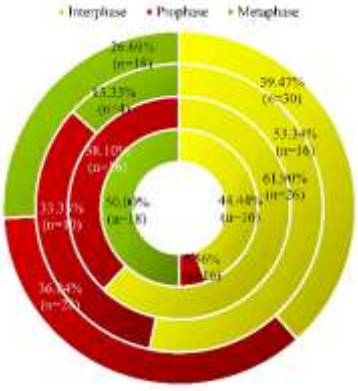


Figure.5. Effect of colchicine concentration 0.50 % for duration 6 hrs with 0.56 % KCL for 1hr on the mitotic division of different tissues *Labeo potail (*1st ring- oral cells ; 2nd ring– kidney; 3rd ring- liver; 4th ring- gill filaments)

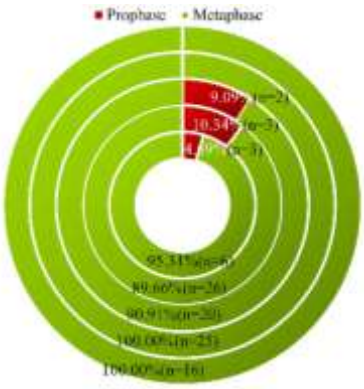


Figure.6. Effect of colchicine concentration 0.50 % for duration 5 hrs with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Pangasianodon hypophthalmus* (1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- gill filaments)

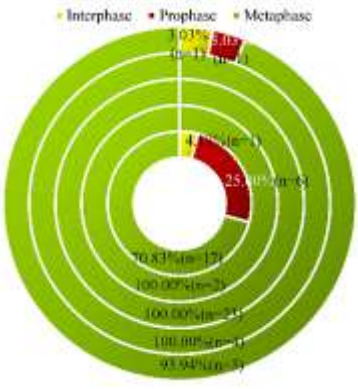


Figure.7. Effect of colchicine concentration 0.50 % for duration 5 hrs 30 mins with 0.56 % KCL for 1 hr on the mitotic division of different tissues *Pangasianodon hypophthalmus (*1st ring- blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- liver; 5th ring- gill filaments)

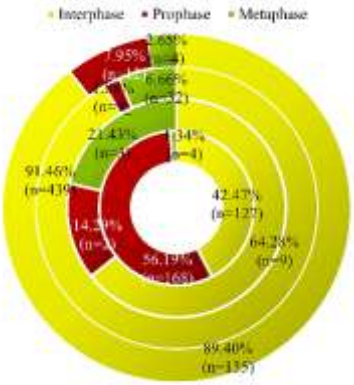


Figure.8. Effect of colchicine concentration 0.50 % for duration 5 hrs 30 mins with 0.56 % KCL for 1 hr 30 mins on the mitotic division of different tissues *Pangasianodon hypophthalmus* (1st ring-blood cells; 2nd ring- oral cells; 3rd ring- kidney; 4th ring- gill filaments)

Discussion

In the present study, the method of chromosome preparation was based on testing with different concentrations of colchicine solutions on various organs of *L. potail* and *P. hypophthalmus*. Mahfuji *et al*. (2014) described the variation of chromosomal characteristics that are largely dependent on methods of chromosome preparation, staining procedure, tissue source of the body where the dividing cells. The optimum colchicine concentration for these fishes was 0.50 %.

When the fishes were treated with colchicine concentration 0.30 % for duration of 0.56 % KCL 25 mins, the blood cells operated largely the early stage of interphase in mitotic cell division. The early interphase stage was observed in blood cells. The lower concentration with short duration of mitotic inhibitors could not be accomplished the complete chromosomal configuration. The concentration of colchicine 0.50 % for 4 hrs 30 mins with hypotonic solution at 1 hr was generated the highest frequency of late metaphase stage of chromosomes in oral cells compared with other different cells. The most frequent distribution of early interphase stage was found in gill filaments of colchicine concentration for 6 hrs and KCL for 1 hr in *L. potail.*

Therefore, the long-term treatment of colchicine duration together with the same concentration 0.50 % was used in *P. hypophthalmus*. The early metaphase stage was the maximum distribution of chromosomal stage for 5 hrs of colchicine duration and hypotonic treatment for 1 hr. Unfortunately, the same results of the early metaphase stage were also recorded by rising 30 mins duration. Therefore, the duration of colchicine solution was fixed at 5 hrs 30 mins and the incubation time of hypotonic solution was 1hr 30 mins. Then, the late metaphase and early interphase stage of chromosomal configurations were also observed in kidney cells.

The hypotonic treatment of 0.56 % KCL for 1 hr was good for explosion of nuclear membrane in kidney tissues of *Oreochromis* spp. (Win Win Mar and Thant Zin, 2020). According to the results of this study, longer treatment of 0.56 % KCL solution for 1 hr generates enough explosion of nuclear envelope and cytoplasmic membrane observed in all tissues and blood cells in *L. potail*.

However, the same technique did not support the explosion of cells from all tissues in *P. hypophthalmus* except the blood and oral cells. When the exposure time was raised up to 1 hr 30 mins, the check point of mitotic division cells was observed. Another important factor for cytogenetic analysis is Carnoyl's fixative (3 methanol : 1 acetic acid). In this study, Carnoyl's fixative was used to treat the extracted cells for 10 mins duration. The good shape of chromosomal configuration of *L. potail* and *P. hypophthalmus* were observed that is leading to count and differentiate the chromosomal structure in detail.

Therefore, the chromosomal characteristics on studied fishes could be generated and standardized by using the optimal checkpoint of mitotic cell division for their population. These

recorded data are highly recommended for further investigation on variations in cytogenetic research and designate the karyotype formula of respective species.

Conclusion

The effect of colchicine concentrations 0.50 % was better than 0.30 % with various durations 4 hrs 30 mins, 5 hrs and 5 hrs 30 mins in *Labeo potail* and *Pangasianodon hypophthalmus.* The different tissues generated the different stages, interphase, prophase, metaphase, anaphase and telophase. The highest frequency of interphase stage was observed in blood cells 93.97 % (n= 187) in *L. potail*, kidney 91.46 % (n=439) in *P. hypophthalmus* and prophase stage was also found in kidney 38.10 % (n= 16) in *L. potail* and blood cells 56.19 %(n=168) in *P. hypophthalmus*. The oral cells, kidney, liver and gill filaments of *L. potail* generated the optimal check point of metaphase stages by treating with 0.50 % colchicine concentration for 4 hr 30 mins with 0.56 % KCL for duration 1 hr, expect the blood cells, however oral cells could not be resolved in colchicine concentration 0.50 % for 5 hrs. The metaphase checkpoints of mitotic cells were more observed in all tissues and cells of *P. hypophthalmus* by treating with 0.50 % colchicine concentration for duration 5 hrs and 5 hrs 30 mins with 0.56 % KCl for duration 1 hr.

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References

Griffiths, D., Van Khanh, P. and Trong, T.O. (2020). *Cultured Aquatic Species Information Programme, Pangasianodon hypophthalmus (Sauvage, 1878),* In: FAO Fisheries and Aquaculture Department (online), Rome. Accessed date February, 2020.

Iwasa, J. and Marshall, W. (2018). *Karp's Cell and Molecular Biology*. John Wiley and Sons, Inc, 111 River Street Hoboken, United States of America, pp. 552-554.

Khamees, N.R., Ali, A.H., Abed, J.M. and Adday, T.K. (2013). First record of Striped Catfish Pangasianodon hypophthalmus (Sauvage, 1878) (Pisces: Pangasiidae) from Inland Water of Iraq, *Basrah Journal of Agriculture Science*, vol. 26(A):184-194.

Mahfuji, M.S.E., Hossain, M.B., Rahman, M.M. and Hoshan, M.I. (2014). Chromosomal studies and quantitative karyotypic analysis of Rohu, *Labeo rohita*, *Pakistan Journal of Biological Sciences,* 17(4): 490-496.

Sarma, K.J., Prajapati, M. and Mankodi, P.C. (2017). Morphological description and taxonomic account of *Labeo* species (Cypriniformes, Family: Cyprinidae) from Gujarat, India, *Journal of Entomology and Zoology*, 5(4): 1120-1125.

Talwar, P.K. and Jhingran, A.G. (1991). *Inland Fishes of India and adjacent countries*. Oxford and IBH Publishing Co. Pvt. Ltd, New Delhi, Bombay and Calcutta, 1:152-331.

Win Win Mar and Thant Zin, (2020) "Karyotype analysis on *Oreochromis* spp. from Thatyetkhone Fisheries Station, Mandalay District" *Meiktila University Research Journal*, 1: 310-315.