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THE GAMETOGENIC CYCLE OF HARD CLAM, *GELOINA EXPANSA* (MOUSSON, 1849) IN SHWE THAUNG YAN, AYEYARWADY REGION*

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Abstract

A total of 692 clams (319 were males and 373 were females) were analyzed. Six different gonad development stages in both males and females were identified. The average gonads socratic index (GSI) value of males was high in May and November (2.02 ±0.6 and 1.88 ±0.7). In females, the maximum GSI value was observed in May and November (1.80 ±0.6 and 1.75 ±0.5). The monthly changes in the gonadosomatic index and the microscopic characteristics of ovaries showed that *G. expansa* was recorded as two times spawning periods, the majority of spawning periods were recorded in the summer months of May, June, and July, and the second spawning (minor spawning) took place in or around November. The present results indicated that *G. expansa* is dioecious and gametogenic development was synchronous between the sexes. Temperature is the major factor in controlling the reproductive cycle in *G. expansa*.

Keywords: *Geloina expansa*, gonadosomatic index, spawning periods.

Introduction

The mud clam *Geloina expansa* from the family Corbiculidae lives semi-infaunally on the soft sediment that is accumulated around the roots of the mangrove trees and spends a considerable portion of its life exposed to air in mangrove swamps where salinity fluctuates greatly (Ingole *et al*., 2002). The coastal area of Shwe Thaung Yan (study area) is located in the western part of the Ayeyarwady Division and about 48 km west far from Pathein. This coastal region is known for its extensive local fisheries and mudflat and mangrove forests, which are home to a vast variety of naturally occurring crustaceans. Despite being a naturally occurring bivalve, the clam is harvested for its edible qualities and has not been commercially farmed.

Reproduction is an important aspect of the life history of any species and having an understanding of reproductive processes is central to the management of any commercial fishery (Barber and Blake 2006). Generally, the reproductive cycle of marine invertebrates, mainly bivalves is mostly influenced by adjacent environmental parameters, and their gonads could vary from place to place over a year (Drummond *et al*., 2006). Inter-area differences in reproductive cycle and breeding patterns have been noticed in bivalve communities and the differences emerge to be linked with variations in food and temperature (Delgado and Camacho, 2005).

Histological preparation of gonads on reproductive cycle studies of clams to date is the most reliable where detailed information can be obtained (Hartati *et al.,* 2005). However, the gonad in Corbiculidae is difficult to distinguish especially in the females during the off breeding season (Morton, 1982). The production of this species from its natural habitat in this area is still unknown and unreported. A poor understanding exists of the underlying mechanisms that influence *G. expansa* reproduction, as well as the potential contribution of these factors to the ongoing clam supply. A thorough understanding of these elements is necessary for the efficient management of this significant marine resource. The goal of this study was to highlight the key elements of the reproductive biology of *G. expansa* is a determining component of the population dynamics in bivalves. Detailed knowledge of these factors is required for the effective management of this important marine resource.

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Materials and Methods

Study area and sampling procedures

Shwe Thaung Yan coastal region (Lat. 17˚ 05' 54" N and Long. 94˚ 28' 52" E) is located in the western part of Ayeyarwady Division and about 48 km west from Pathein. The present study was conducted along the U-To tidal creek. The map of the sample collection area is shown in Figure 1. Physico-chemical properties of adjacent estuarine water such as temperature, salinity, pH, and dissolved oxygen were measured and recorded using a thermometer, pH meter, and refractometer during sampling.

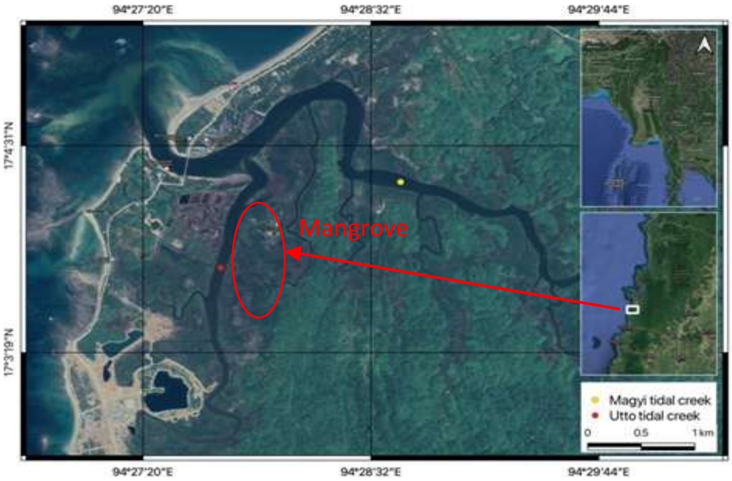

Figure 1. Map showing the sample collection area.

Clam samples were collected monthly by random sampling method from mangrove areas of the Shwe Thaung Yan region between May 2021 and April 2022. During the present study, a total of 692 clam samples (319 males and 373 females) ranging in size from 34.93 mm to 87.10 mm total length and 32 to 149.3 g body weight were examined. The shell was opened by inserting a paring knife between the valves to cut the anterior and posterior adductor muscles. The soft tissues were shucked and the sexes were recorded based on the color of the gonad. The gonad in females is black and in males is creamy white (Fig. 2). When in doubt, a gonadal smear was examined under the microscope for sperm or eggs.

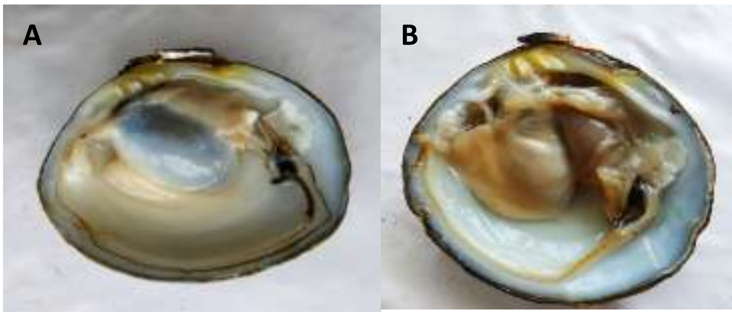

Figure 2. Anatomy of male and female *G. expansa*. A. Female; B. male.

After the samples were dissected to examine sex, the gonads were fixed in 10% buffered formalin for 48 hours and then the gonadal tissues were then embedded in paraffin blocks. After embedding the tissues, the paraffin blocks were trimmed to facilitate accurate sectioning. The blocks were then sectioned at 5 to 7 µm thickness using a microtome. The sections were then mounted on slides and dried overnight in an incubator at around 40˚C. Staining was performed using Haematoxylin and Eosin through standard methods. Histological slides were examined under the microscope, and photographed. Gonadal maturation stages were classified based on the classification system reported by Gimin (2005). The stages of gonad development were classified as primordial, developing, maturing, partially spawned, and spent.

The gonadosomatic index (GSI) was calculated using the formula given by Banerjee (2004).

$$GSI = \frac{\text{weight of gonad}}{\text{weight of fresh meat}} \times 100$$

Results

Environmental Parameters

In the present study, the environmental parameters showed small differences among monthly measurements of salinity and DO for water quality (Table 1). The lowest temperature (27˚C) was recorded in August 2021 and the highest (28.5˚C) in May 2022. Salinity at Shwe Thaung Yan fluctuated between 17 and 32 ‰. The lowest salinity was recorded in November 2021 and the highest in May 2021. The pH of the water was highest in February, and March 2022 (5.3) and lowest in August 2021 (3.3). The pH of the seawater at the sampling site did not show any seasonal fluctuation. The minimum and maximum values for dissolved oxygen (DO) were recorded (4.2 mg/L) in April and (5 mg/L) in October respectively.

Table 1. **The monthly variations of environmental parameters (temperature, salinity, pH, and dissolved oxygen) in the Shwe Thaung Yan region from May 2021 to April 2022**.

Months	**Parameters**			
	Salinity (‰)	**Temperature (°C)**	**pH**	**Dissolved oxygen (mg^{-1})**
May 2021	30	28.5	4.2	4.5
June	20.5	27.3	4.1	4.9
July	21	27.6	4	4.7
August	22.5	27	3.3	4.5
September	18	28	4	4.6
October	18.5	28.5	4.2	5
November	17	28.2	4.3	4.9
December	18	26.5	4.6	4.9
January 2022	22.5	28.3	5	4.8
February	24	27.5	5.3	4.6
March	29.5	28.2	5.3	4.4
April	30	28	4	4.2

Gonad development stages

Six stages of gonad development were identified in *G. expansa* both for males and females are primordial, developing, maturing, partially spawned, and spent (Figs. 3 and 4, A-F). The colors of gonads were grayish in females, and whitish in males, and no hermaphrodite was found in *G. expansa* at Shwe Thaung Yan. Different gonad development stages for both sexes are summarized in Table 2.

Reproductive cycle of *G. expansa*

The reproductive cycle of *G. expansa* from Shwe Thaung Yan mangrove areas is presented in Figures 6 and 7. Most of the sampling months were predominated by maturing and ripe individuals. Only the immature stage (Stage I) occurred mainly from December to April. The maturing stage (Stage III) was encountered in almost all months and the percentage occurrence of ripe individuals (Stage IV) peaked in October followed by a decrease in July and December.

The partially spawned stage (Stage V) started to be found in June, July, November, December, and January with the maximum percentage occurrence in November. The spent stage (Stage VI) was observed from June to July and from November to January, and the maximum percentage was recorded in January.

Table 2. Description and criteria for the gonadal stages of *G. expansa*

Gonadal stages	Histological characteristics	
	Males	Females
Primordial	The Gonad area is fully covered by the connective tissue and present in the lumen (Fig 3, A).	Fully covered by connective tissue and less empty acini appeared (Fig 4, A).
Developing	Acinus forming stage, intensive spermatocytes,and spermatogonia stages. (Fig 3, B).	Acinus is visible and the diameter increased. (Fig 4, B).
Maturing	Ascinus reaches to maximum size and visibility of spermatozoa (Fig 3, C).	Filled with packed oocytes and nucleus was able to be seen (Fig 4, C).
Ripe	Stripes of spermatozoa occupy almost the whole area of the follicle leaving a very narrow strip along the periphery for the spermatogonia (Fig 3, D).	A small number of oocytes are still attached to the follicular wall by a thin stalk. Most oocytes are polygonal (Fig 4, D).
Partially Spawned	The number of spermatozoa decreases in some follicles. Spermatids occupy the area vacated by the spermatozoa (Fig 3, E).	Some follicles contain mature ova. Follicles reduce in size, particularly in those devoid of ova (Fig 4, E).
Spent	Most of the follicles are empty. Empty follicles with irregular and elongated shapes. Phagocytes are active (Fig 3, F).	Follicles are empty and the follicular wall collapses in places. Some residual mature oocytes are still present in the empty lumen (Fig 4, F).

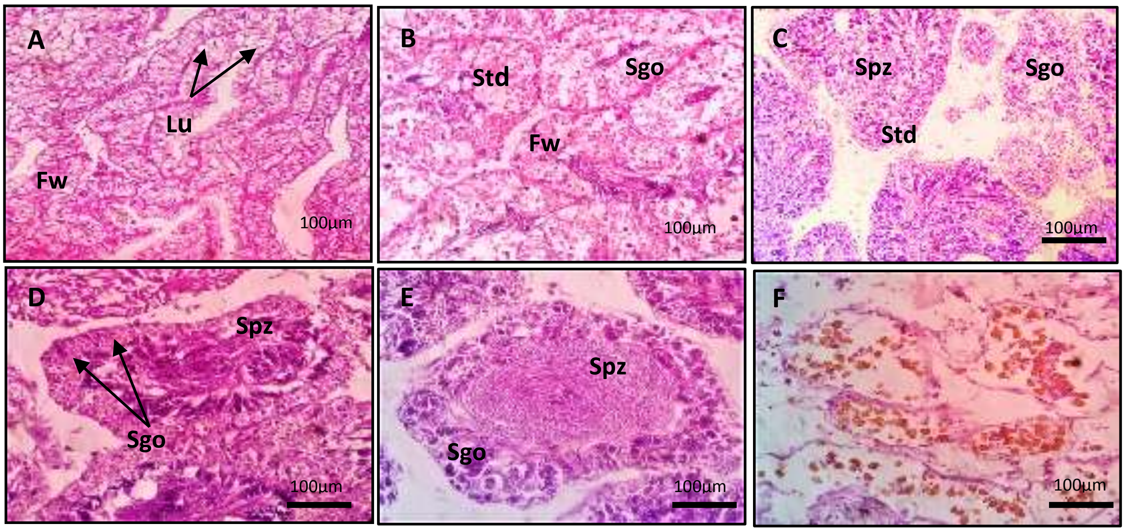

Figure 3. Gonad development stages in females of *G. expansa* (A) Primordial, (B) Developing, (C) Maturing, (D)Ripe, (E) Partially Spawned, (F) Spent. FW=follicular wall; L=lumen; DO= degenerating oocytes; sgo=spermatogonium; std=spermatid; spz=spermatozoa; -spermatozoa about to release from the follicle; MO= developing oocytes.

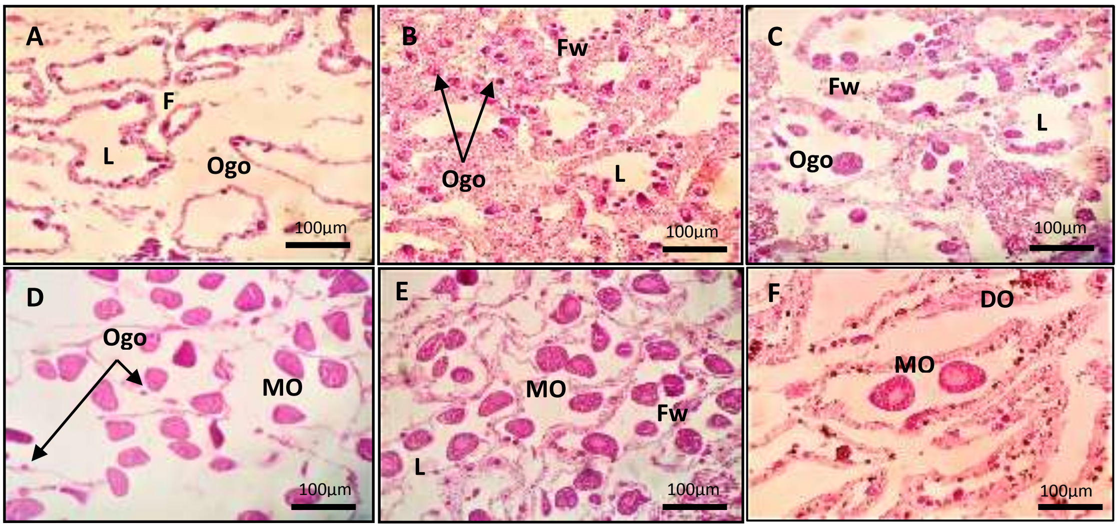

Figure 4. Gonad development stages in females of *G. expansa* (A) Primordial, (B) Developing, (C) Maturing, (D)Ripe, (E) Partially Spawned, (F) Spent. FW=follicular wall; L=lumen; DO= degenerating oocytes; sgo=spermatogonium; std=spermatid; spz=spermatozoa; - spermatozoa about to release from the follicle; MO= developing oocytes.

Gonadosomatic index (GSI)

The result of the monthly trend of the Gonadosomatic Index of males and females was expressed in Table 3 and Fig. 5. An increase in GSI values indicates the development of gonads. It is observed that the highest average GSI values were obtained in May 2021 and November 2021. The value continuously decreased from June to October and reached its peak in November. After that, it decreased from December to February 2022 and increased again in March 2022 and April 2022. It is observed that the lowest average GSI values were obtained in August and September. The monthly average GSI values of males were always higher than those of females in all months.

Table 3. Monthly average GSI values of *Geloina expansa.*

Sex	Months											
	May 2021	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan 2022**	**Feb**	**Mar**	**April**
Male	2.02 ±0.6	1.01 ±0.2	0.83 ±0.6	0.54 ±0.3	0.71 ±0.6	0.97 ±0.4	1.88 ±0.7	1.34 ±0.2	1.15 ±0.8	1.02 ±0.6	1.34 ±0.2	1.40 ±0.6
Female	1.80 ±0.6	0.86 ±0.1	0.72 ±0.4	0.49 ±0.2	0.62 ±0.2	0.85 ±0.3	1.75 ±0.5	1.24 ±0.3	1.06 ±0.2	0.92 ±0.6	1.21 ±0.3	1.22 ±0.6

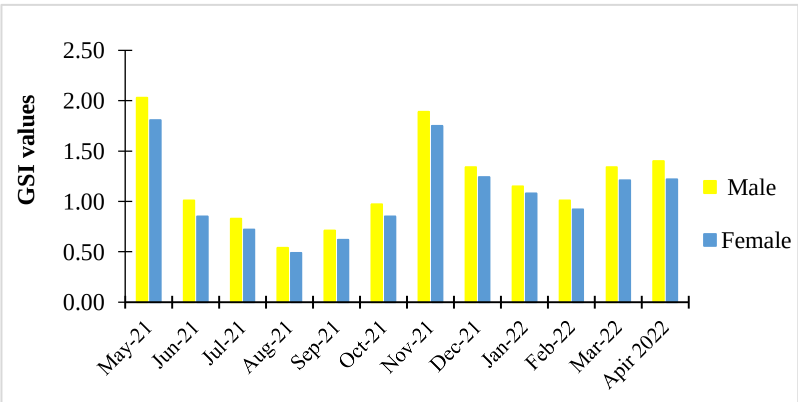

Figure 5. Monthly average GSI values of males and females of *Geloina expansa.*

Relation between Temperature and gonad maturity stages of *G. expansa* in Shwe Thaung Yan mangrove areas

The seasonal variations in water temperature exhibited a gradual increase from the beginning of the post-monsoon months to reach a peak at the end of the pre-monsoon season. A decrease in the temperature during the monsoon season into the post-monsoon season was evident (Fig 6 and Fig 7). At the Shwe Thaung Yan region, gametogenesis of *G. expansa* peaks when temperatures are lowest (Fig 6 and Fig 7). Thus temperature appears to affect reproductive patterns in this clam, at least for part of the year. Water temperature in the present study showed considerable variation with values of 25.5- 28.5 which influenced bivalve reproduction.

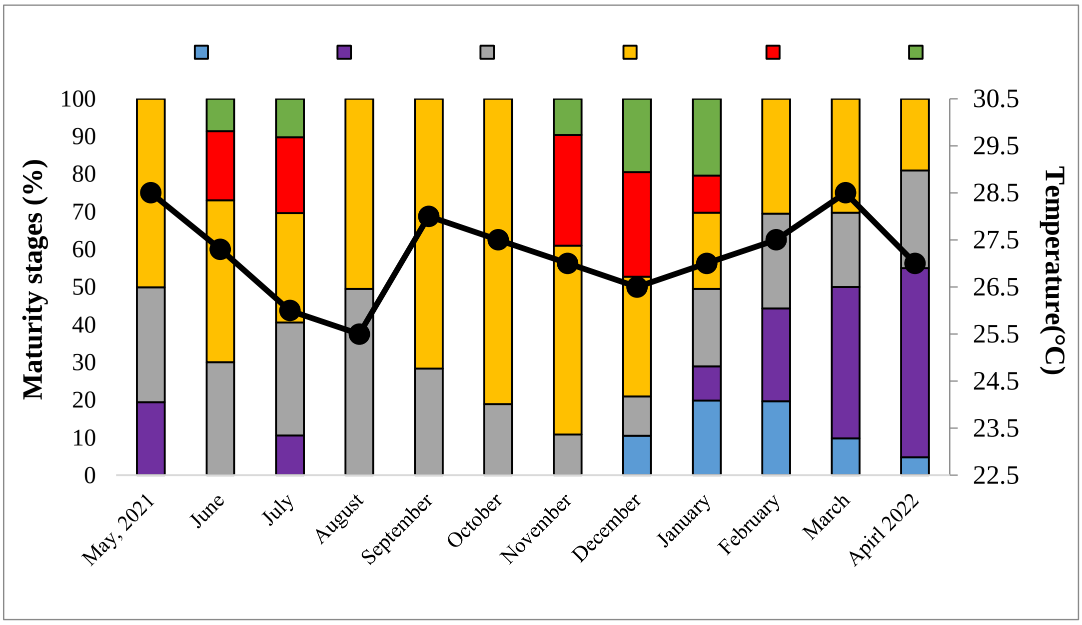

Figure 6. Relation between water temperature and gametogenesis of male *Geloina expansa* in Shwe Thaung Yan mangroves areas.

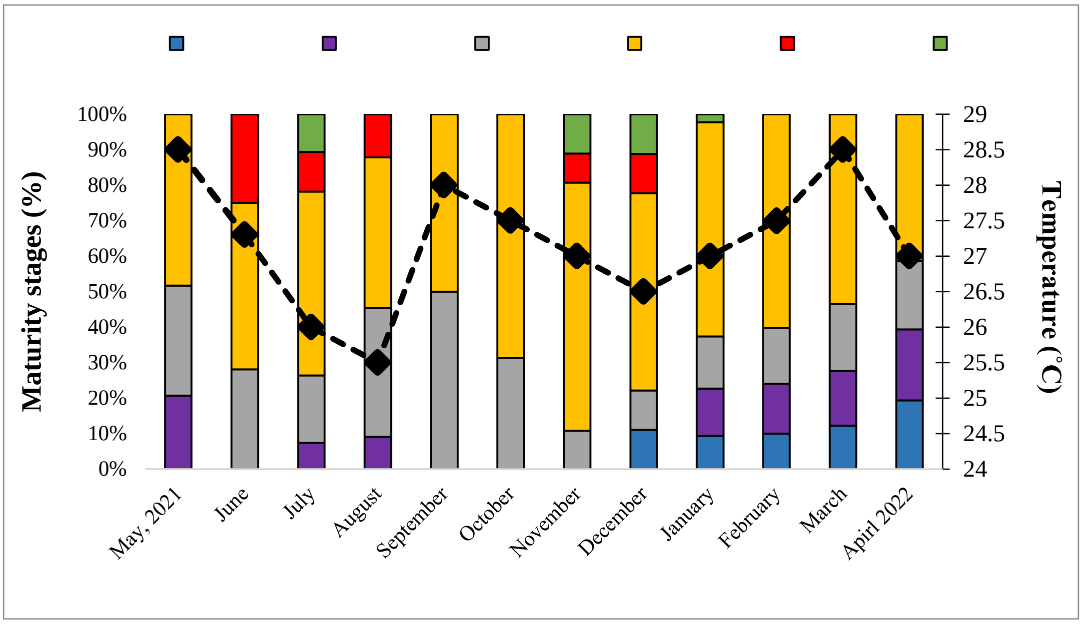

Figure 7. Relation between water temperature and gametogenesis of female *Geloina expansa* in Shwe Thaung Yan mangroves areas.

Discussion

The process of reproduction is the generation of new individuals that have the potential to become members of the population (Clemente and Ingole 2009). In the present study, the maturity stages of gonads were studied on a morphological basis as well as microscopically. Based on the microscopic examination followed by Gimin 2005, the maturity stages of *G. expansa* were recorded and categorized into six stages. From the results of the monthly percentage distribution of male maturity stages of *G. expansa*, most of the sampling months were predominated by maturing and ripe individuals.

In general, the *Geloina expansa* population from Shwe Thaung Yan mangrove areas was continuously breeder as confirmed by both GSI and histological data. However, the population showed some seasonality in spawning intensities. Continuous breeding with different spawning intensities is typical for tropical bivalves (Gimin, 2005). In the present study, the population in Shwe Thaung Yan mangrove areas had two major spawning periods i.e., a short breeding season in November and an extended season occurring between May, June, and July. This gametogenic pattern is similar to the *P. erosa* population in Hong Kong and Chareao island, Indian mangroves which is a seasonal breeder with an extended single breeding period (Morton, 1982, Clemente and Ingole 2007). Gimin (2005) suggested that the spawning season of *P. erosa* is restricted to around 4 months beginning from June to early October, with major events occurring during August-September. It is well known that the duration of the spawning period varies in a species occurring in different parts of its geographic range.

Idris *et al.* (2017) described that from March to November, the gonads were maturing and the spawning started. Gonads were observed to be spawned in September and continued throughout the year becoming less in percentage. They also stated that the spawning season of *P. erosa* in the mangrove site of Kelulit River, Miri, Sarawak, Malaysia was thus characterized by all-year-round spawning, although only in September spawning occurrence maximum frequency.

The gonadosomatic index (GSI) has been used in this study to follow the gametogenic cycle. This index increased before spawning due to the gametogenic development that produced an increase in the size of the gonad, which resulted in a rapid increase of the index throughout the spawning season. In the present study, the monthly gonadosomatic index value of *G. expansa* in the present study showed that it ranged from 0.54 to 2.02 in males and from 0.62 to 1.80 in females. The monthly average GSI values of males were always higher than those of females in all months.

According to Idris *et al*. (2017), the GSI showed a seasonal trend along the year, with high values related to mature individuals while the fall of values was due to spawning activity. In the present study, the GSI values for pooled *G. expansa* were higher in May and November and were lower in June, July, August, September, October, December, and January. Clemente and Ingole (2009) observed that the GSI values decreased from August to September due to the spawning period.

Temperature has been a major factor in controlling the reproductive cycle in various bivalves (Delgado and Camacho, 2005). Many bivalves require a minimum threshold temperature for activation of the oocyte growth phase and although oogonia can develop below this threshold level further differentiation only takes place at warmer temperatures. In temperate areas, temperature plays a very important part in triggering bivalve gametogenesis and spawning (Cross *et al*., 2012; Galimany *et al.*, 2015). At the Shwe Thaung Yan region, gametogenesis of *G. expansa* peaks when temperatures are lowest. Thus, temperature appears to affect reproductive patterns in this clam, at least for part of the year.

Besides that, the changes in water salinity affect a wide variety of biochemical, and physiological processes and growth in marine bivalves. In the present study, spawning occurred immediately when salinity dropped drastically in June 2021 despite large numbers of ripe individuals. Although spawning in November 2021 did not correspond to the lowest salinity, indicated that low salinity did not correspond with the spawning phase.

Conclusions

In the present study, some reproductive parameters such as the maturity stages with histological analysis, and gonadosomatic index (GSI) of *G. expansa* were observed from May 2021 to April 2022 in Shwe Thaung Yan mangrove areas. From the result of this study, it was concluded that *G. expansa* was recorded as two times spawning periods, the majority of spawning periods were recorded in the summer months of May, June, and July and the second spawning (minor spawning) took place in or around November. In addition to the monthly occurrence of GSI values, the peak GSI values were recorded in May and October for both males and females, thus it was the spawning period seemed to occur in June, July, and November. These results revealed that *Geloina expansa* is dioecious and gametogenic development was synchronous between the sexes. Temperature is the major factor in controlling the reproductive cycle in *G. expansa*.

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