AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Gonadal histological characteristics of mud clam (*Geloina erosa*) in the estuary of Reuleung River, Aceh Besar District, Indonesia

[1]Muhammad A. Sarong, [1]Asiah M. Daud, [1]Wardiah Wardiah,
[2]Irma Dewiyanti, [2]Zainal A. Muchlisin

[1] Department of Biology, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia; [2] Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia. Corresponding author: M. A. Sarong, ali_sarong@yahoo.com

Abstract. The objective of the present study was to evaluate the characteristics of mud clam ovaries (*Geloina erosa*) harvested from estuary areas of Reuleung River, Aceh Besar District, Indonesia. The sampling was conducted three times in June, July and August 2014. The clams were measured and weighed for cohort analysis using FISAT software and ovaries of a representative of each group length class was taken randomly for histological analysis. A total of 261 clams were sampled during the study which resulted in one cohort and two groups of shell-length classes i.e. 36.37– 49.09 mm (group A) and 49.09–61.81 mm (group B). Most of the oocytes in group A were at a late developing stage, while mature oocytes were predominant in group B, indicating that bigger clam have higher gonadal development stage. The ovaries of every group had several oocytes development stages, indicating anasyncronous spawner.
Key Words: histology, gonad, cohorts, estuary, Leupung.

Introduction. *Gelonia erosa* is one type of mud clam inhabiting the mangrove forests in tropical and subtropical regions. The clam has oval and dome-shaped shells (Nuryanto & Susanto 2010). The inner part of the shells are white and covered by thick periostracum. The periostracum is yellow with green spots in young spat and dark brown in adult clams (Dwiono 2003). Somatic and reproductive growth occurs from larvae to adulthood (Abdul Rahim et al 2012), and gonado maturity is determined based on the increase in the length of the shells, the total mass, and the age (Widhowati et al 2006).

The mangrove ecosystem of Reuleung River, Aceh Province Indonesia is a home for mud clam (*G. erosa*) populations (Sarong et al 2007). This species has economic value as a protein source for the local people. As a result, this species has been exploited intensely, leading to its population decreasing in the wild (personal communication with fishermen leader of Leupung Subdistrict).

The reproductive behavior plays an important role in the continuity of the population, and therefore, understanding the reproductive biology is central to providing basic information in order to plan a better conservation strategy for *G. erosa*. However, the reproduction cycle of this species is not well understood. Thus, characterization of *G. erosa* gonad is necessary to contribute a basic knowledge of the reproductive biology of this species.

In general, the life cycle of the clam is comprised of four stages i.e. throchophore (larvae), juvenile, spat (young clam) and adult clam. The *G. erosa* is a sexual dimorphism animal (Clemente & Ingole 2009), but the sex of larvae and juvenile clams are difficult to distinguish (Ngo 2010), because no external organ differentiation between male and female and therefore the sex is usually distinguished by the morphological appearance and colour of the gonads (Clemente & Ingole 2009) as the gonadal structure changes as the body and age increase. However, in young spats and adult clams the sex

differences between males and females are clearly distinguishable by the developed gonad characteristics (Widhowati et al 2006).

Sarong et al (2007) reported that the *G. erosa* population in estuaries of the Reuleung Leupung River are grouped into one cohort but vary in length of shells. However, the histological structure of gonads for every length class have not been described previously. Several studies on clams and fish show that the histological structure of gonads vary with gonadal development stages (Widowati 2005; Muchlisin et al 2010; Muchlisin et al 2011). Every stage consists of a nucleus, connective tissues, follicle inter-spaces, oogonia, oocytes and gonad follicles (Widhowati et al 2006; Morton 1988). Hence, the objective of the present study was to examine the characteristics of the cohort histologically according to the composition of oocytes size.

Material and Method

Sampling site. The *G. erosa* samples were collected in an estuary of Reuleung River, Leupung Subdistrict, Aceh Province, Indonesia on June, July, and August 2014. The collected samples were preserved in crushed ice (4^{o}C) and then transported to the Laboratory of Zoology at Syiah Kuala University for further analysis.

Cohort analysis. The *G. erosa* samples were measured and weighed for length of shell, width of shell and total body weight. Based on the minimum and maximum lengths of sampled clams, the cohort was divided into four classes i.e. 35 mm, 44 mm, 53 mm and 57 mm. The data of shell length were analyzed using FISAT software to determine the cohorts.

Histological preparation and gonadal development analysis. A representative of each cohort was chosen randomly for histological examination. The shells were opened and the gonads were taken using tweezers then the gonad was weighed to the nearest gram using a digital balance (Toledo, AB-204, error = 0.01 g). A small piece of each gonad (approximately 1 cm) was preserved in a 10% formaldehyde and 1% gluteraldehyde fixative for 48 hours. After 48 hours, the gonad samples were dehydrated by an ethanol series and soaked in xylene and wax. Next, the samples were embedded in glycol methacrylate plastic and sectioned on a microtome. The 2-3 micron sections were moved on a slide then was kept on the oven for 24 hours. After 24 hours, the sample was stained with Ehrlich hematoxilline and eosine, and observed by a compound light microscope (Muchlisin et al 2010).

Approximately 40-50% of the follicles were selected randomly and then recorded and measured for oocytes size and appearance. The different types of oocytes were grouped into three stages i.e. early growing oocyte, late growing oocyte, and mature (ripe) oocyte (Clemente & Ingole 2009). The early growing stage contains abundant, round alveoli with very little interalveolar space. The follicles vary in form and contain empty lumens. The late growing oocytes are recognizable by their increased size of follicles and decreased size of lumen. The prominent oocytes can be seen attached to the inside of the follicle wall or attached to the wall. The mature or ripe oocytes have reached their maximun size. They are spherical and unattached to the follicle packed inside the lumen (Clemente & Ingole 2009).

Results and Discussion. A total of 108, 80 and 80 individual mud clams were collected during the sampling in June, July and August 2014, respectively, belonging to one size class. The class was determined by 103 clams with a shell length of 34.9 mm to 57.84 mm (46.37±11.47 average) in June, and from 56 clams with a shell length of 49.25 mm and 67.17 mm (58.21± 8.96 average) in July, and from 74 clams with a shell length of 29.18 mm to 50.90 mm (40.04±11.86 evarage) in August 2014.

The study revealed that only one size class of mud clam was found in the Reuleung River from June to August 2014. The mean value of the size class was 49.09±12.72 mm with two ranges of shell length, namely 36.37–49.09 mm (group A) and 49.09–61.81 mm (group B) (Figure 1). A previous study in 2009 by Sarong (2010)

recorded six cohorts of *G. erosa* in this river. Therefore, the number of cohorts has declined significantly during the last four years. Setyobudiandi et al (2004) recorded five cohorts of lamis clams (*Meatrix meatrix*) in Marunda waters, Jakarta where most clams in the 32.08 mm shell length group. In additon, Bakhtiar et al (2008) recorded four cohorts of pokea clams (*Batissa violacea celebensis*) in the Pohara River in Southeast Sulawesi. Therefore, the number of cohorts of mud clams in the Reuleung River is very low compared to the other locations described above.

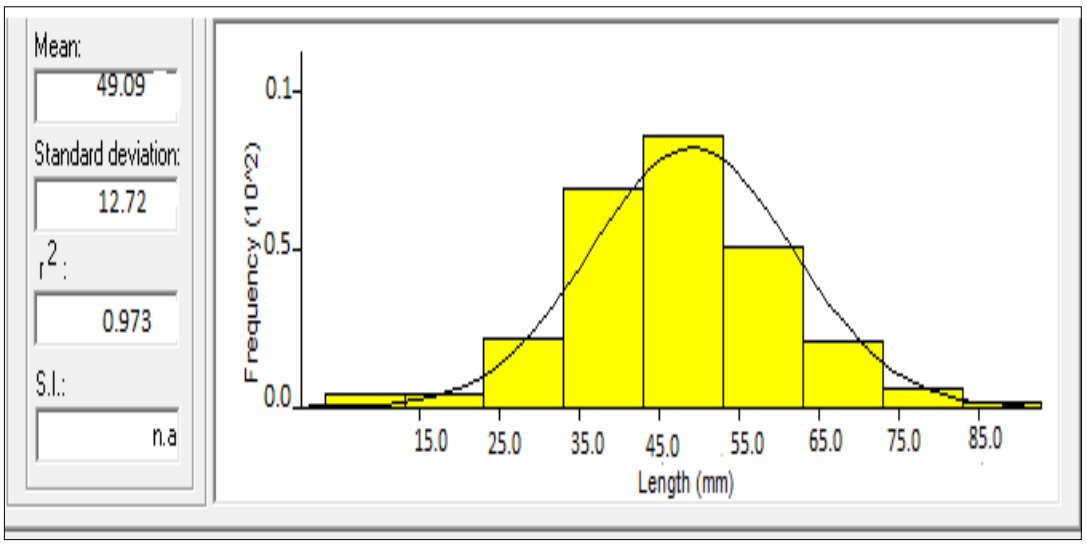

Figure 1. Cohort of *G. erosa* of data collected in June, July, and August 2014.

Two clam samples were selected as representatives of group A, the first clam had a 35 mm shell length and the second clam had a 44 mm shell length. The samples had 5 follicles and 6 follicles, respectively, where 3 follicles from each respective group were selected randomly prior to the maturity stage analysis. The results showed that 24.24% of the oocytes were at an early growing stage, 51.52% (late growing stage) and 24.24% (mature); and 24.33% oocytes (early growing stage), 45.94% (late growing stage), and 29.73 (mature stage) for respective shell lengths. The histological characteristics of female gonads of group A are shown in Figure 2. In addition, two clams from group B at different length classes were chosen randomly, with shell lengths of 53 mm and 57 mm. Five and six types of follicles were observed in the clam samples, respectively.

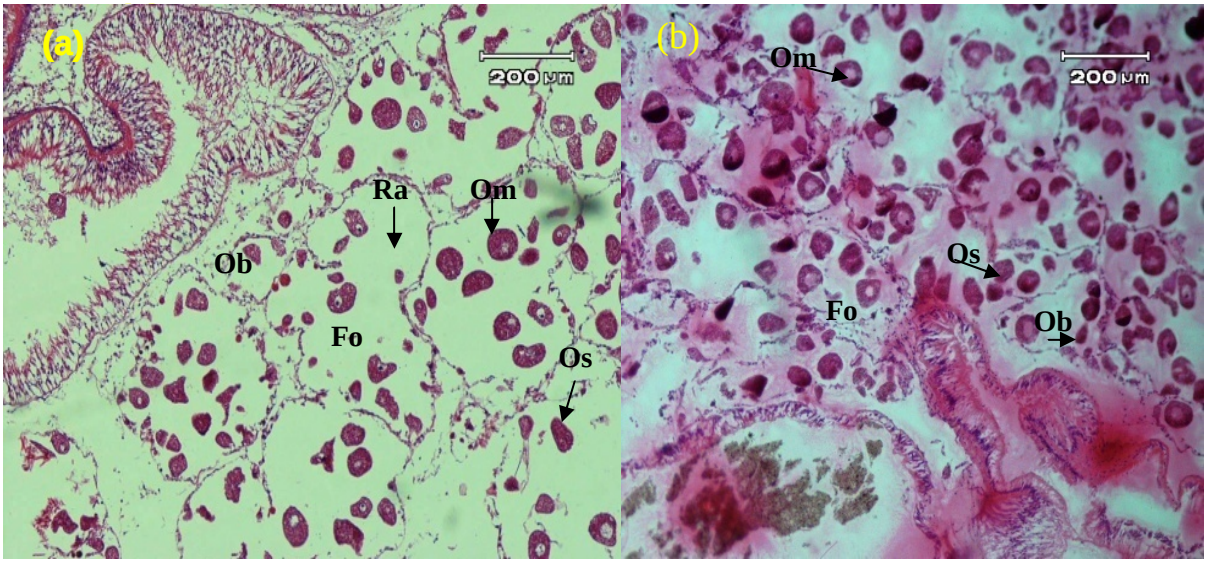

Figure 2. Histological characteristics of ovaries of *G. erosa* at shell lengths of 35 mm (a) and 44 mm (b). Fo = Follicle, Ra = Intercellular spaces, Ob = early growing oocyte; Os = late growing oocyte, Om = mature oocyte.

Three follicles belonging to the first and second clam were selected randomly for maturity stage analysis. The follicles of the first clam consisted of early growing oocytes (27.40%), late growing (35.62%), and ripe oocytes (36.96%), while the follicles of the second clam (57 mm shell length) consisted of early growing oocytes (28.82%), late growing (31.18%), and ripe oocytes (40.0%). The histological characteristics of the female gonads of group B are presented in Figure 3.

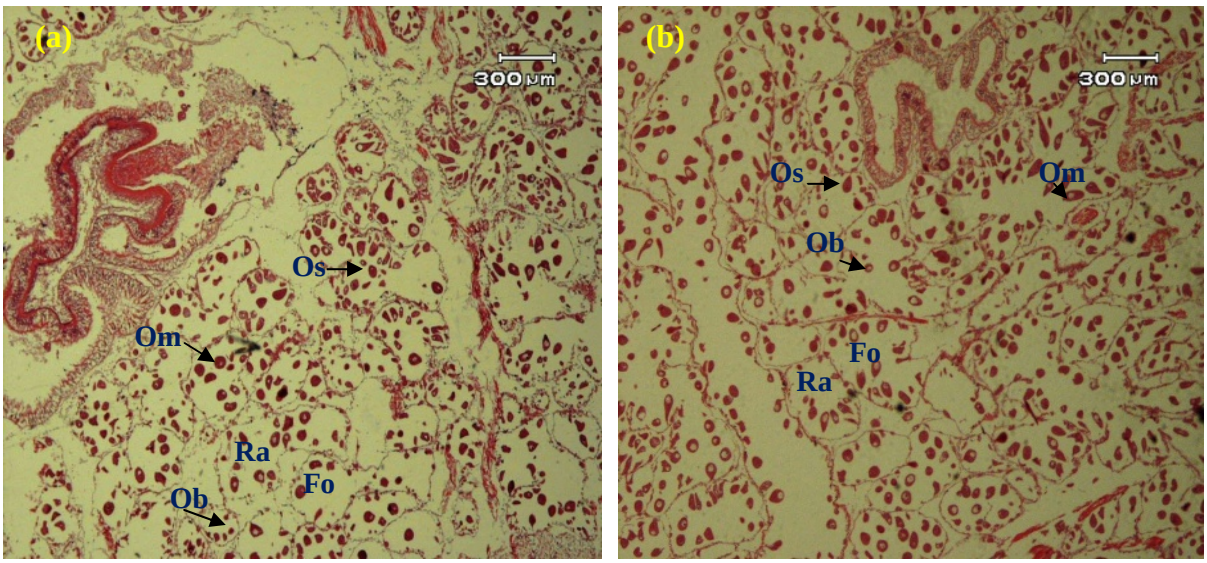

Figure 3. Histological characteristics of ovaries of *G. erosa* with shell lengths of 53 mm (a) and 57 mm (b). Fo = Follicle, Ra = Intercellular spaces, Ob = early growing oocyte; Os = late growing oocyte, Om = mature oocyte.

The study revealed that late growing oocytes were predominant in the group A clams (36.37-49.09 mm in length class), while the mature oocytes were predominant in group B clams (49.09-61.81 mm in length class). This finding indicates that the proportion of mature oocytes are higher as clam size increases. This also shows that the *G. erosa* ovary has several oocyte development groups and therefore this species can be classified as an asyncronous spawner. A similar reproductive pattern was also recorded with freshwater pearl mussels, *Hyriopsis bialatus* (Chatchavalvanich et al 2006). In additon, Hartati et al (2005) recorded three oocyte development stages in totok clams (*Polymesoda erosa)* in Segara Anakan waters, Central Java.

Accoding to Muchlisin (2014) there are three types of ovarian development: synchronous, where all of the oocytes develop and ovulate in unison, where there is a single size distribution in the ovary, and where there is no replenishment of early stage oocytes (West 1990); group synchronous, where there are at least two populations of oocytes at any developmental stage (Murua & Saborido-Rey 2003) and which allows for multiple, distinct ovulatory events that typically follow seasonal or diurnal cycles (Redding & Patino 2013); and asynchronous, where the ovaries contain oocytes at all stages of maturity without dominant populations. The ovary appears to be a random mixture of oocytes at every conceivable stage as recorded in the *G. erosa* of this study. Aquatic organisms with asynchronous ovarian development are also known as multiple spawners, where only a portion of the yolked oocytes are spawned in each batch (Murua & Saborido-Rey 2003). According to Lambert & Ware (1984) the batch spawning is a strategy to release eggs over a long period of time, increasing the survival probability of offspring.

Environmental factors and predators significantly affected the growth and distribution of the clams. The common predators for clam are crabs, lizards and beavers, aside from the fact that human shave significantly pressured the clam population by using it as protein source. The study indicates that the mud clam populations are under threat and therefore a conservation plan is necessary to conserve the mud clams in the Reuleung River particularly and Aceh waters in general. Sarong (2015) proposed three

management strategies for mud clams (*G. erosa*): (a) a biological approach of determining the minimum size for harvesting, (b) a technological approach of applying friendly harvesting practices (non-destructive), and (c) a human resources approach of improving the practices of the local people.

Conclusions. This study concluded that the clam *G. erosa* population in the mangrove forests of the River Reuleng Leupung, Aceh Besar District has one cohort with two length classes. The ovaries have three oocyte development groups, which indicates that the mud clam is an asyncronous spawner. The study also indicates that it is crucial to find a better management strategies to conserve the clam population.

Acknowledgements. The authors would like to thank DGHE, Ministry of Education and Culture Republic of Indonesia and Syiah Kuala University for providing the research grant for this study.

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Received: 9 July 015. Accepted: 27 September 2015. Published online: 02 October 2015.
Authors:
Muhammad Ali Sarong, Department of Biology, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: ali_sarong@yahoo.com
Asiah Muhammad Daud, Department of Biology, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: asiah.daud@yahoo.com
Wardiah Wardiah, Department of Biology, Faculty of Teaching Training and Education, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: wardiah.fkip@gmail.com
Irma Dewiyanti, Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: irma_alfian@yahoo.com
Zainal Abidin Muchlisin, Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh 23111, Indonesia, e-mail: muchlisinza@unsyiah.ac.id

How to cite this article:
Sarong M. A., Daud A. M., Wardiah W., Dewiyanti I., Muchlisin Z. A., 2015 Gonadal histological characteristics of mud clams (*Geloina erosa*) in the estuary of Reuleung River, Aceh Besar District, Indonesia. AACL Bioflux 8(5):708-713.