SPAWNING PERIOD OF BLOOD COCKLE *TEGILLARCA GRANOSA* (LINNAEUS, 1758) IN MYEIK COASTAL AREAS

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Abstract

The spawning period of blood cockle *Tegillarca granosa* was studied by using the histological analysis of the gonads. Five maturity stages of gonads were noticed as the immature, developing, mature, spawning, and spent stages. The spawning period was from July to February with high in November and December. Males and females attain maturity in the same period. The observed mean length at first maturity was 29mm total length in males and 31mm in females. The number of females was predominant than males. Sexual dimorphism could not be noticed in species of *Tegillarca granosa.*

Keywords: blood cockle, histological analysis, maturity stages, *Tegillarca granosa*

Introduction

The blood cockle *Tegillarca granosa* (locally known as Gyin) is a commercially exploited bivalve species for human consumption. They belong to the Family Arcidae that is thick and solid shelled marine bivalves. It is commonly known as blood cockle because of the presence of haemoglobin that enables it to colonize habitats of low oxygen concentration (Poutiers 1998). The diagnostic characteristics of *T. granosa* is the presence of strong nodules on the radial ribs, about 18 radial ribs with wide interstices at both the left and the right shell valves, slightly longer shell than high and hinge area composed of small teeth (Souji and Radhakrishnan 2015). They are widely distributed in the Indo-West Pacific region and inhabit intertidal and sub-tidal mudflats areas and seaward of mangrove forests (Poutiers 1998).

Blood cockles are harvested from a wide population and caught as by hand or drag during the low tide from the mudflat. Nowadays, the sowing culture of blood cockle is very popular in Myeik coastal areas in which blood cockle spats obtained from the natural are used for seeds to sow culture. Therefore it is important to get a stable supply of spats for sustainable blood cockle aquaculture. Overexploitation will lead to diminishing the supply of natural spats. Thus, the understanding of the reproductive period of this species is essential for cultivation, management, and conservation strategy on their resources. The present study aimed to analyze and find out the maturation period and size at first maturity of blood cockle. It is also tend to fill the gap of the biological information in the literature about the blood cockle of Myeik coastal areas.

Materials and Methods

The monthly collections of blood cockles were made from the landing site of Myeik from January to December 2018 (Figure 1). Total length (maximum distance along with the anterior and posterior valves) and total weight were measured to the nearest 0.01mm using vernier calipers and to an accuracy of 0.01g by using the digital balance respectively. Identification of blood cockle was followed on the classification systems of Poutiers 1998, and Souji and Radhakrishnan 2015.

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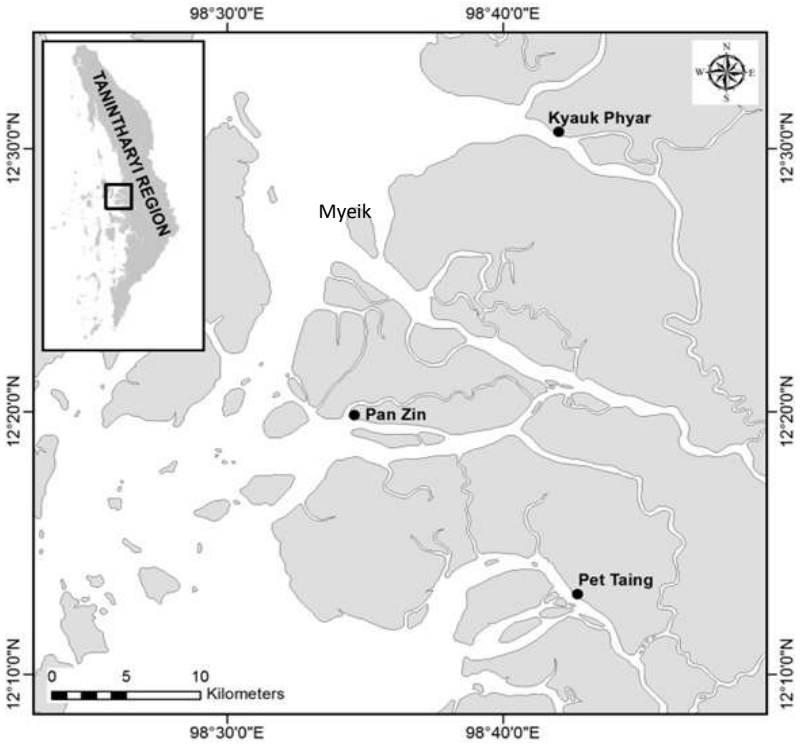

Figure 1. Map showing the sample collecting area

A total of 240 blood cockles samples with the range of 26-35mm shell length and 8.1-33 g shell weight were histologically examined for the determination of sex and stages of gonad maturity. For histological analysis, the whole soft tissues were removed from the shell and the flesh containing most parts of the gonads were fixed in formalin, dehydrated in upgraded series of ethanol, and cleared in xylene. After that the fixed tissue was embedded in paraffin, cut seven micrometer thick sections with a rotary microtome, stained using hematoxylin and eosin procedure, mounted on the microscopic glass side, and examined under the microscope. The classification of the gonad stages was followed by the systems used by Yurimoto *et al*. (2014). Five maturation stages of gonadal development: immature (Stage I), developing (Stage II), mature (Stage III), spawning (Stage IV), and spent (Stage V) stages were distinguished and presented in Table 1.

The sex of cockles was confirmed by the color of gonad and histological examination of the gonads. Sex ratio was tested for the expected ratio of 1:1 by chi-square (χ^2) analysis according to the formula:

$$\chi^2 = \frac{\sum(O-E)^2}{E}$$

where O= observed frequency of males or females, E= expected frequency of males or females

The length attains sexual maturity L50 was estimated by fitting the point where the total length of cockle (X-axis) and 50% level of maturity (Y-axis) are met.

To obtain a quantitative value that represents the reproductive activity, the gonad index was calculated as follows: GI= (sum of specimens number ascribed to each category*category score)/total number of cockles according to Ceballos-Vazquez *et al.* 2000 by using a numerical grading system. Three categories were assigned according to the development of the gonad, with 1=spawning and spent, 2= developing and 3= mature.

Table 1 Description and criteria for the gonadal stages of *Tegillarca granosa*

No.	Gonadal stages	Histological characteristics
1	Immature	Sex is indistinguishable in the gonads of immature stage and difficult to observe germ cells in the gonads. The wall is mainly occupied by connective tissue or an empty genital tube (Fig. 3A)
2	Developing	Male- Spermatogonia appear along the wall of the follicle wall. Spermatocytes and few spermatids can be seen. (Fig. 3B)
		Female- Oogonia appear along the wall of the follicle wall. Immature oocytes are attached to the tube wall. (Fig. 3F)
3	Mature	Male- Follicles are full of spermatozoa with their tails pointing towards the center of the tube. (Fig. 3C)
		Female- Follicles are full of mature oocytes that are irregular or polygonal shapes with the oval nucleus. (Fig. 3G)
4	Spawning	Male- Some spermatozoa are released that causing the empty space in the follicle wall. Many spermatozoa still remain in the genital tube. Some of the genital tubes are contracted and partially collapsed. (Fig. 3D)
		Female- Mature oocytes decrease in number causing the space in the follicle wall. Many oocytes with late-developing mature stages still remain in the genital tube. Mature oocytes exhibit nuclear disappearance because of germinal vesicle breakdown. (Fig. 3H)
5	Spent	Male- The genital tube is deformed and devoid of spermatocytes, which have completely spawned. Some spermatozoa still remain. (Fig. 3E)
		Female- The genital tube is deformed and degenerated. Much of the tube is empty. (Fig. 3I)

Results

The gonad is situated in the basal region of the body and envelops the dark green digestive gland. The morphology of the cockle gonad shows that the area of the gonad increases according to the increased levels of gonad maturity. The coloration of the gonad tissue layer in the blood cockle varies from orange-red to pale orange in females and from white to grayish-white in males for different maturity stages (Fig 2). The histological changes of the gonad in males and females during the reproductive cycle are illustrated in Figure 3.

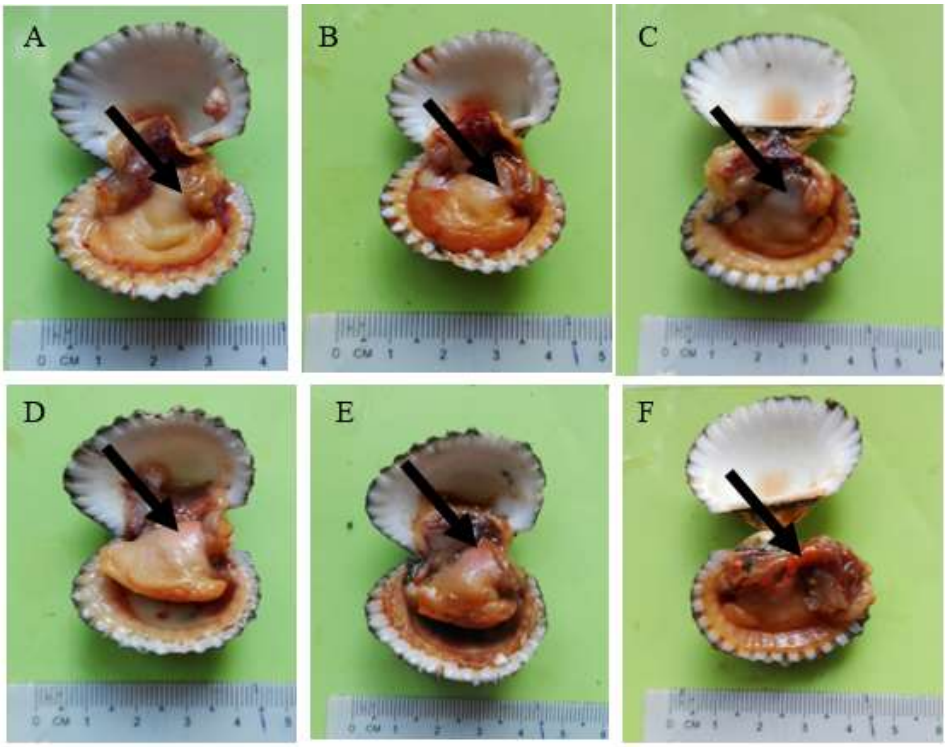

Figure 2. The coloration of *Tegillarca granosa* gonad morphology A-C) Males and D-F) Females

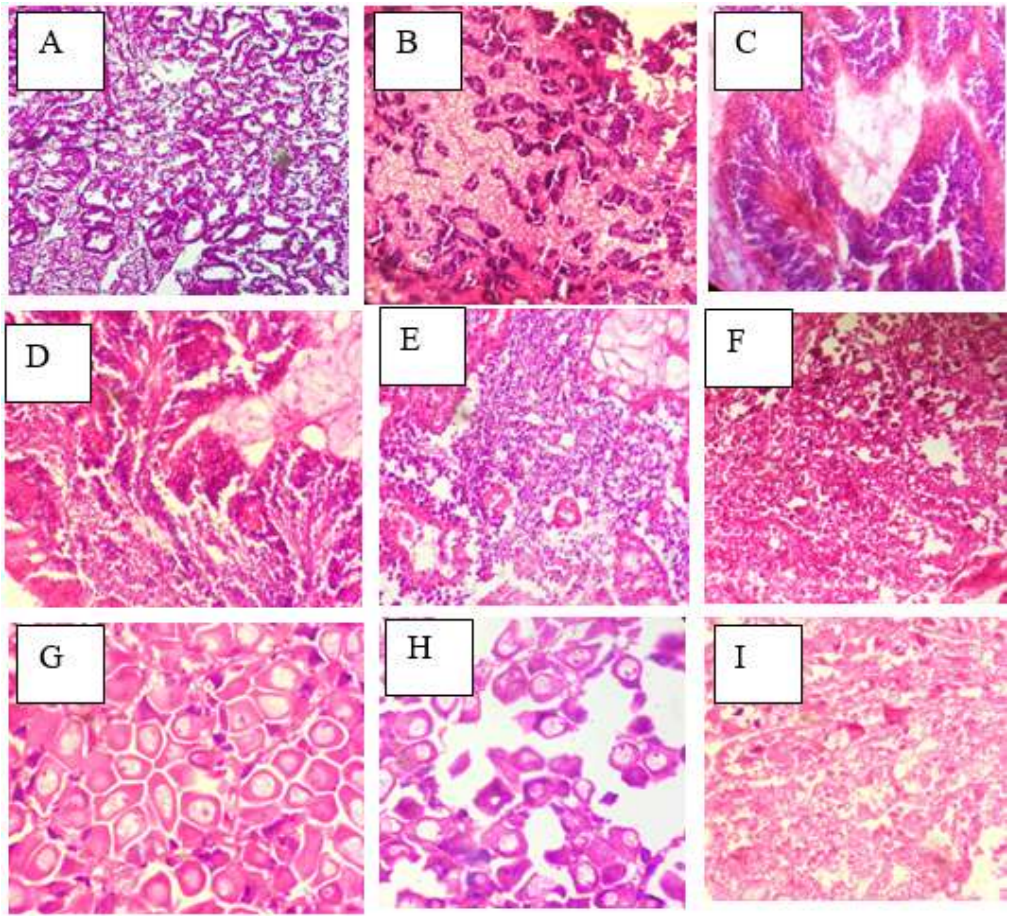

Figure 3 Gonadal stages of *Tegillarca granosa* A) Immature, B-E) Males and F-I) Females, (B, F) developing, (C, G) mature, (D, H) spawning and (E, I) spent, Scale bar=100µm

Occurrence of different maturity stages

The occurrence number and percentage of males and females in different maturity stages were recorded and described in Table 2 and Figure 4. Stage I (immature) occurred in all months except April, May, June, and September, comprising the range of 5% to 15% of all monthly samples. Immature cockles (Stage II) were recorded from January to June for males and from January to July for females. The percentage of immature males and females was high in March, April, and May. Males and females cockles with mature gonads (Stage III) were noticed from March to October. Percentages were varied from the lowest 5.5% (August) to the highest 40% (June) for mature males and from 10% (September) to 50% (June) for mature females. The spawning stage of males and females (Stage IV) occurred in January, February, and July to December. The highest percentage of spawning stage cockles were recorded in November and December, accounting for 44.4% and 47.3% for males and 38.9% and 42.1% for females respectively. The percentage of spent stage cockles (Stage V) was high in February and low in October for males and high in January and low in November and December for females. No spent stage of males and females were observed in April, May, June, and July.

Table 2 Monthly occurrence number of different maturity stages in *Tegillarca granosa*

Month	I	Males				Females			
		II	III	IV	V	II	III	IV	V
Jan	3	2		3	3	1		3	5
Feb	3	1		3	5	1		4	3
March	1	4	3		1	5	4		2
April		4	4			5	7		
May		5	6			3	6		
June		1	8			1	10		
July	2		4	3		2	6	3	
Aug	2		1	4	3		3	3	4
Sep			2	5	4		2	4	3
Oct	1		3	5	1		3	4	3
Nov	2			8	2			7	1
Dec	1			9	1			8	1

I: Immature, II: Developing, III: Mature, IV: Spawning, V: Spent

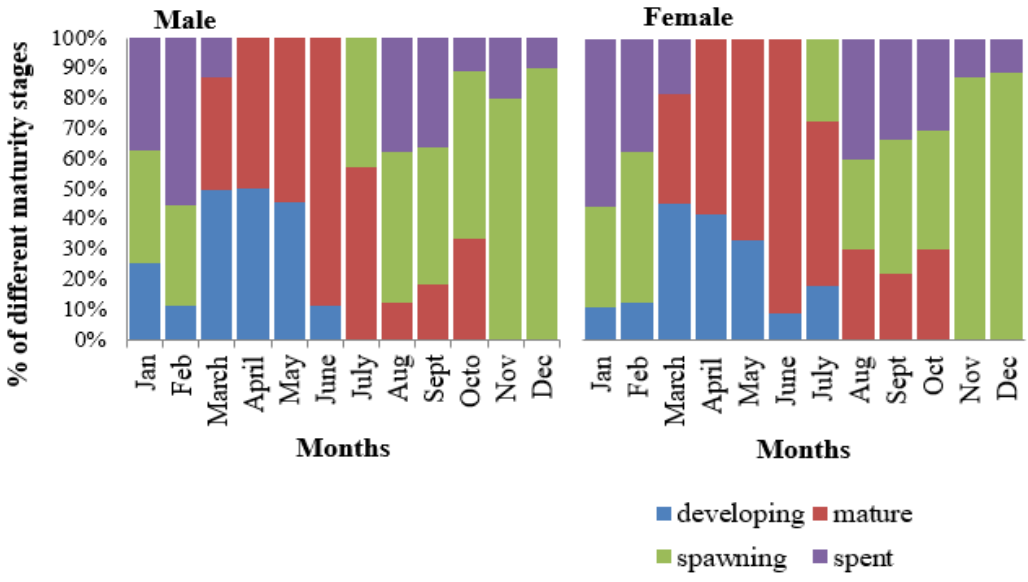

Figure 4 Percentage occurrence of different maturity stages in *Tegillarca granosa*

Length at first maturity

Examination of the percentage maturity stages of different size groups revealed that the percentage of mature cockles increased with the increase of length for both males and females. The percent of 28 in males and 25 in females were mature at 26-28 mm length, 55% males and 40% female at 28-30 mm length, 56.2% males and 57% females at 30-23 mm length, 65.5% males and 62.5% females at 32-34 mm length, 80% males and 78.2% females at 34-36 mm length, 87.5% males and 90.3% females at 36-38 mm length, 90% males and 97% females at 38-40 mm length and 100% for males and females at 40-42 mm length. Thus, the mean size group at first maturity (50%) was considered as 29 mm for males and 31 mm for females (Fig 5).

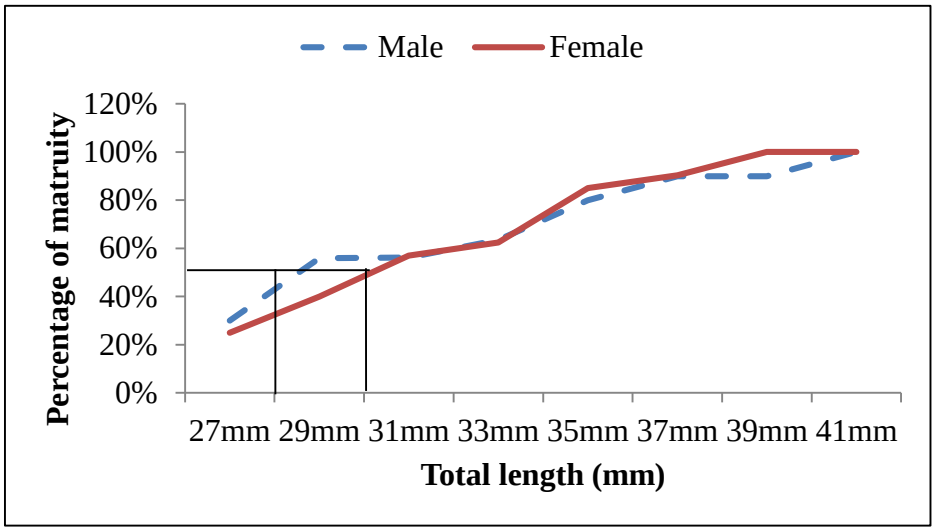

Figure 5 Length at first sexual maturity of males and females

Sex ratio

The sex was determined based on the gonad color and histological analysis of the gonad. The overall result of the sex ratio determined from the histological slides was 0.9 males: 1 female. Males were more abundant in January, March, April, June, July, August, and October (Table 3). The range of chi-square values (0.04 to 0.8) showed that there was no significant difference in males and females for all months from the expected 1: 1 ratio.

Table 3 The monthly sex ratio of *Tegillarca granosa*

Month	Male: Female	Chi-square (χ^2)	Month	Male: Female	Chi-square (χ^2)
Jan	0.9:1	0.06	July	0.6:1	0.8
Feb	1.1:1	0.06	Aug	0.8:1	0.2
March	0.7:1	0.5	Sep	1.2:1	0.2
April	0.7:1	0.8	Oct	0.9:1	0.04
May	1.2:1	0.2	Nov	1.25:1	0.2
June	0.8:1	0.2	Dec	1.1:1	0.04

Gonad index

Monthly quantitative assessments of histological reproductive condition (GI values) were varied from 1 to 2.8 for males and from 1 to 2.9 for females (Fig 6). High GI values occurred in March, April, May, and June coinciding with the occurrence of mature cockles. Low GI values were observed in November, December, January, and February, coinciding with the spawning activity. The average GI values obtained for males and females were 1.7 and 1.8 respectively.

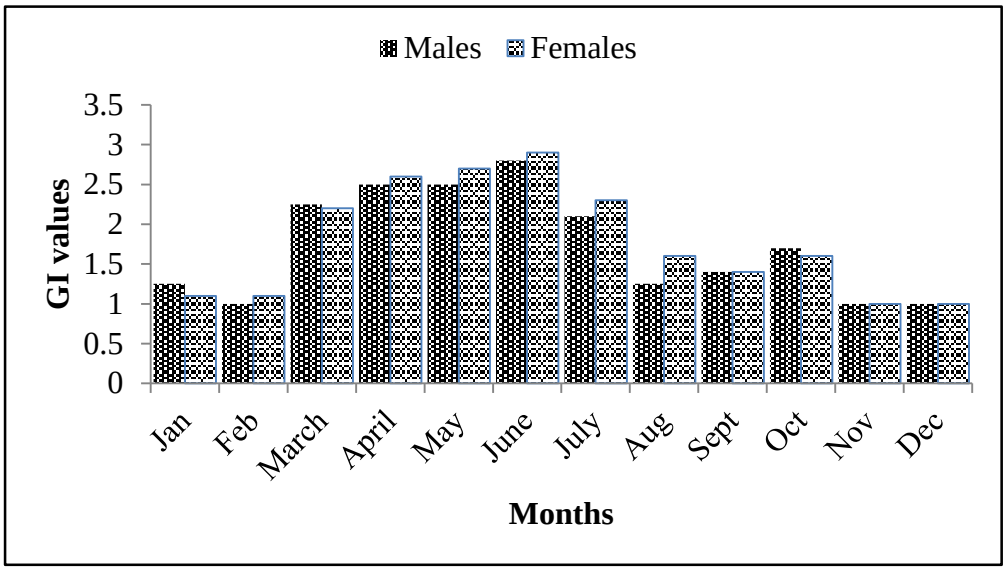

Figure 6 Monthly gonad index (GI) values of males and females *Tegillarca granosa*

Discussion

Tegillarca granosa (formerly known as *Anadara granosa*) belonged to the family Arcidae is a commercially valuable species due to human consumption and market demand for exportation. They are exploited from both natural populations and as a sowing culture. To sustain long-term exploitation and protection, the knowledge of the reproductive cycle of the blood cockle is essential. Gonad maturation and sexuality in the population of *Tegillarca granosa* can be studied through the macroscopic examination of visceral mass and microscopic examination of gonad by histological technique. The most precise results of the stage of the gonad can be obtained through histological examinations.

Little is known about the sex-determining mechanism of bivalves and mollusks in general. So far it is known that there are no morphologically distinguishable chromosomes (Afiati 2007). Different schemes of classifications of gonadal maturity are available in bivalves. There are five maturity stages of blood cockle gonad identified as immature, developing, mature, spawning, and spent stages in the present study. The identification of maturity stages was followed by Yurimoto *et al* (2014). Four stages of gametogenesis: developing, ripe, spawned out, resorbing were identified in male and female of *Anadara antiquata* from Pakistan by Jahangir *et al.* 2014.

During the present study, no hermaphrodites were found in any of the gonads of *Tegillarca granosa* section examined. There is no sexual dimorphism could be noticed in *Anadara senilis* from west African by Yankson (1982), *Anadara rhombea* from Porto Novo coast by Natarajan and John (1983), in *Anadara antiquatea* from Philippine by Toral-Barza and Gomez (1985), and *Anadara antiquata* from the northern Arabian Sea by Jahangir *et al.* (2014). But some hermaphrodite individuals were encountered in *Anadara granosa* from central Java, Indonesia (Brotohadikusumo 1994). Afiati (2007) also reported that hermaphrodites occurred in *Anadara granosa* and *A. antiquatea* with a percentage of 1.43% and 1.45% in the specimens of Central Java respectively.

Based on histological analysis of gonad and observed GI values, spawning stages of males and females cockles were observed from July to February with a peak in November and December during the present study. Yurimoto *et al.* (2014) stated that the spawning season of the blood cockle in the tropical region is very long. Different spawning period of the blood cockle from the different regions was reported by various authors. Spawning of cockle *Anadara antiquata* occurred

continuously throughout the year, with peak spawning from July to September (Toral-Barza and Gomez 1985). According to the four-year study on the spawning behavior of *Anadara granosa* in Kakinada bay, Narasimham (1988) described that the blood clam spawns throughout the year and their major spawning months vary between years. Muthiah 2004 also stated that *Anadara granosa* breeds throughout the year with peak spawning from January to April in Malaysia. Suwanjarat *et al.* (2009) stated the breeding season of *Anadara granosa* in Pattani Bay was mainly from July to August.

The present result of the spawning period that occurred in blood cockle *Tegillarca granosa* from Myeik areas was nearly similar to the report of Khalil (2013), Yurimoto *et al.* (2014), Jahangir *et al.* (2014), and Saputra *et al.* (2019). Khalil (2013) reported that the spawning period of blood cockle *Anadara granosa* was continuous throughout the year in the northern Straits of Malacca, peak spawning from October until January. Yurimoto *et al.* (2014) reported that the spawning period of *Anadara granosa* along the Selangor coast, Peninsular Malaysia was from November to February. Jahangir *et al.* (2014) and Saputra *et al.* (2019) also stated the dominant of the spawning stage of blood cockle in December.

Determination on first maturity length is the basic requirement for the protection and sustainable exploitation of the stock. *Tegillarca granosa* mature first at a mean length group of 29 mm in males and 31 mm in females in the present study. The observed size at first maturity of the present study was smaller than that of Saputra *et al.* (2019) in which the estimated length at first maturity of blood cockle from Banjar Kemuning river as 35.73mm in males and 37.21mm in females and larger than that of Narasimham (1988) in which male cockles from Kakinada bay attain the first maturity at 20mm and female at 24mm in length.

Sex ratio studies show information on the proportion of male to female fish in a population and are expected to be 1: 1 in nature. Any deviation from this ratio may indicate the dominance of one sex over the other. Pathansali and Soong 1958 reported that the equal sex number of *Anadara granosa* in the culture of Malaysia. The present study showed that the occurrence number of females was high than males. However, the analysis of the chi-square method showed that there was no significant difference at the 5% probability level. So, it can be concluded that the sex ratio of male and female cockle based on the one year study period is in a balanced condition. Females were also dominant in the population of blood cockle *Anadara inaequivalvis* in the southeastern Black Sea Coast (Sahin *et al.* 2006) and the population of *Anadara antiquatea* from the northern Arabian Sea (Jahangir *et al.* 2014). However, Natarajan and John (1983) and Brotohadikusumo (1994) indicated the predominance of males in the population of *Anadara rhombea* from the backwaters of Porto Novo and the population of *Anadara granosa* from Indonesia respectively.

Conclusion

According to the histological observation on the gonad, it would be concluded that the spawning period of *Tegillarca granosa* was high in November and December. Thus, intensive harvesting of blood cockles should be avoided during this time to protect the production of spat and to fulfill the demand for human consumption from year to year. Yet the present result still provides important biological information to produce the artificial blood cockle spat. More studies are still needed to do research on breeding biology for the long term period and to assess the stock of this area for successful aquaculture and management.

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