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by Endang Dm

Submission date: 08-Apr-2023 01:29PM (UTC+0800)

Submission ID: 2058875951

File name: Prosiding_13._The_Analysis_of_Cockle.pdf (710.27K)

Word count: 3265

Character count: 16220

The Analysis of Cockle (*Anadara inaequalvis*) Gonad Maturity Level in the Estuary of Banjar Kemuning River, Sedati, Sidoarjo

R F Saputra, E D Masithah, and P D Wulansari
Fisheries and Marine Faculty, Universitas Airlangga, Surabaya, Indonesia

Abstract. Cockle (*Anadara inaequalvis*) is aquatic biota belonging to the mollusk group. Cockle has a high potential because all parts of its body can be processed and economic value. Cockle can be used for consumption and the shells can be used as jewelry. According to the data from WWF Indonesia (2017), the production of cockle in the estuary of Banjar Kemuning River from 2006-2016 decreased in average by 0.84% every year. One of the efforts in maintaining the availability of cockle in the waters is conducting research on the analysis of gonad maturity level on cockle. The purpose of this research is to learn the morphological structure and histological structure of cockle gonad (*Anadara inaequalvis*) in the estuary of Banjar Kemuning River at different levels of gonad maturity and to learn the maturity level of cockle gonad (*Anadara inaequalvis*) in the estuary of Banjar River Kemuning through morphological and histological observation. This research used descriptive method with data collection by field observation. The sampling technique used the method of quota sampling. The analysis result shows that gonad maturity level of cockle in the estuary of Banjar Kemuning River in December is dominated by TKG IV, in January is dominated by TKG I, and in February is dominated by TKG II. The male cockle's first mature gonads are at the shell length of 35.73 mm (34.55-36.91 mm) and female cockle's first mature gonad are at the shell length of 37.21 mm (36.06-38.37 mm).

Keywords. cockle, gonad maturity levels, index maturity levels, the estuary of banjar kemuning river.

1. Introduction

Cockles are aquatic biota that belong to the mollusk group. Cockle live in tidal areas or in estuarine regions with mud substrates to areas bordering with mangrove forests (Pattikawa, 2007). Cockles are used in some food preparations and are special attraction for the community. Cockle taste good and contain high nutrition. The production of cockles at the Banjar Kemuning River estuary from 2006-2016 has decreased by an average of 0.84% per year (WWF Indonesia, 2017). One of the efforts in maintaining the availability of cockle in the waters is conducting research on the analysis of the level of gonad maturity in cockle.

The level of gonad maturity is a certain stage of gonad development before and after organisms spawn (Effendie 2002). According to Jayadi (2011), the level of gonad maturity is needed to determine the comparison between organisms that have matured gonads and those that are immature, size or age of the organism when first gonads mature to determine whether the organism has spawned or not, the spawning period, and the frequency of spawning. The level of gonad maturity is needed to determine the size of the first mature gonad in the cockle. The determination of the first size of the gonad maturity



is used as a reasonable measure of capture on cockle, so that the size of the cockle with immature gonad is not caught and availability of cockle in the waters will be more preserved.

2. Method of implementation

2.1. Research place and time

This research was carried out at the estuary of the Banjar Kemuning River, Sedati, Sidoarjo from December 2017 to February 2018. Morphometric measurements, histological making and analysis of gonad cockle were carried out at the Microbiology Laboratory, Faculty of Fisheries and Marine, Universitas Airlangga, Surabaya. Substrate type analysis was carried out at the Soil and Stone Mechanics Laboratory, Faculty of Civil Engineering, Sepuluh November Institute of Technology, Surabaya.

2.2. Research tools and materials

The materials used in this study were cockle (*Anadara inaequalis*), water samples, and substrate. The ingredients for making gonad histology preparations are Davidson solution, multilevel alcohol (60%, 70%, 80% and 96%), xylol, paraffin, entellan neu, haematoxylin and eosin (HE) solutions. The equipment used in this study were motorboats, scallop fishing gear (garit), cool-box, Eikman grabs, label paper, baskets, plastic clips, permanent markers, and GPS. Water quality measurement equipment include thermometer, refractometer, DO meter, and pH meter. The morphometric measurement equipment include a shear period with an accuracy of 0.05 mm and an O-Haus scale with an accuracy of 0.0001 grams. The gonad histology manufacturing and analysis equipment include a set of section, cassette, hotplate, microtome, tissue processing, object glass, cover glass, and light microscope tools.

2.3. Work procedure

2.3.1. Determination of sampling points

Determination of sampling points for shells, water, and substrate was carried out by a preliminary survey in advance to determine the geography and activities around the research environment (Hadi, 2007). Based on the results of the preliminary survey, the research location is the fishermen's daily catchment area. The location point for cockle, water and substrate sampling was divided into five points which were considered to represent the research area, namely the estuary of the Banjar Kemuning River, Sedati, Sidoarjo.

2.3.2. Sampling of cockle, water, and substrate

The number of samples of cockle (*Anadara inaequalis*) needed in this study was 100 cockles. The cockle samples were collected by using garit fishing gear. There were 20 cockles taken at each point, and with all five points, the total samples were 100 samples. The collected cockles were put into a plastic bag, then stored in the cool box and analyzed in the laboratory. Water sampling was done using a water sampler. The measured water quality parameters consisted of temperature, salinity, DO, and pH. Substrate retrieval was conducted by using Eikman grab. The collected substrate was put into a plastic bag, then stored in the cool box and taken to the laboratory for analysis.

2.3.3. Morphometric measurements

The shell of cockle sample was measured by morphometric measurements, including shell length (mm) and shell width (mm), using calipers with a precision of 0.05 mm. Cockles that have been measured in length were grouped in 3 class range of shell length that were previously determined by the researcher, namely 27.1-33.0 mm (small); 33.1-39.0 mm (medium); and 39.1-45.0 mm (large). For scallop samples were then measured for total weight (g), weight of meat (g), and weight of gonads (g) using O-Haus scales with 0.0001 gram accuracy.

2.3.4. Observation of gonad maturity levels of cockle

Gonad observation began with sex determination. Female cockle are characterized by the color of the reddish orange gonad and egg grains, while the male cockles have a milky white gonad color (Widyastuti, 2011). Morphological observations on the level of cockle's gonad maturity is done by using visual observations based on the extent of gonad closure on visceral mass (Hartati et al., 2005). Observation of gonad maturity level was carried out histologically by preparing gonad tissue and observing the tissue structure.

2.4. Data Analysis

The data collected in this study were qualitative and quantitative data. Data analysis was carried out descriptively. Data analysis can also be used to draw a conclusion in the study.

3. Result and discussion

The following are the results and discussion of research on the maturity level of the cockle (*Anadara inaequalis*) gonad at the estuary of the Banjar Kemuning River.

3.1. Gonad maturity level of cockle, morphologically.

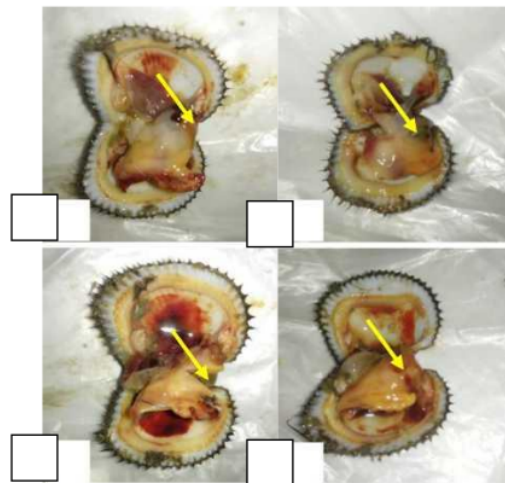


Figure 1. Gonad maturity level of male cockle, morphologically. (a = TKG I, b = TKG II, c = TKG III, d = TKG IV).



Figure 2. Gonad maturity level of female cockle, morphologically.
 (a = TKG I, b = TKG II, c = TKG III, d = TKG IV).

Figure 1 and Figure 2 show that at TKG I (resting), gonads of male and female cockle appear very small and have not covered the visceral mass area. Gonads begin to develop in TKG II (developing). Male cockle's gonads are white and females' are light orange. The area of gonads in the visceral mass area is around <60%. TKG III (mature) shows that the gonad is growing. The male cockle's gonads are white and the females' are slightly brownish orange. The area of the gonads in the visceral mass area is around 60-90%. TKG IV (spawning) shows a mature gonad. Male cockle's gonads are white and females' are brownish orange. The area of gonads in the visceral mass area is around 90%.

3.2. Gonad maturity level of cockle, histologically

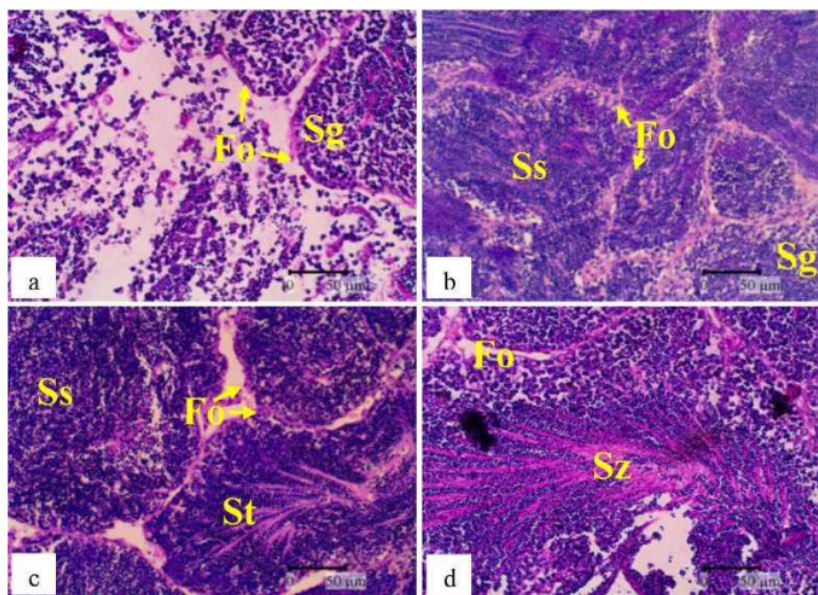


Figure 3. Gonad maturity level of male cockle, histologically, with 400x magnification
 Description: a = TKG I, b = TKG II, c = TKG III, d = TKG IV
 Fo = Follicle, Sg = Spermatogonia, Ss = Spermatocyte,
 St = Spermatids, Sz = Spermatozoa

Figure 3a shows that at TKG I (resting), the follicular cavity contains spermatogonia but not too many. Figure 3b shows that in TKG II (developing), the spermatogonia then begin to develop into spermatocytes. Spermatocytes then develop into spermatids in TKG III (mature) (Figure 3c), which then develops into spermatozoa. This is indicated by the presence of grooves in the follicle. Figure 3d shows that in TKG IV (spawning), there are many spermatozoa that come out of the follicle. The follicular cavity appears to be empty, marked by the number of grooves in the follicle

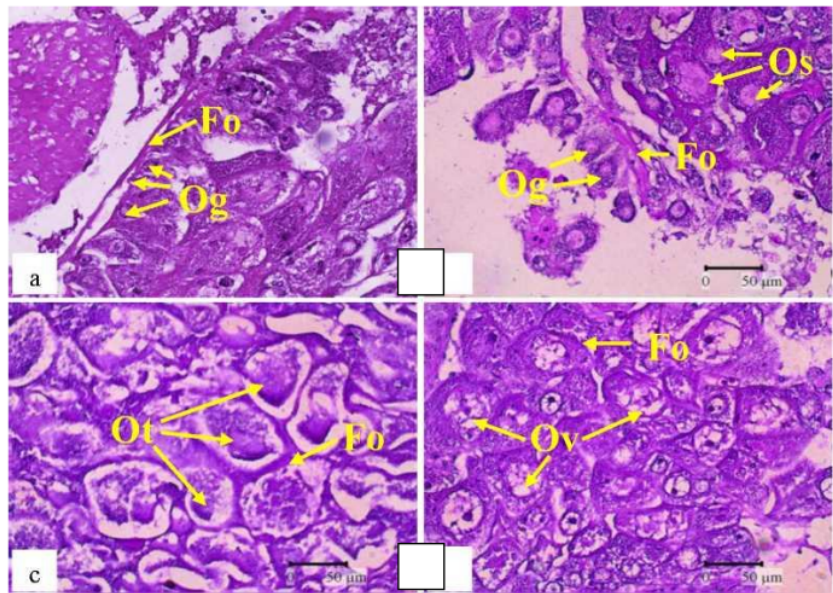
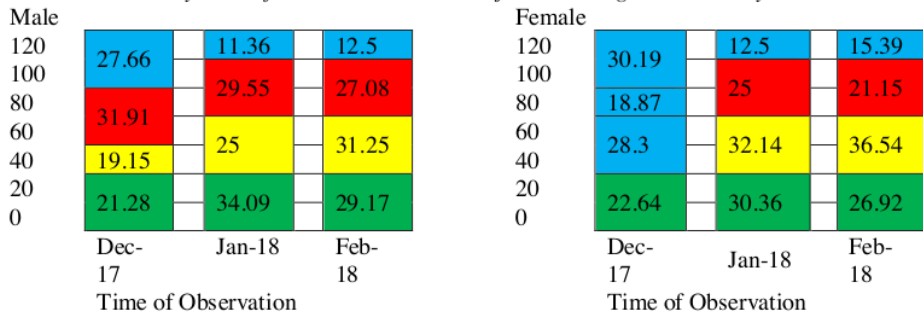


Figure 4. Gonad maturity level of male cockle, histologically, with 400x magnification
 Description: a = TKG I, b = TKG II, c = TKG III, d = TKG IV
 Fo = Follicle, Og = Oogonia, Os = Oocyte, Ot = Ootids, Ov = Ovum

Figure 4a shows that at TKG I (resting), the follicle wall contains a lot of oogonia. Oogonia then develops into Oocytes in TKG II (developing) (Figure 4b), which then develop into ootids in TKG III (mature) (Figure 4c). Ootid then develops into an ovum and is ready to be released and then fertilized by spermatozoa. Figure 4d shows that in IV TKG (spawning), a lot of the ovum has been removed. It is indicated from the follicular cavity that starts to be empty.

3.3. Gonad maturity level of male cockle in the Banjar Kemuning River Estuary



4 TKG I TKG II TKG III TKG IV 4 TKG I TKG II TKG II TKG IV

Figure 5. Percentage of gonads maturity based on time of observation.

11 The observation of gonad maturity level of male cockle and female cockle in the estuary of the Banjar Kemuning River in December was dominated by TKG IV which was equal to 57.85%; in January, TKG

I was dominated at 64.45%; and in February TKG II was dominated at 67.79%. Mackie (1984) claimed that gonad shellfish maturity was influenced by several factors, namely exogenous and endogenous factors. Exogenous factors include: temperature, month period, depth, mechanical factors, abundance and availability of feed, and light intensity, while endogenous factors, including genetics and hormones.

3.4. First Measurement of Cockle's Gonad Maturity in Banjar Kemuning River Estuary

The first measurement of mature gonads was determined based on the maturity level of the cockle's gonad at each shell length interval using the Spearman-Karber method (Udupa, 1986).

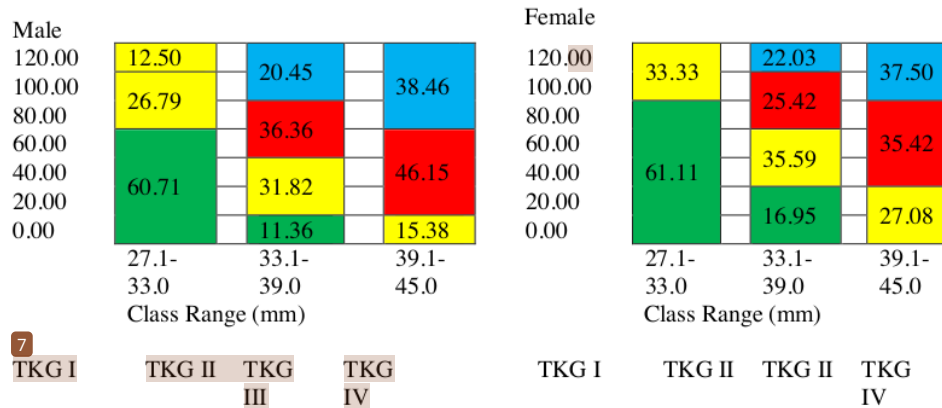


Figure 6. Percentage of gonad maturity based on the range of length class.

The results of the determination of the first size of mature gonad in male cockle and female cockle were determined by using the Spearman-Karber method. The male cockle's first gonads matured at a shell length of 35.73 mm (range 34.55-36.91 mm) and female cockle gonad matured at 37.21 mm in length (range 36.06-38, 37 mm). The first size of ripe gonad shells is male fur and female cockle are in the class range the size of the shell length 33.1-39.0 mm. Moresco and Bemvenuti (2006) state that the size of the first gonads mature differently; it is a reproductive strategy to restore the balance of the population caused by changes in conditions, abiotic factors and over-catch.

3.5. Index of cockles' gonad maturity in Banjar Kemuning River Estuary

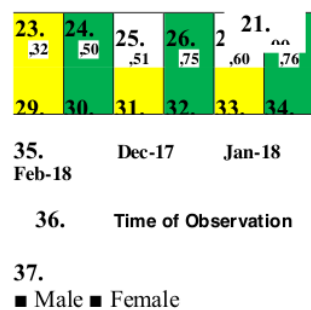


Figure 7. Index of cockles' gonad maturity based on time of observation

The IKG value in December has the highest IKG average value compared to January and February since in December, cockles were dominated by TKG IV. The average IKG score decreased in January because cockle were dominated by TKG I. The average IKG score rose again but was not significant in February because cockles were dominated by TKG II. According to Nurohman (2012), the higher the

level of gonad maturity, the greater the IKG value, which is related to the size of the gonad that increases along with the development of the gonad. As the gonad develops, the gonad will get bigger and heavier, then when it reaches gonad maturity, the value of IKG will be even greater.

3.6. Sex ratio of cockles in Banjar Kemuning River Estuary

Table 1. Sex ratio of cockles based on the time of observation.

Time of Observation	Frequency		Ratio	chi square		Remark
	Male	Female		x2 calculated	x2 table	
December 2017	47	53	1:1.11	0.36	3.841	balance
January 2018	44	56	1:1.21	1.44		balance
February 2018	48	52	1:1.08	0.16		balance
Total	139	161	1:1.14	1.613		balance

The sex ratio of cockle based on observation time (Table 1) shows the results of the x-test (Chi-square) with a confidence level of 95% in December to January having a calculated x value lower than the x table value. It can be said that the sex ratio of male cockle and female cockle based on the observation time is in a balanced condition. This condition means that each mother shell has its own pair and is predicted to guarantee the success of fertilization during spawning provided that food and environmental conditions support this process (Natan, 2008).

3.7. Aquatic conditions of the Banjar Kemuning River Estuary

Table 2. Conditions for the estuary of the Banjar Kemuning River.

Parameter		Time of Observation		
		Dec 17	Jan 18	Feb 18
Water Quality	Temperature (oC)	28.6	28.4	29.6
	pH	8.0	8.2	7.7
	Salinity (ppt)	35	35	26
	DO (ppm)	4.33	6.82	2.34
Substrate	Gravel (%)	0.14		
	Sand (%)	2.80		
	Mud (%)	97.06		
	Particle Size (mm)	<0.075		

The water parameters measured in the Banjar Kemuning River Estuary include water quality and substrate type. Water quality parameters measured include temperature, pH, salinity and dissolved oxygen (DO). Overall, the environmental conditions at the Banjar Kemuning river mouth can support the development of the cockle gonad.

4. Conclusion and Suggestion

The morphology of cockle gonad shows that the area of the gonad will increase along with the increased levels of gonad maturity. Histology of male cockle gonad in TKG I is dominated by spermatogonia, TKG II is dominated by spermatocytes, TKG III is dominated by spermatids, and TKG IV is dominated by spermatozoa. Histology of female cockle gonad in TKG I is dominated by oogonia, TKG II is dominated by oocytes, TKG III is dominated by ootid, and TKG IV was dominated by ovum. The maturity level of the gonad of cockle in the estuary of the Banjar Kemuning River in December 2017 was dominated by TKG IV, in January 2018 it was dominated by TKG I, and in February 2018 it was dominated by TKG II.

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