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Original Research Article

Comparison of Some Aspects of Morphological and Reproductive of Blood Cockle (*Anadara granosa* L.) in the Intertidal of Kupang Bay, West Timor, Indonesia

Aludin Al Ayubi[1], Ricky Gimin[2], Yahyah[2]
[1,2]Master's Degree Program in Environmental Sciences, Postgraduate Program, University of Nusa Cendana, Kupang, Indonesia 85001
[2]Faculty of Marine Science and Fisheries, University of Nusa Cendana, Kupang, Indonesia 85001

***Corresponding author**
Aludin Al Ayubi
Email: aludinalayubi@ymail.com

Abstract: Blood cockle (*Anadara granosa* L.) is the one commodity that is important for coastal communities in Kupang Bay, West Timor, Indonesia. This study aimed to compare some aspects of morphology and reproductive blood cockle between locations that have been long period exploited (Villages of Oebelo and Tanah Merah) and location that have been short period exploited (Village of Noelbaki) in the region of Kupang Bay. The method used in this research was surveyed by using line transect technique. The results of this study found that morphological aspects of blood cockles based observations covering its morphometric i.e. size shell length, weight, condition index, the gonadal index in the Oebelo Village and the Tanah Merah Village has a lower value than in the Village Noelbaki. Based on observations of aspects of reproductive known that blood cockles in the Oebelo Village and the Tanah Merah Village were dominated by the blood cockle males, more rapid in the process of sex maturation and gonad maturity, and more rapid transition gender from male to female, compared with blood cockle in the Noelbaki Village.
Keywords: Morphology, reproduction, blood cockle, comparison, exploitation, Intertidal

INTRODUCTION

Blood cockle (*Anadara granosa* L.) is one of bivalves that live in the intertidal area of Kupang Bay, West Timor, Indonesia. This bivalve was an important commodity for local communities in the coastal area [1]. Particularly in the region of Kupang Bay, there are three coastal village which is the blood cockle fishery areas i.e. Oebelo Village, Tanah Merah Village and Noelbaki Village. Since the 1990s, the village of Tanah Merah and Oebelo has been started the business of blood cockle fishery, whereas the Village Noelbaki had been since the 2000s. Fishery production of blood cockle in this area was taken traditionally for consumption them and marketed.

Bivalve populations in intertidal areas experiencing changes in the size and reproductive vary with the length or often exploited [2-6]. Especially in blood cockle population, shell size decrease due to the difference in intensity of fishing was resulting in differences in the size of blood cockle and the size of the mature sex [7]. Additionally, populations of blood cockle in an area with higher exploitation have a smaller body size and sex maturity earlier than in area with lower exploitation [8].

This study examines the relationship between resource utilization period difference blood cockle blood to change aspects of the morphology seen from its morphometric, as well as aspects of reproduction as seen from the sex ratio, the size of the first mature sex and the size of a sex change. The comparison is based on samples of cockle taken from different area's utilization rate of the Village and Village Oebelo Tanah Merah (representing the area with long period of exploitation) and Village Noelbaki (representing the newly exploitation area).

MATERIAL AND METHODS

This research has been conducted in October 2014 to January 2015. The Sampling location was an intertidal area in Oebelo Village, Tanah Merah Village and Noelbaki Village, with a sampling interval every two weeks. Observation and analysis of blood cockle samples conducted at the Laboratory of Marine Science and Fisheries, Faculty of Marine Science and Fisheries, University of Nusa Cendana.

The materials used in this study were alcohol for preservatives blood cockle gonad samples of blood, and serotonin for stimulation of blood cockle spawning. While the equipment used in this study were caliper (dialmax type) for measuring the length of shell; pipette 1 ml and 5 ml and Beaker glass 1000 ml for measuring the volume of blood cockle; aquarium 100 L and aerators for accommodating samples of the live blood cockle; dissecting sets and surgery board for surgery of blood cockle sample; flask (5 mL and 100 mL), syringe (1 mL) and syringe (tuberculin 3G: 15 mm), pasteur pipette and plunger (stirrer) for making serotonin solution; analytic balance for considering weight of blood cockle; oven and desiccators for drying soft tissue of blood cockle; and sartorius balance (0.0001 g in precision) for weighting of blood cockle soft tissue.

The parameters measured in this study are the morphological and reproductive aspects of blood cockle. Morphological aspects of blood cockle based on its morphometric i.e. length, weight, dry weight of shell, dry weight of gonad, volume of body, dry shell volume, gonadal index, and condition index. As the reproductive aspect, i.e. sex ratio, size of the first sex matures, and size of sex change.

Observation of the morphological aspects of blood cockle based on its morphometric i.e. shell length, body weight, dry weight of shell, dry weight of gonad, volume of body, dry shell volume, gonadal index, and condition index. The shell length was measured based on the maximum distance between anterior to posterior using calipers. The Body weight and the dry weight of shell measured using an analytic balance, while the dry weight of gonad using analytic balance (precision 0.0001 g). For measuring the volume of body and volume of dry shell have been done based on the volume of water displaced after shells inserted into a measuring glass that containing water [8]. Furthermore, for measuring and calculating the condition index and the gonadal index of blood cockle have been done by using the formula.

Observations reproductive aspects of blood cockle can be done by observing the sex ratio, the size of the first sex matures and the size of a sex change from male to female. Determining the sex ratio has been by counting the number of individual males and females in the length class specified. The blood cockle of each sex, then grouped into shell length classes 3 cm to see whether there is any difference in male-female ratio for a particular shell length [8]. Furthermore, the size of first sex matures determination have been done by blood grouping by blood cockle size for most small to largest size, then surgery the blood cockle to observing their sex. If sex has been observed that the blood shells had started to mature sex. While the determination of the size of the male to female sex change can be done by determining the length of the class at the time the number of female individuals reached 50% [9].

The value of sex ratio was analyzed using chi-square. Differences in shell length distribution can be accomplished using a frequency distribution chart analysis, index difference between the condition and gonad index location, use Variety analysis (ANOVA), that if there is a real effect followed by LSD test. Meanwhile, to determine the relationship of the length and weight of the body shells of blood can be used linear regression analysis. The difference value gradient (slope) and intercept of the regression equation between continued with test F. All statistical analyzes performed using SPSS 16.0 software.

Determining a sex change from male to female was starting from observation of blood cockle sex on each length class. The length class has been determined in the preliminary study. Observation of blood cockle sex, maturity in the Oebelo village and the Tanah Merah village, have been done in the class of shell length: 13.0-15.9 mm and 16.0-18.9 mm, while in the Noelbaki village: 13-18.9 mm and 19-21.9 mm.

RESULT AND DISCUSSION

The Length and Weight

There was different in modes of distribution of shell length between the three villages producer blood cockle. Noelbaki village has highest of the length frequency mode on the class of length 8 (37-39.9 mm), followed by the Tanah Merah village on the class of length 5 (25- 27.9 mm). While the lowest of the length frequency mode was in Oebelo village in the class of length 4 (22-24.9 mm) (Figure 1). This condition indicating that the shell length of blood cockles in the Oebelo village and the Tanah Merah village has the shell length lower than in the Noelbaki village.

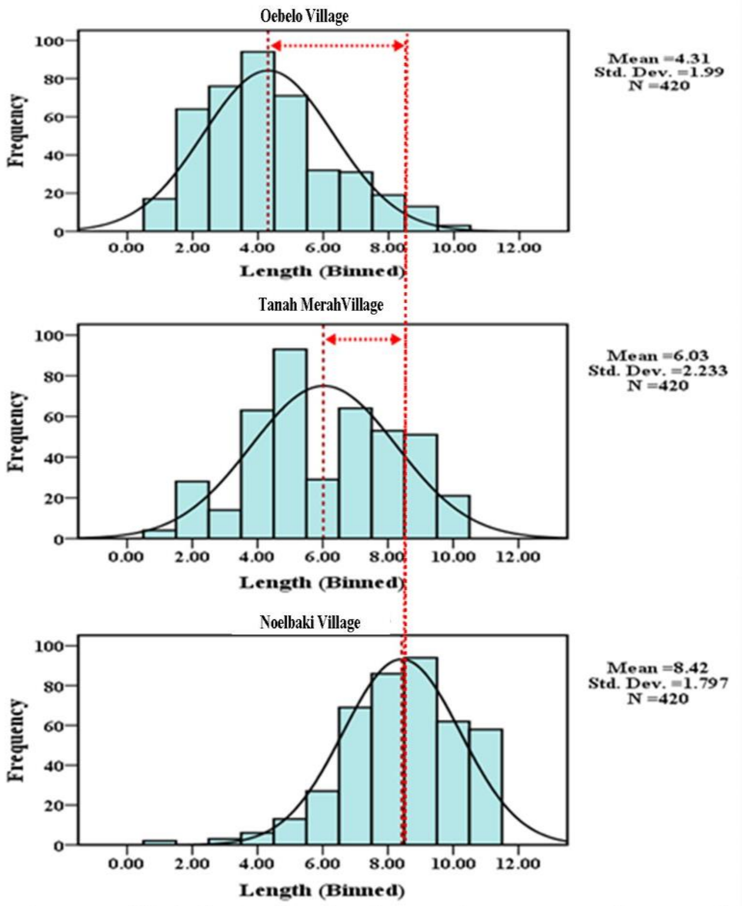

Fig-1: Mode of the Length Frequency Distribution of Blood Cockle

The linear regression equation between the three villages i.e. Y = 0.50X - 5:57 (R2 = 0.66) for the Oebelo Village, y = 0.76X - 12 072 (R2 = 0.427) for The Tanah Merah Village, and y = 0.34X - + 1.02 (R2 = 0:17) for the Noelbaki Village. It means that based on the diversity of length and weight of blood cockle, the model can explain the relationship between the length and weight of blood cockle equal to 66%, 42,7% and 17.0% respectively. The result of the student's t-test showing that the intercept and the gradient of the regression equations was significant (P <0.05) between one village to another (Figure 2).

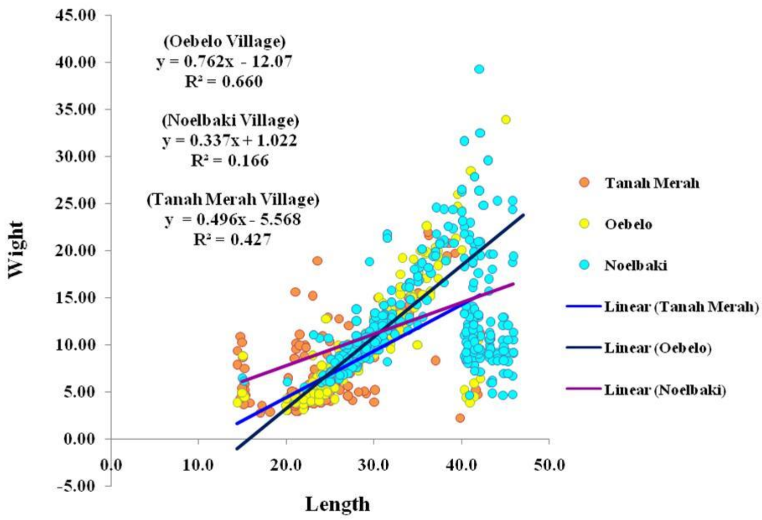

Fig-2: Relationship between the Length and Weight of Blood Cockle

The result of analysis of variance showed that the average value of the total length and also weight of blood cockle significantly different (LSD, P<0.05) among the three locations in Kupang Bay (Figure 3). Whereabouts length and also weight of blood cockle in the Noelbaki Village were significantly higher than in the Tanah Merah Village. Similarly, blood cockle in the Tanah Merah Village were significantly higher than in the Oebelo Village.

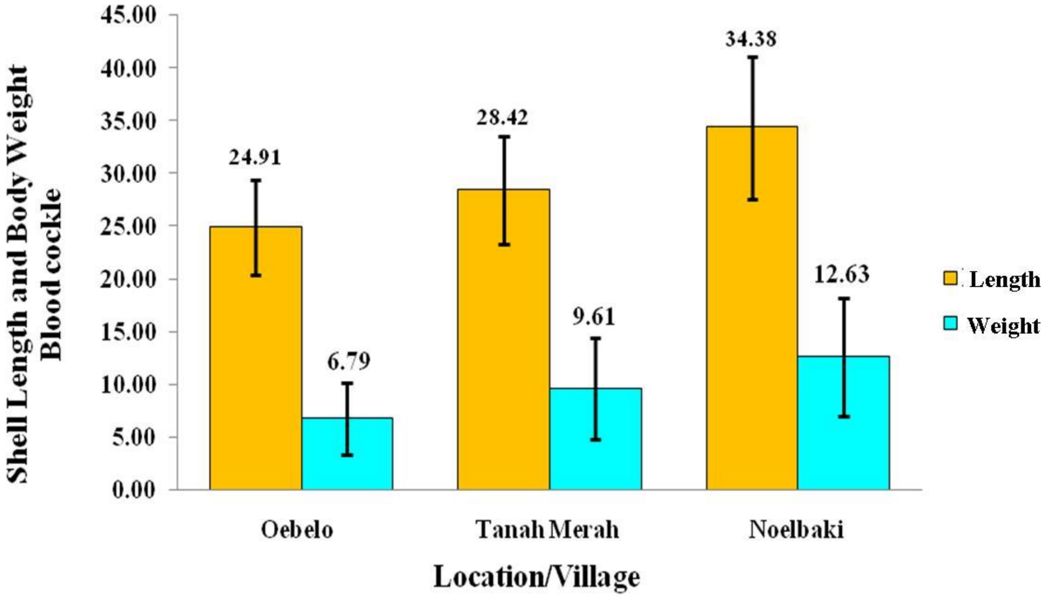

Fig-3: The Average Values of the Length and Weight of Blood Cockle among Three Locations

This result indicated that the length size of blood cockle reduced in line with the longer of exploitation period. Reduced by blood cockle length is negative impact of exploitation [3]. The negative impact happened at least three reasons, i.e. under conditions no conservation action, and over-exploitation of large size blood cockle that decreased in the population and disadvantaged its smaller [4]. The other reason is genetic decreased, that led to a decrease in blood cockle size due to the selective fishing that targeting only large-sized, while the most of large blood cockles have a pre-eminent phenotype. This condition was causing degradation in genetically quality [10].

Condition Index

Results of analysis of variance (ANOVA) showed that the conditions index of blood cockles was significantly different (P<0.05) among the three locations in Kupang Bay. The conditions index of blood cockles in the Noelbaki Village was higher than in Tanah Merah Village or Oebelo Village (Figure 4).

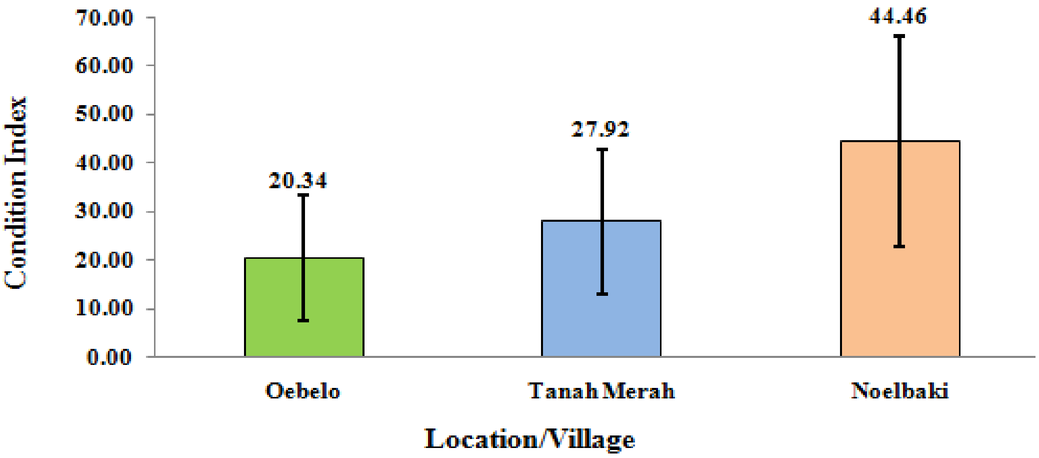

Fig-4: Condition Index of Blood Cockle among Three Location

The differences from conditions index are exempted cause by differences in the availability of blood cockle food. There is a positive correlation between the availability of food in the waters and the condition index of blood cockle *Anadara inaequivalvis* [11]. This assumption is strengthened by data of particulate organic matter (POM) that showing the highest of the gradient range in the Noelbaki Village (0.0168 ± 0.0090 mg/l) followed by Tanah Merah Village (0.0029 ± 0.0008 mg/l), and the lowest in Oebelo Village (0.0028 ± 0.0004 mg/l). When POM or chlorophyl-a concentration was high, hence blood cockle accumulates nutrient reserve especially glycogen and protein. The other factors that influence the index condition are water quality, stress, disease, reproduction, and sex. The disease in blood cockle is not efficient in managing energy and the budget deficit, which impact on the decline of index condition. Similarly, the stress associated with extreme changes in water quality or pollution, often makes bivalves reduce food-making activity resulting in lower of index condition, although food is available in abundance in nature [8].

Gonadal Index

The results of analysis of variance (ANOVA) showed that there was significantly different (P <0.05) of blood cockle gonadal index both in male and female among the three villages in the Village Noelbaki Kupang Bay Village, Tanah Merah and Village Oebelo. The highest of gonadal index values were in Noelbaki Village (13.783 in male and 16.094 in female), followed in Tanah Merah Village (11.358 in male and 13,268 in female), and the lowest is in Oebelo Village (9.728 in male and 11.935 in female) (LSD, P <0.05) (Figure 5).

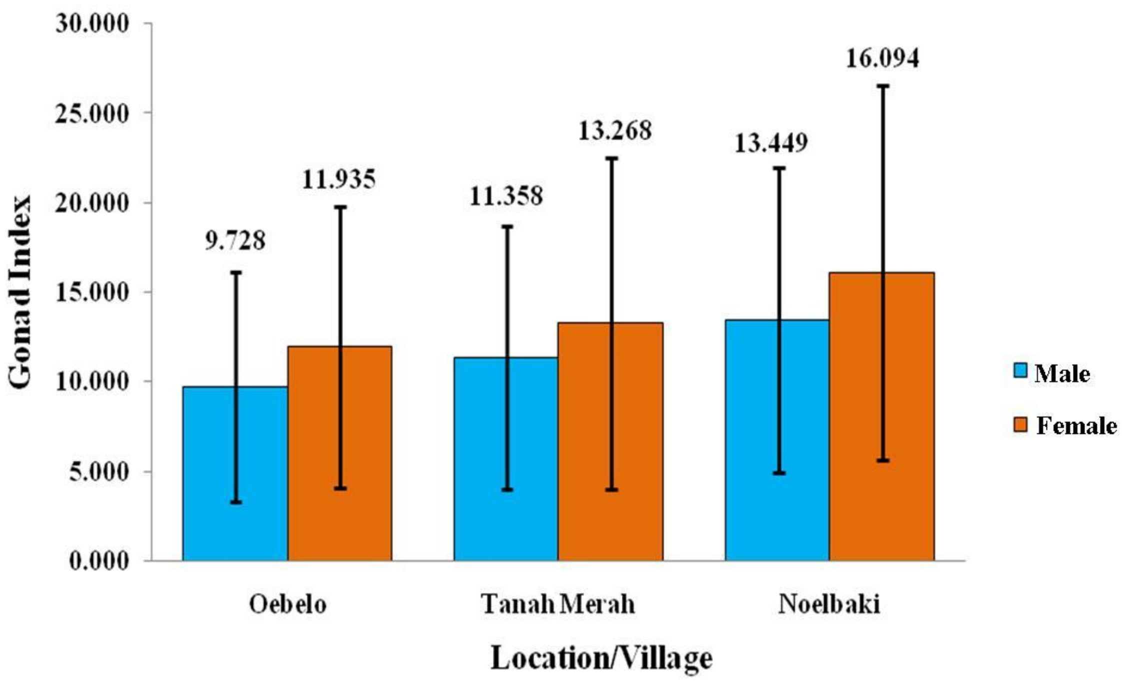

Fig-5: Gonadal Index of Blood Cockle among Three Locations

Differences in gonadal index value indicate the level difference of gonadal maturation. The higher of gonadal index value indicating that it's getting closer to the level of maturity before spawning [8]. Gonadal maturation in bivalves is determined by environmental factors such as temperature and food availability. However, in tropical regions usually relatively uniform temperature throughout the year, so it was not a major factor for reproduction in blood cockle [12]. Therefore, in this study the availability of food for the main role of the intensity of the gonadal index. The highest of gonadal index in the Noelbaki Village along with the content of POM that was higher than other blogs.

Sex Ratio

There are differences in the amount of blood cockle blood of males and females ($\chi 2$, P <0.05) between the three locations in Kupang Bay. The sex ratio of males and females of various length classes generally in the ratio 1:1 (Table 1). Although there is a tendency that the males of blood cockle more on the little size of shell length and the females more on the large size.

Table-1: Sex Ratio of Blood Cockle among Three Location

No	Shell Length (mm)	Oebelo				Tanah Merah				Noelbaki			
		U	♂	♀	χ^2	U	♂	♀	χ^2	U	♂	♀	χ^2
1	13 – 15,9	17	0	0	0.0	4	0	0	0.0	1	0	0	0.0
2	16 – 18,9	0	53	11	11.8*	0	21	7	7.8*	1	0	0	0.0
3	19 – 21,9	0	57	19	5.6*	0	10	4	2.9	0	3	0	3.4*
4	22 – 24,9	0	67	27	3.5*	0	44	19	11.4*	0	6	0	4.2*
5	25 – 27,9	0	40	31	0.9	0	52	41	2.0	0	9	4	0.6
6	28 – 30,9	0	16	17	2.5	0	16	13	0.5	0	15	12	0.1
7	31 – 33,9	0	9	22	14.2	0	25	39	2.3	0	43	26	0.3
8	34 – 36,9	0	6	13	7.4*	0	17	36	5.8*	0	44	42	2.1
9	37 – 39,9	0	2	11	10.8*	0	11	40	14.9*	0	39	55	11.7*
10	40 – 42,9	0	0	3	4.9*	0	6	15	3.4*	0	41	21	1.4
11	43≤	0	0	0	0.0	0	0	0	0.0	0	46	12	10.0*
Total		17	250	154	61.6	4	202	214	51.0	2	246	172	33.8

*) Significantly affected (χ^2, P<0.05)

Where:
U : Undetermind
♂ : Male
♀ : Female
χ^2 : Chi-Square

The result of the study supports the theory that the blood cockle species is sequential hermaphroditism or changed sex from male to female (protandry). Nevertheless, this change is unclear in limit so both sexes are always present in the same size [13]. Based on the data in three location was known that the shell size 13.0 to 27.9 mm towards males. Instead, the female of blood cockle was dominant in shell length > 31 mm. Deviation of sex ratio is the natural consequence of species that undergo sex reversal [9, 14].

Size of the First Sex Mature

The results of histological analysis in three locations showed that the existence of gonads in various phases of maturity, i.e. active beginning (phase I), active (phase II) and active end (phase III) (Figure 6, 7 and 8). In male, the active beginning (phase I) is a resting phase, where the gonad only covered by connective tissue and only empty follicles are visible and cannot be determined gender; the active (phase II) is a developing phase, which at this stage the presence of spermatozoa can be seen clearly; and the active end (phase III) is a phase of maturation of gametes (maturing phase), which the spermatozoa occupies the entire follicle, reaching entire the tissue, and the connective tissues are start to disappear. In females, the active beginning (phase I) is a resting phase, where the gonad is covered by connective tissue and no reproductive activity, so it impossible to determined sex; the active (phase II) is a developing phase, which is histologically characterized by the development of the follicle and oocytes is begin developed; and the active end (phase III) is the stage of maturation of gametes (maturing phase), that is histologically the size of follicle growing larger and containing the mature oocytes [5, 12].

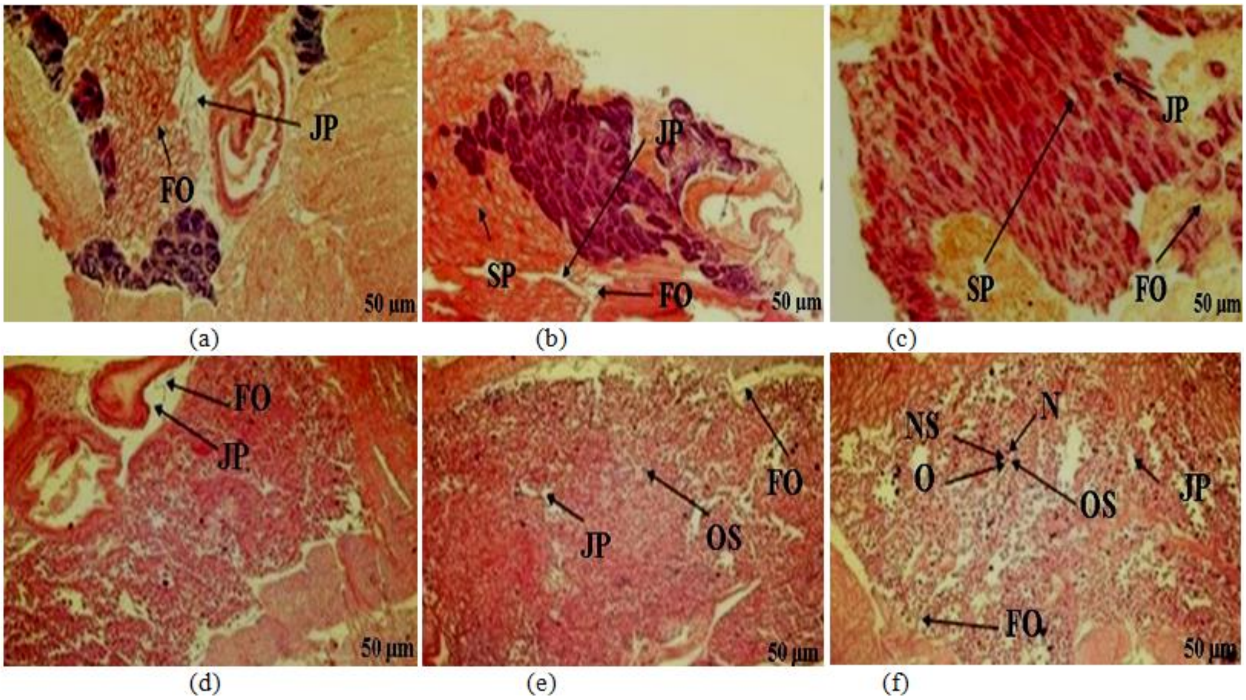

Fig-6: Gonad Maturity of Blood Cockle in the Oebelo Village

Where:

(A) Males in active beginning /phase I (17 mm in length), (b) Males in active/phase II (21 mm in length), (c) Males in active end/phase III (25 mm in length), (d) Females in active beginning/phase I (31 mm in length), (e) Females in active/phase II (33 mm in length), (f) the Female in active end/phase III (31 mm in length)

FO = follicles, JP = connective tissue, SP = sperm, O = Ovum, OS = oocytes, N = Nucleus, NS = nucleolus

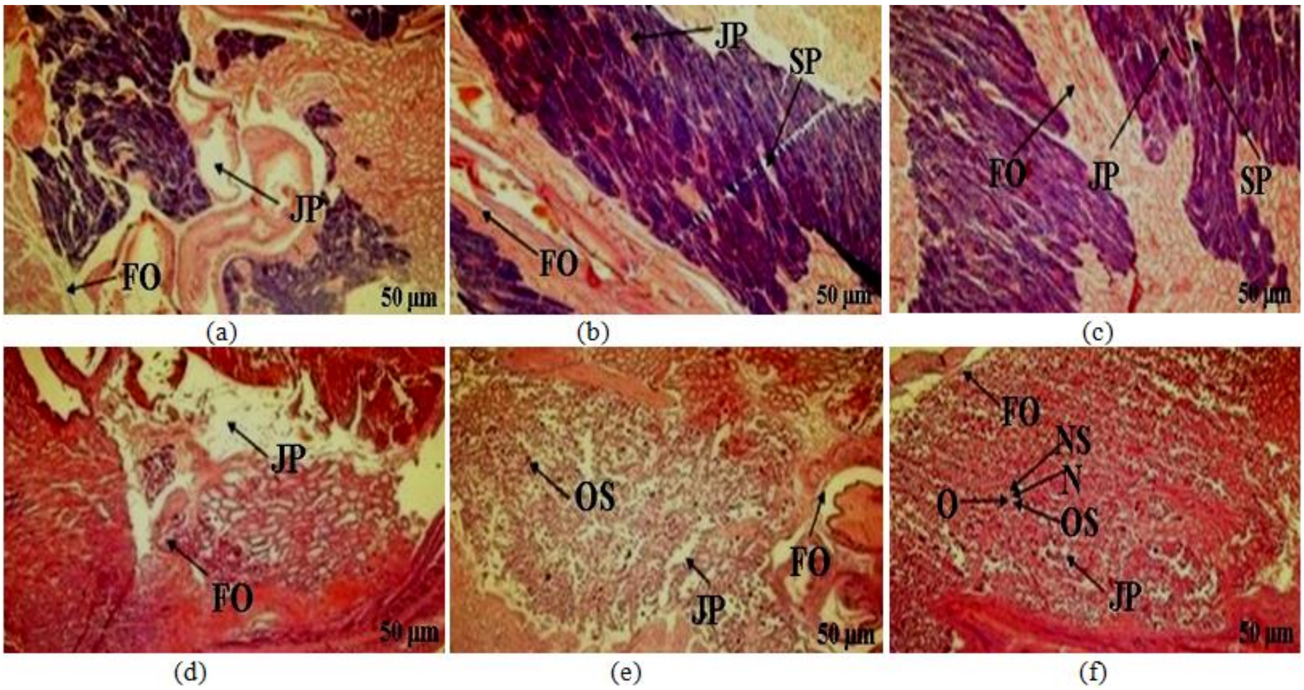

Fig-7: Gonad Maturity of Blood Cockle in the Tanah Merah Village

Where:

(A) Males in active beginning /phase I (19 mm in length), (b) Males in active/phase II (26 mm in length), (c) Males in active end/phase III (28 mm in length), (d) Females in active beginning/phase I (31 mm in length), (e) Females in active/phase II (33 mm in length), (f) the Female in active end/phase III (37 mm in length)

FO = follicles, JP = connective tissue, SP = sperm, O = Ovum, OS = oocytes, N = Nucleus, NS = nucleolus

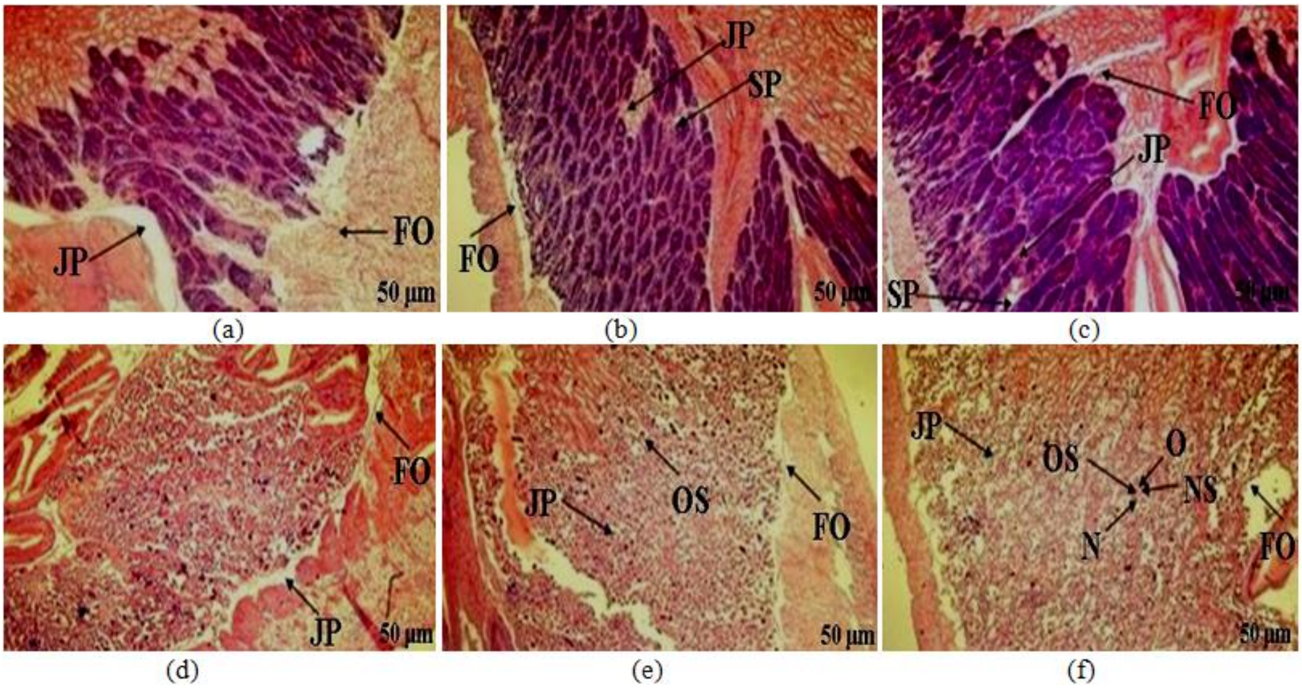

Fig-8: Gonad Maturity of Blood Cockle in the Noelbaki Village

Where:

(A) Males in active beginning /phase I (23 mm in length), (b) Males in active/phase II (31 mm in length), (c) Males in active end/phase III (34 mm in length), (d) Females in active beginning/phase I (34 mm in length), (e) Females in active/phase II (38 mm in length), (f) the Female in active end/phase III (41 mm in length)

FO = follicles, JP = connective tissue, SP = sperm, O = Ovum, OS = oocytes, N = Nucleus, NS = nucleolus

The results of histological analysis of blood cockle in these three locations showed the size of the first sex maturity. The blood cockles in Tanah Merah Village and Oebelo Village were recognizable their sex in the length class 13.0 to 15.9 mm, while in Noelbaki Village was in the length class 19.0 to 21.9 mm. Under that length class, the gonad was not yet distinguishable. It showed that in Tanah Merah Village and Oebelo Village (older in exploitation), cockles were maturing earlier than in Noelbaki Village (newer in exploitation). These results are similar with blood cockle in Central Java, where in order of exploitation location sex, maturity was in length class 15.6 to 15.7 mm [5].

Pressure on cockle fishing, especially on large individuals was triggering the smaller individuals increasing their reproductive investment and early gonadal maturity [15]. In the other species like crab, when the large size of males decreased by fishing activities, then the smaller size males effort to increase their reproductive frequency through early sex maturity [16].

Sex reversal in blood cockle occurs when the proportion of female population at least 50% [9]. Related to that statement, hence the genital transition of blood cockle from female to male in Kupang Bay occur in the length class 28.0 to 30.9 m. Under that length class, males of blood cockle dominate the population. Instead, above of that length class the females of blood cockle were dominant than males. However sex reversal also depended on a genetically [13], where the same of length class in sex reversal indicated that blood cockle at three locations come from the populations or sub-populations.

CONCLUSION

This study found that aspects of morphology, blood based on the observation size its morphometric covering shell length, weight, condition index, index of gonad in the village Oebelo and the village of Tanah Merah (long period of exploitation) have the size or value lower than blood cockle blood in the village Noelbaki (not long exploitation period). In addition, based on observations of aspects of reproduction, also found that the blood cockle blood in the Village Oebelo and the village of Tanah Merah that had long exploited more dominated by blood cockle males of the shells females, undergo a process of mature sex and mature gonads early and intermediate gender from male to female faster than blood cockle blood in the village Noelbaki recently exploited.

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