Aspects of reproduction biology of blood cockle (*Anadara granosa*) in Pasir Limau Kapas waters

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Abstract. Blood cockle (*Anadara granosa*), has important economic value and in various regions, caught, consumed and exploited commercially. The bivalve has been cultivated in some parts of intertidal areas of Pasir Limau Kapas waters, Riau, Indonesia, since 2015. This work studied reproduction biology aspects of blood cockle in the Pasir Limau Kapas waters. Samples were collected, counted, measured (length, width and thickness). The sex and the gonads were examined macroscopically and microscopically, included sex ratio, gonad maturity, gonadosomatic index, fecundity and oocyte diameter. We found that sex ratio fluctuated monthly, with an average ratio of 1:0.95. Three gonad maturity levels were noted, included the developping (immature), the maturing (mature), and the spent (spawned). The gonadosomatic index ranged from 3.76 to 11.61 % and varied monthly, although not significantly different. Fecundity ranged from 186.687 to 2.363.843 eggs per female. The diameter of oocyte fluctuated monthly, and ranged from 60 to 105 μm. It appeared that biological reproduction aspects of blood cockle in this area were in normal state. The cockles grow, lay eggs and spawn throughout the year, meaning that they can be harvested regardless of time. However, the precise calculation on sustainable catch is not fully studied yet, and this data is critically required.
Key Words: fecundity, gonadosomatic index, sex ratio, shellfish.

Introduction. Blood cockle (*Anadara granosa*), also known as blood clam (*Tegillarca granosa*) spreads in the Indo-Pacific region, from Africa to Australia, Polynesia and Japan. They live mainly in the intertidal zone of the sea to a depth of two meters, immersing themselves in sand or mud (Donadi et al 2014; Clarke & Tully 2014). This invertebrate has important economic value as they have high protein content and are consumed by all levels of society. These animals are hunted and exploited in large number by fishermen due to increased demand (Troost et al 2010).

Pasir Limau Kapas waters is one of potential area for blood cockle farming in Riau Province, Indonesia. Along with the increasing demand on the market, it has led to exploitation of these shellfish resources, which tends to undermine the principles of sustainable exploitation and conservation of natural resources (Yulinda et al 2020). Fortunately, during the last five years, the local community have been cultivating the animals by spreading seedlings of blood cockles in intertidal areas controlled shallow ponds (Riza et al 2021). The animals are left for about six months and then harvested. The activity turned out to have provided benefits for the community. To support the conservation of these blood cockles resources, it is necessary to learn basic information about their reproductive biology to set a proper exploitation management. Some researchers have studied some biologic aspects of cockles reproduction.

Afiati (2007) compared gonad maturation of two intertidal blood clams *Anadara granosa* (L.) and *Anadara antiquata* (L.) in Central Java. Ferreira et al (2006) reported morphological and morphometric aspects of *Crassostrea rhizophorae* oocytes. Herrmann et al (2009) studied reproductive cycle and gonad development of the Argentinean *Mesodesma mactroides*. While others (Sahin et al 2006) reported seasonal variations in condition index and gonadal development of the introduced blood cockle *Anadara inaequivalvis* in the Southeastern Black Sea Coast. This work aimed to study reproductive biological aspects (sex ratio, gonad maturity index, and fecundity) and management of blood cockle live stock and farm in the coastal waters of Pasir Limau Kapas, Riau Province, Indonesia.

Material and Method

Sample collection. This research was conducted in intertidal zone of Pasir Limau Kapas waters, Riau Province, Indonesia. Wild blood cockles were collected every month, from February to July 2020, by hand and by using a rake. All individuals were counted, measured (length, width and thickness) using calipers and weighed with analytical scales. The sex and the gonad maturity were determined in the laboratory both macroscopically and microscopically.

Sex ratio. A number of 100 shells were collected for the sex ratio determination. All reproductive organs samples were observed by opening the blood cockle shell. Blood cockles gonads were observed visually and microscopically by smearing gonadal tissue on the slide and observed under a microscope. Furthermore, the sex of the animals were classified as male, female, and indistinguishable.

Gonad maturity. The gonad maturity stage more accurately was determined by carrying out a histological observation. A total of 10 individual shellfish were dissected for soft tissue removal (including gonads, digestive tracts and legs). The samples were then fixed in 10% formalin fixative solution for 24 hours, and continued with 4% formalin fixative solution. Then the samples were dehydrated with multilevel concentration ethanol, clarified with xylol, blocked in paraffin and cut with 6–8 µm thick microtome, then stained with hematoxylin and eosin. The resulting preparations were observed under a microscope by assigning 30 selected follicles.

Gonadosomatic index (GSI). A total of 30 individuals were weighed (gonad and meat weight) every month to calculate the gonadosomatic index (GSI) which will be used to estimate the spawning season. Determination of the GSI was based on the criteria stated by Sahin et al (2006).

Fecundity and oocyte diameter. Fecundity was examined monthly by counting the number of eggs in every sample. As many as 15 individuals were dissected and their fecundity was determined. The oocyte diameter was also measured by using an ocular micrometre in a binocular microscope. A number of 30 oocyte per gonad were examined.

Results and Discussions

Sex ratio and gonad condition. A total of 448 individual blood cockles were examined during the study. The shell lengths ranged from 16.5 to 41.9 mm. The colour of the male gonad was milky white and the female blood cockle was orange. The colour was more clearly seen as the blood cockle gonad stage increased. Determination of sex was processed through a visual observation of the gonads easily when the shells are matured gonads. Determination of sex in relatively young shells sometimes required an observation under a microscope.

It was noted that male and female gonads were different individuals (dioecious). Afiati (2007) stated that *A. granosa* male gonads were smooth, white to semi-transparent, while females gonads were fine-grained and reddish orange colour. According to Mzighani (2005), the mature female gonad is bright orange, while the male is white. Nabuab and del Norte-Campos (2006) stated that male and female gonads of *Gari elongata* shells were creamy.

Microscopic observations were carried out by placing gonadal tissue to the glass object (smear) and observed under a microscope. No hermaphrodite blood cockle was scored. Shells that have developed their gonads can be sexually distinguished microscopically. Male and female gonads blood cockle are in different individuals (dioecious). This is consistent with what was stated by Limpanont (2010) and Yang et al (2011), that most bivalves are gonochoristic.

Genital or sex ratio is the ratio between the number of males and females in a certain location. Comparison of males and females largely determines the success of fertilization by males against eggs revealed by females. The sex ratio of male and female blood cockle in Pasir Limau Kapas waters fluctuated every month, with an average of 1:0.95 (Table 1).

Table 1

Blood cockle genital or sex ratio per month in Pasir Limau Kapas waters

Months	*Male*	*Female*	*Sex Ratio*
February	48	41	1:0.85
March	43	32	1:0.74
April	45	24	1:0.53
May	40	40	1:1.00
June	27	33	1:1.22
July	26	48	1:1.84
Average	229	218	1:0.95

In this study, male and female genital ratios in the area was considered balanced. Balanced sex ratio (1:1) was also determined by Natan (2008) in mud shells (*Anodontia edentula*) in the inner Ambon Bay. Similar result also reported by Alexandre et al (2005) in razor clam (*Siliqua patula*). Sahin et al (2006), determined *Anadara inaequivalvis* sex ratio was 1:1.04. Flores and Licandeo (2010) studied sex ratio of a subpopulation of the mangrove blood cockles *Anadara tuberculosa* and *Anadara similis* from natural beds in the Cayapas-Mataje Ecological Mangrove Reserve, Ecuador. These authors reported that the sex ratio of *A. tuberculosa* and *A. similis*, regardless of month during the study, was dominated by females. Overall, the sex ratio (female:male) was 2.6:1. However, there is still variation in the monthly sample, which was between 1.2-5.9:1 for *A. tuberculosa* and between 1.9-5.3:1 for *A. similis*. Serdar et al (2010) showed that the sex ratio of male and female clam *Tapes decussatus* was 1:1.06 and 1:1.10 for inside and outside part of the lagoon, respectively. Nabuab and del Norte-Campos (2006) obtained 1:1.04 sex ratio of *Gari elongate* shells in the Banate Bay Area, Philippines. This sex ratio determines the survival of a population. If the number of males is very small, the possibility of sperm cells to fertilize eggs is getting smaller, and many eggs in the water column are not fertilized. The blood cockle genital ratio in Pasir Limau Kapas waters is currently considered normal.

Gonad maturity. The observations of the blood cockle gonad maturity stage (60 samples) referred to Sahin et al (2006). Three stages of gonad maturity were noted in this study, including: the developping (immature) stage, maturing stage (mature) and spent (spawned) stage. The developing (immature) stage was marked by the condition of diameter of larger follicles and all follicles were filled with immature oocyte (Figure 1a, Figure 1b). The maturing stage (mature) was characterized by larger follicles and all follicles were filled with mature oocytes (Figure 2a, Figure 2b). The spent (spawned) stage is the stage where the follicles have lost their structure (Figure 3a, Figure 3b). Jahangir et al (2014) reported four stages of gonad maturity in *A. antiquata* shells in Pakistan, namely 1) developing, 2) ripe, 3) spawned out, and 4) reabsorbed.

From histological preparations we observed that there were no stages of spawning (full spent) and resting (resting). This is thought to be related to the nature of the blood cockle spawning, in which these animals release only part of their eggs or sperm each time they spawn. Some stages of oocytes or spermatogonia, in different portions of all the existing follicles were observerd. This is also evidence that blood cockles do not release oocytes or sperm all at once (full spent), but these were released gradually (partially spawned). The same result was also reported by Brien and Keegan (2004) in the *Abra alba* shells, that at all stages of gonad maturity contains some different stages of oocytes.

Gonadosomatic index (GSI). The average of GSI of blood cockles during the study ranged from 3.76 to 11.61% (Table 2). GSI varied every month, although not significantly different. The average GSI was rather low in March, higher in April and down again in June and the following month. Based on this data, it is tempting to speculate that blood cockles spawned throughout the year. When the shells have spawned, the gonad's volume is reduced, because the content of gonads are mostly immature eggs or sperm. The increase in spawning activity in certain months could have happened incidentally, namely the influence of climate at that time, however it would not necessarily take place in the same month in the following year.

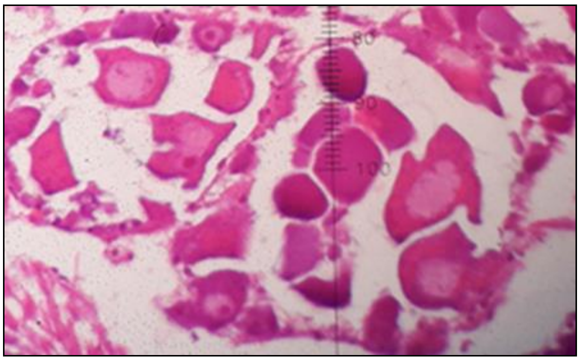

Figure 1a. Female. Developing (immature): diameter of follicles increased and all follicles were filled with oocytes.

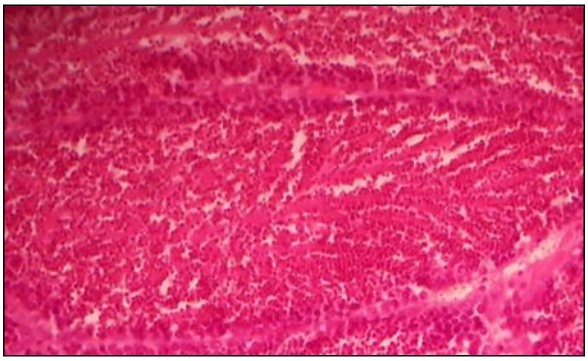

Figure 1b. Male. Developing (immature): groups of spermatozoid were formulated and connecting tissues were reduced.

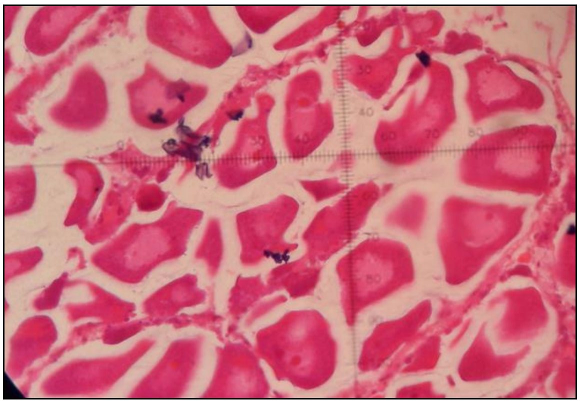

Figure 2a. Female. Maturing (mature): follicles increased and all were filled with mature oocyte.

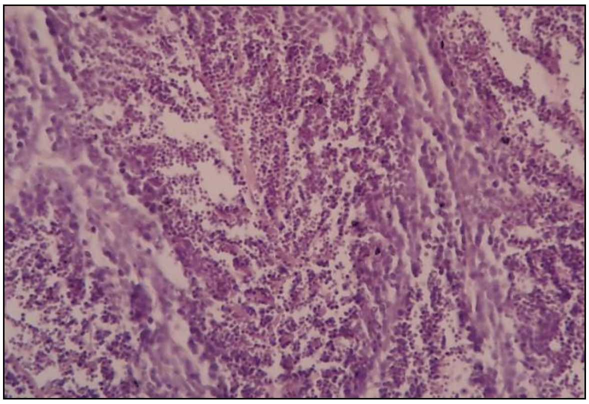

Figure 2b. Male. Developing (immature): groups of spermatozoid were formulated and connecting tissues reduced.

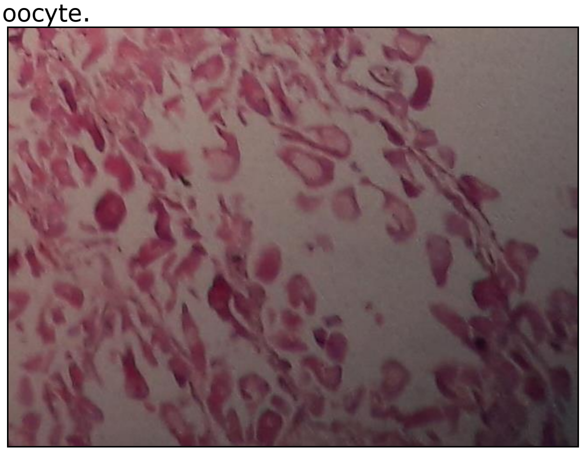

Figure 3a. Female. Spent (spawn) : the follicle begins to lose its structure.

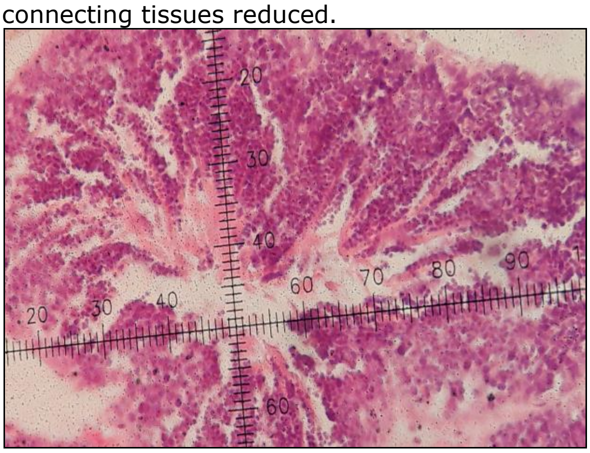

Figure 3b. Male. Spent (spawn): follicular wall thinning, the structure begins to form and the number of spermatozoa increased.

Table 2

Blood cockle average GSI for each month in Pasir Limau Kapas waters

Months	*GSI (%)*		
	Average	*Female*	*Male*
February	4.00	3.40	5.23
March	4.25	3.76	5.65
April	9.04	10.06	7.80
May	5.91	5.25	6.27
June	9.93	8.37	11.61
July	6.94	6.14	7.41

GSI changes according to the level of gonad maturity and reaches a peak at a moment of spawning. Therefore, it can be used to determine the spawning season. The GSI of each species of shell varied according to the number of the fecundity and the egg diameter of a shell. The higher the fecundity and egg diameter the higher the GSI of the clam. From Table 2, in general it can be seen that the male GSI was relatively higher than females. Similar result was reported by Natan (2008) who obtained an average GSI of *A. edentula* mud shell of 33.22%. In addition he found that the female GSI was higher than the male. The GSI fluctuated during the 6 months of the study. Clemente and Ingole (2009) reported that GSI of *Polymesoda erosa* shells were 20–22% when the gonads were matured. Nabuab and del Norte-Campos (2006) reported a 26.74–37.92% male and female 27.91–37.24% GSI of elongata sunset clam, *Gari elongate* in the Banate Bay Area, Philippines. They stated that based on the GSI level, it can be concluded that spawning takes place throughout the year.

Gonadal maturation analysis in shellfish is critical in the aquacultural industry of noble scallops (Sigang et al 2021). GSI studies show that gonad maturity is influenced by some environmental parameters. According to Araujo and Nunes (2006), temperature and salinity have a close correlation with the reproductive aspects of bivalves. Laudien et al (2001) stated that the reproductive cycle of *Donax serra* is related to sea surface temperature every year. McKindsey et al (2007) mentioned that the increase in GSI of *Argopecten irradians* reaches the peak of gonad maturity followed by optimum exogenous factors such as temperature and food availability in waters.

Fecundity and egg diameter. Blood cockle fecundity during the study ranged from 186.687 to 2.363.843 eggs per female, with a body total length of 23.7–44.1 mm. This figure was lower than *Anadara* shellfish reported by Mzighani (2005), who recorded that the average *A. antiquata* fecundity was 1.652.000 ± 562.000 eggs per individual female. The number of mature eggs ranged from 549.001 to 5.756.211 eggs per individual female of small size (22.67 mm long) and of large size of (69.01 mm long). This author added that fecundity increased with increasing of shell length. The number of eggs produced has a very important meaning to every species, as many external fertilization results are lost due to predation, pollution and other environmental factors (Tenjing 2017; Malik et al 2018). Litaay and De Silva (2003), reported fecundity has a linear relationship with the length and weight of the abalone. Abalone *Haliotis rubra* L. fecundity ranged from 1.09 to 7.5 million eggs per individual with a length of 12–14.5 cm and a weight of 115–487 g.

The diameter of the egg or oocyte of aquatic biota will reach its maximum size when it is mature or ready to spawn. Therefore, by studying the development of the oocyte diameter size of these shells for one year, the spawning season can be predicted. The diameter of blood cockle oocytes obtained during the study ranged from 60 to 105 µm, with an average ranging from 72–95 µm. The result of this study was higher than that of the mature *Crassostrea rhizophorae* oocyte diameter of 35.27 µm (Ferreira et al 2006). Gribben (2005) reported the size of oocyte razor clam (*Zenatia acinases*) of 5–60 µm. Based on egg production and egg diameter, as stated by Pina et al (2009), the blood cockle larvaes is planktotrophic, and this is commonly found in bivalves.

The average diameter of oocytes during the study fluctuated every month. In a month where the egg size was smaller it is estimated that many shells spawned, so the remaining oocytes in the gonad were mostly immature oocytes. Mostly eggs were at low development stages with smaller diameters. In the months with a high average oocyte diameter, many blood cockles were in the mature stage. Whereas when the average diameter of oocyte is lower, it is expected that they generally are immature or spawned. The shells spawn by partially removing their eggs (partially spawned). Herrman et al (2009) reported differences in oocyte diameters according to the stage of gonad maturity. The average diameter of *Mesodesma mactroides* oocytes in the active stage was 27.83 μm, mature 33.61 μm and spawning 32.37 μm.

Sex ratio, GSI, fecundity, and oocyte diameter are parameters that can be used to analyze and take decisions in managing aquatic resources in certain areas (McKindsey et al 2007; Bantoto-Kinamot 2016). In this study it appeared that these indicators were still in a normal state. This can mean that in Pasir Limau Kapas waters, blood cockles grow, lay eggs and spawn throughout the year. This data can be interpreted that the blood cockle can be harvested regardless of time. However, the precise calculation on sustainable catch of the blood cockle is not fully studied yet, and of course this data is critically required.

Conclusions. In Pasir Limau Kapas waters, the sex ratio (male and female) of blood cockle is considered to be balanced (1:0.95). The average gonadosomatic index (GSI) during the study ranged from 3.76 to 11.61%, rather declined in March, inclined in April and down again in June. Fecundity ranged from 186.687 to 2.363.843 eggs, with a length of between 23.7–44.1 mm. Female gonad colour was orange, while the male was milky white. The diameter of oocytes fluctuated monthly and ranged from 60 to 105 μm. Judging from the sex ratio, GSI and fecundity, and oocyte diameter it can be interpreted that blood cockles spawn throughout the year and can be harvested any time. However, precise calculation on carrying capacity of this waters must be carried out in a further study.

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Conflict of Interest. The authors declare that there is no conflict of interest.

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