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Reproductive characters of fresh water shellfish (*Corbicula sumatrana* Clessin) In Singkarak Lake West Sumatra Indonesia

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Abstract
Corbicula sumatrana is one of shellfish that could be used as one of water biota in Indonesia. However, until now it has not got good attention This species was caught a lot and used by people as food source. It is hard to get this shellfish now. Mostly shellfish with small size are caught, while the big ones are very rare now. For this reason, a research was done to understand how to culture this kind of shellfish by studying characters of its reproduction in Singkarak lake.Shellfish samples were taken from Singkarak lake, Solok Regency West Sumatera. Analysis of males and females frequency, and histology was done in laboratory. Results showed that the ratio of males and females frequency was 0.95:1 (1 : 1) with the frequency of males 40.80%, females 42.69%, and hermaphrodite individuals 16,51%. Reproductive cycle found in its habitat consisted of 4 phases, early active, active, spawning, and post spawning.Gonad index value ranged 0.78 – 1.30.The peak of spawningof this species occured in June – July 2013.

Keywords: *Corbicula sumatrana*, reproductive character, gonad maturity

1. Introduction
Various kinds of Pelecypoda live in water of Indonesia. Some of them live in fresh water (rivers and lakes)called pensi, remisor shellfish and some shellfish live in ocean.The use of shellfish as protein source has attracted many people in Indonesia mainly which belong to Pelecypoda having economical value [9, 15].

One species of Pelecypoda found in fresh water is *Corbicula sumatrana.* This species present in Lake Singkarak, Diatas Lake and Dibawah Lake West Sumatra. Other species, *C. moltokiana* is found in Maninjau lake. *C. javanica* and *C. rivalis* are found in Lido lake, West Java.

Shell of *Corbicula* in general was very small, almost symmetrical, from rounded triangle to irregular oval form. It was in general hard, little or not transparent, andumbois convect in several species. The outer part was hard, little fibrous, concentric side, and outer ligament was hard [8]. Shell of shellfish consists of three layers: periostracum, prismatic and nakreus. Outer surface of shell is usually smooth but some show growth and radial lines or combination of both [4].

In Pelecypoda, gonad which determines `reproductive system is located on body surface between above ventricular and epithel in the outside. A matured gonad has fine canalis genitalis tissues and it is shown on body surface because at the time the body surface becomes thin. In matured shellfish it is shown on follicle that egg cell and spermatozoa have been formed in small amount [11].

In shellfish reproductive cycle, maturity of gonad is main factor in its reproduction. Gonad maturity phase is started at the time shellfish produce eggs and spermatozoa. The first maturity phase of shellfish gonad could be observed in shell length [9]. *C. manilensis* reached sexual maturity at 10 mm shell length [1]. Qualitatively, sexual maturity phases could be observed from gonad development [3]. Maturity phases of gonad could be seen by observing its histology. There were 6 phases of gonad maturity [14]. Phases of gonad maturity in individual male and female of *C. japonica* was studied by Baba [3].

Pelecypoda have different reproductive periodes and spawning time. *C. manilensis* has reproductive cycle14-17 months [1]. In *C. australis* spawning and its embryo emerge from October until May [5]. Shellfish spawning is influenced very much by environmental factors. Spawning of *C. japonica* was very low at very low environmental temperature [2]. Based on the above reasons thus a research has been done about reproductive characters on *C. sumatrana* Clessin in Singkarak Lake.

2. Materials and Methods

This research was conducted from September 2012 until December 2013 in Singkarak Lake X Koto Singkarak and Junjung Sirieh subdistrict in Solok district West Sumatra Indonesia. Samples were taken and data were analized in Basic laboratory of Zoology and Botanical Laboratory of Botany, Biology Education and Laboratoryof animal Ecology and Laboratory of animal development structure, departement of Bology Faculty of mathematics and natural sciences Andalas University Padang Eswt Sumatrana Indonesia.

Histological analysis on gonad and sex of shellfish was done every month. Sex was determined by looking at gonad color. Male individual was marked by white color of gonad and female individual by yellow or orange color of gonad. Other markers for sex determination from external morfology were length, width, and height of shell as well as number of growth lines.

Shellfish gonad obtained from field was sorted based on length and then analized histologically. Shell of shellfish was disected using cutter knife, then gonad located at dorsal of visceral organ and anterior of legs was taken[12]. Gonad was taken from visceral organ by cutting the point of its attachment to digestive organ. Gonad was then placed in a film bottle containing Bouin's solution.Then histological preparate of *C. sumatrana*gonad was made in reference of Mc Manus research [16].

Isolated gonad was fixationed in fixative solution for 2-10 hours, then washed using 70% alcohol.Then it was dehidratedin series of alcohol concentrations and was bleached with xylol and then was paraffin infiltrated. Next step was embedded and continued with slicing cross section 6-7µ

After bands were formed it was then placed on object glass after being smeared with Meyers albumin then given distilled water. Slicing that had been placed on object glass was deparafined using xylol, and colored with HE. The ready preparate was documented using microscope and photo camera to facilitate observation on gonad structure.

Gonad maturity analysis was done descriptively by observing gamet structureinfolicle cells, then gonad development phases were observed with reference to Sahin *et. al.* [14]. In the same individual, parameters measured were gonad mature phases and morphological characters, length, width, and height.

Analysis on gonad maturity index use this equation:

$$d\text{GI} = \frac{d\text{GW}.\ 10^6}{\text{H}^3}$$

3. Results

The results showed that the appearance of males was 40.80% (n = 424) and that of females was 42.69% (n = 424), and hermaprodite individuals was 16.51% (n = 424). Analysis of gonad existence in visceral mass when compared to gonad histological observation, thus the males and females frequency ratio in one year was 0.95:1 (1: 1). Numbers of males found was 48.87% (n = 354) and that of females was 51.13% (n = 354).

Based on observation done on gonad histological preparates and anatomical observation, percentage of males fresh water shellfish was 40.80% (n = 424) and that of females was 42.69% (n = 424). The emergence of hermaprodite individuals was 16.51% (n = 424). Analisys of gonad existence in visceral masswhen compared to gonal histological observation, the ratio between males and females for one year was 0.95:1. Numbers of males found was 48,87% (n = 354) and that of females was 51.13% (n = 354). The ratio of males and females was insignificantly different where the number of males individuals was 173 and that of females was 181 (χ^2 test, $p < 0.05$). In general, shellfish were found in males, females, and hermaprodite individuals.

According to Morton [10], hermaprodite individuals from shellfish *C. fluminea* di Pearl River RRC was found 4.5%. In addition, the one found in Hong Kong was 29.5% male, 39.3% female and 31.2% hermaprodite individuals. This difference could happen because of environment factors. Hermann research [7] reported that ratio of males and females of shellfish *Mesodema mactroides* found in North Argentina was 1:1, but hermaprodite individual was not found. Further, Xu research [17] reported that shellfish *C. fluminea* in Shanghai Diansan lake was mostly found in heterogametic and very little in hermaprodite.

Sexual maturity phases is started at the first time shellfish produce eggs and spermatozoa. Early sexual maturity of shellfish could be observed in shell length[9]. *Corbicula manilensis* reached sexual maturity at shell length 10 mm [1]. Qualitatively, sexual maturity phase of shellfish can be observed from gonad development. Gonad maturity phases can be observed from gonad histology. In this observation, early maturity phase of gonad was found at shell length 12 mm which was different from result found by Aldrige and McMahon [1] in *C. maniliensis* in which early sexual maturity was found at shell length 10 mm.

Observation on gonad histological preparates of *C. sumatrana* indicated thatthere were 4 gonad development phases in its life cycle (Table 1).They were early active, active, spawning and post spawning.

Results of analysis on gonad maturity index were presented in Fig. 1. In December 2012 until February 2013 shellfish were found in immature phase with dGI value < 1.00 the same as in periode from September 2013 until Desember 2013. Mean while, shellfish found from Marchuntil Agustus 2013 were in early maturity phase with dGI value > 1.00. The lowest gonad maturity index value was found in November 2012 i.e. 0.78 ± 0.11 (n = 9) and the highest one was found in July with gonad maturity index 1.3 ± 0.13 (n = 6). Result on gonad dry weight and gonad maturity index indicated that qualitatively the peak of spawning also occured in Juli. This could be observed from the fluctuation of gonad dry weight average at that periodes. This result also showed the same pattern as the one showed histologically.

Gonad developmental cycle is shown in Table 1. Histological study indicated that gonad developmental phases were early active, active, spawning and post spawning. In active phase, numbers of spermatogonium and ovary were very few and small size. At the following phases the numbers increased and the size was bigger. Alveols wall had been clear. In spawning phase spermatogonium and oosit sizeswere gettting bigger and filled with spermatozoa and matured ovary. In the last phase, post spawning, size of spermatogonium and oosit was smaller, loose and new folicles were shown to replace the old ones.

Result of research done by Baba [3] on spawning and post spawning phases in *C. japonica* in Abashiri Lake in Japan showed the same histological characters with *C. sumatrana* found in Singkarak lake. Spawning phase in female gonad of *C. japonica* showed genital tubulus filled with matured oosit, and in male gonad filled with matured spermatozoa. In postspawning phase, genital tubuluswas empty otherwise oosit (female gonad) or spermatozoa (male gonad) were not foundand oosit or spermatozoa would be degenerated throughphagositosis.

Based on gonad index value (dGI), *C. sumatrana* found in Singkarak lake were in maturity preparation phase (preparing for spawning). This was indicated by dGI value> 1. From March – Juli 2013 shellfish were found in early mature phase, while from September 2012 until February 2013 and from August untill December 2013 they were in immature phase because dGI value < 1. There were 5 levels of gonad index classification: 1) GI I, gonad index value < 1.0, the animal gonad was in immature phase, 2) GI II, gonad index value was 1.0 – 5.0 the animal gonad was in preparation for maturing), 3) GI III, gonad index value was 5.1 – 10.0 the animal was in early mature phase, 4) GI IV,gonad index value 10.1 – 20.0 the animal was in mature phase, and 5) GI V gonad index value was > 20.0 the animal was in mature phase and had spawned repeatedly.

From this reserch it was found that spawning peak of *C. sumatrana* was in June until July 2013. However, Doherty research[6] reported that spawning peak of *C. fluminea* was found from June until early July, end of Augustandearly October with spawning duration 2 – 6 weeks.

Gonad maturity level could be determined by observing gonad histology. If gonad maturity stage was related to gonad position inviscera mass,the presence of matured gonad clearly was found at at shell length 12 mm.When shell length <12 mm, the difference between gonad and viceramass was not clearly observed. When the shell length was > 20 mm, repeated spawning phase could be seen by bigger gonad than the former one and more dominant to fill viscera mass.

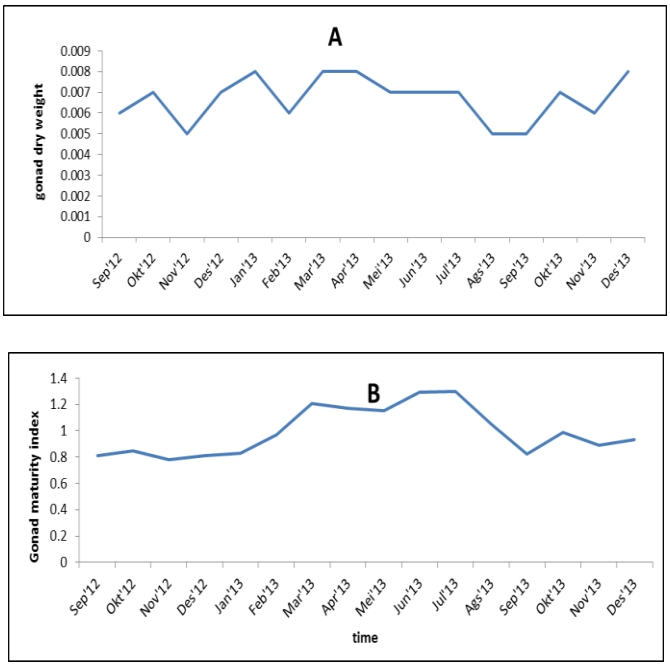

Fig 1 A: average dry weight of gonads (dGW); B: gonad maturity index (dGI) of *C. sumatrana* in Singkarak lake

Table 1: Gonadal developmental phase of male and female shellfish, *C. sumatrana*in Singkarak lake

Gonade developmental phase	Description	
	Males	**Females**
Initial active	Number of spermatogonium was very few and small. Alveolus wall was not clearly seen	Number of ovary was very few and small size. Alveolus wall was not clearly seen
Active	Numbe of spermatogonium was more and bigger size. Alveolus wall was clear. There was goups of spermatosit and spermatid in spermatogonium. Numbers of spermatozoa was few	Number of ovary was more and bigger size. Alveolus wall was clear. Oosit had been attached alveolus wall. Number of oosit was few.
Spawning	Size of spermatogonium was bigger and filled full with spermatozoa. Group of spermatid and spermatosit became less	Size of oosit was bigger and genital tubulus had been filled with matured ovary. Alveolus wall was wider
Post spawning	Size of spermatogonium was smaller and loose. Number of spermatozoa and spermatid decreased with irreguler size. Spermatozoa remnant was clearly seen	Ovary had been loose. Alveolus wall damaged and not clear. New follicles began to be seen to replace old ones.

4. Conclusion

Ratio of male and female appearance of *C. sumatrana* during research period was 0.95:1 (1 : 1). Male frequence was 40.80% and female 42.69% and hermaprodite individuals was 16.51%. Reproductive cycle consisted of early active, active, spawning and post spawning with gonad index value 0.78 – 1.30. Spawning peak occured from June – July 2013.

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