ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

HERMAPHRODITISM IN A*nadara granosa* (L.) and *Anadara antiquata* (L.) (BIVALVIA: ARCIDAE) FROM CENTRAL JAVA

Norma Afiati *)

Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, 50275
Central Java, Indonesia

Received : April, 25, 2007 ; Accepted : May, 25 , 2007

ABSTRACT

Gonad maturation and sexuality in Central Java populations of A. granosa and A. antiquata were studied by means of macroscopic examination of the visceral mass, microscopic examination of smears of gonadal products and by histological technique. In this study, hermaphrodites occurred extremely rarely, i.e. less than 1.5% for A. granosa and less than 1% for A. antiquata, were observed with both male and female gametes present within the same individual follicles. Unbalanced sex ratios derived from size frequency distribution of the sample revealed that the increased percentage of females with increasing size suggest the occurrence of sequential protandric hermaphrodite with only a single sex change during their life history, i.e. from male to female. The preponderance of juvenile males within the Wedung population should be seen as the advantage of firstly being male, since some energy could be saved and redirected towards somatic growth because in Bivalvia there is a trade-off between growth and reproduction.

Keywords: *A. granosa*, *A. antiquata*, sequential protandric hermaphroditism.

***Correspondence:** Phone :+62-24-76480685, fax: +62-24-7474698, e-mail : normaafiati@yahoo.com

INTRODUCTION

Sex change in lamellibranchs has been a subject of scientific interest since the 17th century (Coe, 1943). Information regarding the basic reproductive biology of the family Arcidae has rarely been published. The recognition of the potential of species in this family for aquaculture has lead to investigation of basic reproductive biology followed by attempts at artificial induction of spawning. The main subjects of such studies have been *Anadara broughtoni* (Kan-no, 1963; Kim & Koo, 1973), *A. subcrenata* (Ting et al, 1972) and *A. senilis* (Yoloye, 1974; Yankson, 1982). However, probably the most important species in this genus, in terms of quantity landed annually in many countries, is *A. granosa* which is the subject of both fishery and ecologically important species. Brief accounts provided by Pathansali (1966) and Broom (1983) for *A. granosa* and by Toral-Barza & Gomez (1985) for *A. antiquata* being the most recent contribution - yet did not describe the possibility of hermaphroditism which may occur in these particular species. An investigation of the two species *A. granosa* and *A. antiquata* – common arcids in many beaches and estuaries in Indonesia – was therefore undertaken to determine the possible occurrence of sex changes among these relatively primitive bivalve.

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

MATERIALS AND METHODS

Brief Description of Study Sites

A. granosa were collected from two sites, *i.e.* Wedung in the Demak Regency, some 50 km from Semarang, and Tirang Cawang Island in the Tapak village, *ca* 20 km from the city of Semarang. Both sites are semi-sheltered water body within a large bay with some rivers and rivulets opening into the bay, thus resulting in considerable local deposits of mud and high turbidity. The bottom sediment is composed of fine mud and surrounded by a dense mangrove swamp consisting mainly of *Avicennia* sp and *Rhizophora* sp. For *A. granosa*, each site was purposely selected in order that comparisons between disturbed population but relatively undisturbed environment (Wedung) and undisturbed population but disturbed environment (Tapak) could be made (Afiati, 1994). Meanwhile, *A. antiquata* was taken from Bandengan in the Jepara Regency. Sampling area in Bandengan was sandy-muddy sediments mixed with coral rubble. The average depth during low tide was 0.6m. *A. antiquata* which burrow in the sediments with their weak byssus, are found at a depth of approximately 2-6cm.

Sampling of those blood clam populations was conducted at approximately monthly intervals at low water and daytime over 24 months within the period of August 1991 - August 1993.

Treatment of Samples of Blood Clams for Histology Preparation

Since the smallest individual with distinguishable reproductive tissues was 15.7mm and 23 mm for *A. granosa* and *A. antiquata* respectively, any undifferentiated clams longer than 20 mm for *A. granosa* or 25 mm for *A. antiquata* presumably represented mature individuals (Afiati, 1994). For determination of the sex ratio in the adult populations, 50 randomly selected adult clams ranging from 15-40mm in shell length were opened at the hinge by cutting the adductor muscles, and sexed by microscopic examination of fresh smears of the gonad. No spawning occurred between the time of collection and the subsequent processing of the tissues (6-8 hours). Adult specimens whose sexes could not be established by the above procedures were processed along with those (20-25 individuals) selected purposively for histological examination. Following the routine protocols in Disbrey and Rack (1970), the whole tissue was fixed for 8 hours in saline (30%) Bouin's solution. A piece of tissue was excised transversely through the body mass comprising the digestive gland, reproductive tissue and muscular foot, dehydrated using increasing concentrations of ethanol, then transferred to xylene prior to embedding in paraffin wax (56°C MP). Sections were cut at 7µm, re-hydrated in descending ethanol series before staining in aqueous Haematoxylin-Eosin and mounted in Canada balsam (DPX). Following microscopic examination, histology of the reproductive tissues was photographed.

RESULT AND DICUSSION

General Observation on Gonad Maturation

Some hermaphrodite individuals were encountered for *A. granosa* and *A. antiquata* in the present study. All had male and female tissue simultaneously in the same follicle rather than a separate testis and ovary. Plate 1 illustrates that hermaphrodite of both *A. granosa* and *A. antiquata* discharged sperm whilst starting to develop oogonia. Quite often this commencement is not apparent, as only one or two oogonia slightly bigger than the spermatogonia and less intensely stained appeared marginally in the follicle wall. At

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

this stage, the animals seemed not to produce a new layer of spermatogonia, but continued with the ripening process of spermatozoa. By the time the spermatozoa had been almost totally discharged, the oogonia reached stage two developing (Afiati, 2007). This is the last stage evident from the sections in this study. Since no evidence is available for the converse, *i.e.* females becoming male, they presumably then continued the progress as pure female individuals, because all large and the largest specimens of both species (70-83.5mm shell length) were all females. They retained their reproductive tissue even though specimens of smaller size were completely empty (Afiati, 1994, 1999a & 1999b).

Sexuality and Sex Ratio

Both species of *A. granosa* and *A. antiquata* are dioecious (Afiati, 2007). In this study hermaphrodites occurred in only 1.43% and 1.45% (6 out of 420 and 4 out of 276 individuals) for the populations of *A. granosa* in Wedung and Tapak respectively. For *A. antiquata* in Bandengan the figure is somewhat lower, i.e. 0.84% or 3 out of 356 individuals.

Apparently both species have a protandric type of development in which a primary male phase precedes the adult stages until both sexes are approximately equally represented, after which sex reversal takes place. Figure 1 supports this suggestion at the population level. In Wedung and Bandengan, the overall ratio of males to females is 1.49:1 (χ^2= 120.08; $P<0.001$, df= 7, n= 2336) and 1.47:1 (χ^2= 51.13; $P<0.001$, df= 6, n= 2704) respectively whereas in Tapak it is 0.76:1 but in favour of the females (χ^2 =49.90; $P<0.001$, df= 6, n= 508). So, in all populations there was a significant departure from a 1:1 ratio. Only when *A. granosa* attained a size of 30-35 mm in shell length did the sex ratio approach 1:1; for *A. antiquata* this occurred when the animals were larger in size, i.e. 40-45 mm long (Figure 1).

The histograms in Figure 1A show that in the Wedung population, the mode for male individuals is 25 mm, thereafter the number of male *A. granosa* decreased rapidly at 30 mm onwards, whereas the number of females remained constant from 25mm until they are at about 40 mm long. Similarly in Tapak, the mode for males is at 35 mm, and 40 mm for females (Figure 1B). In Bandengan, although the mode for male and female *A. antiquata* appeared to be the same at 35 mm, but only when they attained a size of 40-45mm did the sex ratio approach 1:1 (Figure 1C). It was also noteworthy that in Wedung and Bandengan, the number of male blood clams is always higher than that of the females but this was not the case for the Tapak population where samples always consist of large, female individuals. This may be attributed to the low recruit of natural spat of *A. granosa* in Tapak due to the disturbed environment as the result of industrial discharge to the River Tapak (Afiati, 1994, 1999a).

Sex Changes in A. granosa and A. antiquate

The blood clam *A. granosa* (Pathansali & Soong, 1958; Pathansali, 1964; Broom, 1983; Wong et al, 1985) and *A. antiquata* (Toral-Barza & Gomez, 1985) has separate sexes but is not sexually dimorphic. However, all these authors reported that the sexes appear to be in an approximately 1:1 ratio and no sex changes have been reported thus far. The results of the present study found that the overall ratio of males to females in Wedung is 1.49:1 but in Tapak it was 0.76:1 in favour of females. Apparently a protandric type of development occurred in Wedung and Tapak as there was evidence of a change in sex ratio with size. In both populations, the majority of 15-30mm clams are males. The sex ratio shifts to become 1:1 when the animals were between 30-40mm in length and by the time they attained a size over 45mm the

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

populations were dominated by female individuals (Figures 1A, 1B).

Approximately 96% of bivalves have been reported as being gonochoristic (Morton, 1963), the remaining 4% exhibit one or more of the following grades of hermaphroditism: ***1)*** simultaneous hermaphroditic (ambisexual) species in which: **a)** sperm and ova are formed in different region of the same gonad, *e.g*. most Pectinidae, some Tridacnidae and many members of the Unionidae, and **b)** a distinct ovary and testis open separately on either side for instance in many members of the order Anomalodesmata, ***2)*** Sequentially hermaphroditic species have one sex change in their life history (or many, *e.g*. Ostreidae) and are generally protandric, *e.g. Venus mercenaria* (Loosanoff, 1937) and *Astarte sulcata* (Saleuddin,1964).

Lucas (1975) showed that the percentage of hermaphrodites amongst juvenile bivalves may be quite high, for instance, 23% for *Venerupis decussata* (L.), 44% for *Venus striatula* (da Costa) and 72% for *Glycymeris glycymeris* (L.). These proportions are all relatively large and therefore important. However, it was not reported whether those hermaphrodite juveniles survived to become adults. Subsequently, Broom (1983b) found evidence of only 0.33% (1 out of 300 specimens, size not indicated) hermaphrodites amongst *A. granosa* in West Malaysia. In comparison to that, the figures of 1.43% and 1.45% for hermaphrodite individuals in the Wedung and Tapak populations were still within the range reported for other populations.

Narasimham (1988) in India found no hermaphrodite *A. granosa* individuals over 4200 specimens examined throughout a four years study (3840 were from smear and 360 from histological preparation) and that the sex ratio did not differ from 1:1. From the data presented by Narasimham (1988) it is obvious that for small mature individuals ranging from 22-28mm, the occurrence of male clams was much higher than females (3:1 or 99:33 individuals), and that 82.1% clams of 18-20mm were male as no female individuals were encountered. Furthermore, in accordance with the findings in the present study, males outnumbered females in the first 22-38mm groups, females dominated in the rest of the length groups (40-62mm) and at 70mm all were females. Yet, somehow the author calculated that the sex ratio was not different from 1:1.

Kastoro (1978) reported an occurrence of 1.2% hermaphrodite individuals with a male to female sex ratio of 1:1.4 in a population of *A. antiquata* in West Java, Indonesia. On the contrary, the male/female sex ratio recorded for *A. antiquata* in the present study is 1.47:1. Surprisingly, of the 1,040 of *A. antiquata* examined, Toral-Barza and Gomez (1985) in the Philippines did not find any hermaphrodites. They noted that there were more males than females although the sex ratio did not differ from 1:1. Similarly, Kayombo and Mainoya (1987) in Tanzania established that the ratio for *A. antiquata* did not depart from 1:1 (1:1.13, 253 individuals). Yet from one of their graphs (Figure 6 in Kayombo & Mainoya, 1987), it is obvious that over the size range 26-30mm the percentage of male individuals was >90% whilst the female was <10%. The proportions approached each other as the clams grew to a larger size and the ratio becomes exactly 1:1 amongst clams of 41-45mm shell length. These observations were remarkably similar to the findings of the present study.

Moreover, Kayombo and Mainoya (1987) have also found a higher percentage of hermaphrodites in *A. antiquata* (i.e. 9.88% compared to only 0.84% in Bandengan). They reported that examination of gonad smears misidentified these hermaphrodites as functional females, before detailed histological sections revealed that they were in fact hermaphrodites. Here, the hermaphrodites were macroscopically misidentified as male clams of 32-42mm with whitish coloured gonads, but the sections revealed that a set of oogonia develop simultaneously and the blood clams rapidly make a quick transition to females without the follicles being in regression.

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

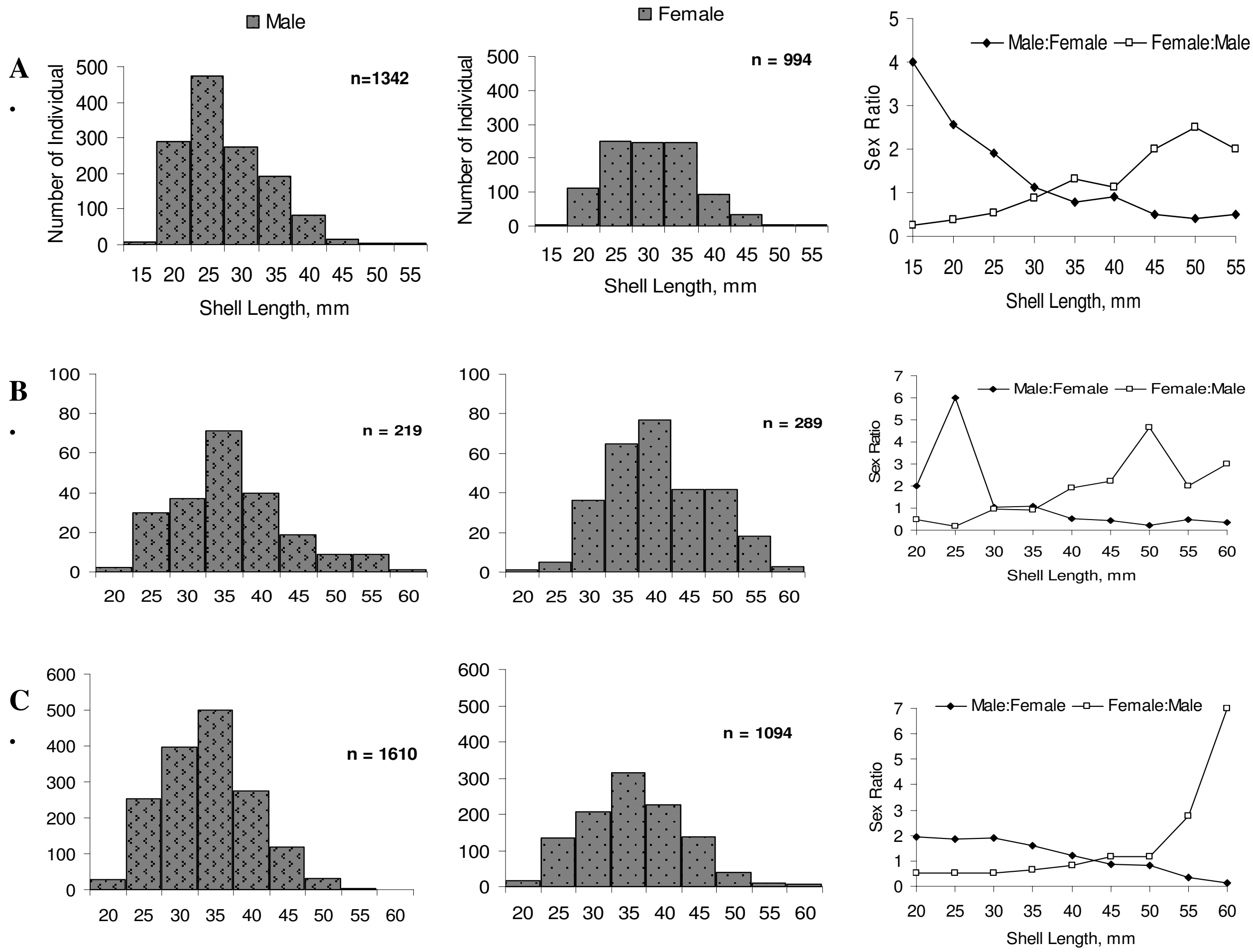

Fig 1. Size frequency distribution and sex ratios of male and female *A. granosa* and *A. antiquata* collected during 24 month period from the populations in: A) Wedung, *A. granosa* (n = 2336) , B) Tapak, *A. granosa* (n = 508), C) Bandengan, *A. antiquata* (n = 2704). Only individuals whose sexes can be confidently determined from external appearance of their gonads were used to construct the histograms. For all populations, the sex ratios depart from 1:1 with 99% level of confidence.

So, despite their small number which is insufficient to satisfy a hypothesis of occasional random sex change by many or all members of the population, these simultaneous hermaphrodites in both *A. granosa* and *A. antiquata* could be interpreted as transitional phases of a protandrous sequential hermaphrodite.
Yoloye (1974) working on specimens of *A. senilis* from the Nigerian coast, reported that this Species is a protandrous hermaphrodite and that all young specimens become functional males when only one month old, and, judging from the information given, 5-9mm in length. From the sixth month, many specimens appeared to be hermaphrodites and from the end of the first year the sex ratio had become 3.17:1 and remained more or less constant in the adult populations. By contrast, Yankson (1982) who studied

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

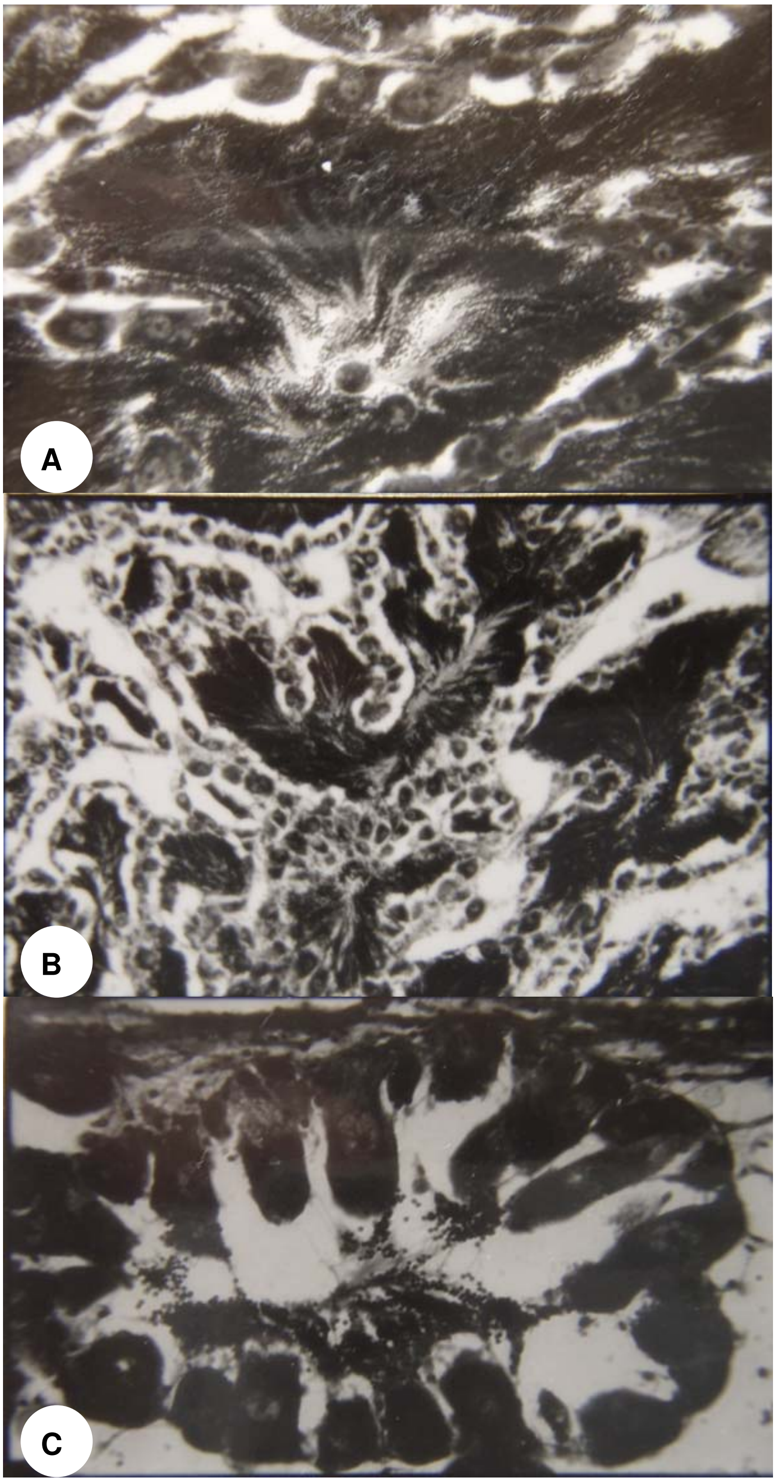

Fig 2. Photomicrographs of sectioned gonads of *A. granosa* and *A. antiquata*. A) Hermaphrodite individual *A. granosa* discharging its sperm whilst starting to develop the female reproductive tissue, x250. B) The appearance of hermaphrodite *A. antiquata* with a layer of fusiform oogonia flattened to the follicle wall, x100. C) Further stage in sex changing in hermaphrodites *A. granosa*. Oogonia become elongated whilst a few residual sperms were still in the centre of the follicle, x400.

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

Ghanaian populations of *A. senilis*, reported a completely different situation. Hermaphrodites occurred extremely rarely, *i.e.* 5 out of 1,448 and 3 out of 313 individuals in the two lagoon populations studied. There was no evidence of protandric nor, under the normal conditions of salinity, did the sex ratio deviate significantly from 1:1. However, in closed lagoons where, in the hot, dry season salinities rose to 50‰ and surface water temperatures were 32-34°C, the sex ratio did deviate significantly from 1:1, the actual observed ratio being close to 1:2 in favour of the females. Furthermore, primary gonads in this species begin to differentiate when the animals are 10-12mm long and the first spawning takes place at a length of approximately 20mm.

Little is known about the sex determining mechanism of bivalves and molluscs in general. So far it is known that there are no morphologically distinguishable sex chromosomes and it is thought that hormone balance determines whether an individual changes sex (A. Beaumont, pers. comm.). Allen & Guo (1994) suggested that the dwarf surf-clam *Mulinia lateralis* is more likely to have an XX and XY (like many vertebrates) than an XX: 2A and XY: 2A (as in the fruit fly *Drosophila* sp.) sex determining mechanisms. In line with other findings of Allen and Guo (1994), karyotypes in both *A. granosa* and *A. antiquata* have shown no evidence of heteromorphic sex chromosomes (Afiati, 1999a, 1999b). Nevertheless, development as a male is considered to require less energy than as a female (Calow, 1983; Russell-Hunter & McMahon, 1975). For example, one germ cell will produce four sperm which largely consist of a nucleus surrounded by cytoplasm specialised for locomotion and penetration of the egg. In females, one germ cell will develop only to become one large oocyte with energy-rich yolk and cytoplasm. So, presumably the advantage of firstly being male is that some energy could be saved and redirected towards somatic growth because there is a trade-off between growth and reproduction (Calow, 1983; Seed & Brown, 1977b). This could perhaps explain the preponderance of juvenile males within the Wedung population.

CONCLUSION

It could be concluded that sex ratio varied from one to another Anadarinid populations. Whereas hermaphroditism in Bivalvia showed a tremendous discrepancy, *i.e.* from 72% for *Glycymeris glycymeris* to 0.33% in *A. granosa* from West Malaysia; even in some populations it was reported as none. In this study hermaphroditism occurred extremely rarely, *i.e.* only 1.43% and 1.45% for the populations of *A. granosa* in Wedung and Tapak respectively, and for *A. antiquata* in Bandengan the figure is even lower, *i.e.* 0.84%. Both species studied performed a sequential protandric hermaphroditism in which species have one sex change in their life history, in particular from male to female.

ACKNOWLEDGMENT

Part of this paper covers the work conducted for the author's PhD degree at the University of Wales Bangor, UK. I wish to express my sincere gratitude to Prof. R. Seed, Dr. C. Richardson, and Prof. G. Walker of the School of Ocean Sciences University of Wales Bangor for their encouragement and fruitful discussion throughout the study. My sincere thanks to the Late Prof. Nurjaman of the School of Medicine Diponegoro University, Semarang, for his suggestions and expertise on histological preparation during the course of the study. The author is also grateful to the Marine Science Education Project for financing most of this study. All the photographic works was conducted by the author.

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

REFERENCES

Afiati, N. 1994. The Ecology of Two Species of Blood Clams *Anadara granosa* (L.) and *Anadara antiquata* (L.) (Bivalvia: Arcidae) in Central Java, Indonesia. Unpubl. PhD Thesis, Univ. of Wales Bangor, 260pp

Afiati, N., 1999a. Cytoplasmic Granules in the Red Blood Cells and the Karyotype of Rounded Ecomorph of *Anadara granosa* (L.) (Bivalvia: Arcidae) from Central Java, Indonesia, *Majalah Ilmu Kelautan IV* (14): 51 - 59

Afiati, N., 1999b. The Chromosomes of *Anadara antiquata* (L.) (Bivalvia: Arcidae) from Central Java, Indonesia, *Majalah Ilmu Kelautan IV* (15): 136 - 144

Afiati, N., 2007. Gonad Maturation in *Anadara granosa* (L.) and *Anadara antiquata* (L.) (Bivalvia: Arcidae). Journal of Coast.Dev. 10(2).

Allen Jr., S.K. & X. Guo, 1994. More Light on Sex Determination in Bivalves from all-female, Gynogenetic *Mulinia lateralis* Say. Abstract of Technical Papers in Annual Meeting of National Shellfisheries Assoc., Charleston, South Carolina 24-28 april 1994. J. Of Shellfish Res. 13 (1): 269-306

Broom, M.J., 1983. Gonad Development and Spawning in *Anadara granosa* (Bivalvia: Arcidae), Aquaculture 30: 211 – 219

Calow, P. 1983. Life-cycle Patterns and Evolution, _in_: The Mollusca vol. 6 Ecology (W.D. Russell-Hunter ed). Academic Press, Inc. London, p: 649-678

Coe, W.R. 1943. Sexual Differentiation in Molluscs. I. Pelecypoda. Quart. *Rev. Biol* 18: 154-164

Disbrey, B.D. & J.H. Rack, 1970: Histological Laboratory Methods. E & S Livingstone, London, 414pp

Kan-no, H., 1966: Bottom Environment of the Ark Shell, *Scapharca broughtoni* (Schrenck), in Sendai Bay, Report from the North East Aquatic Centre 26: 55 – 75

Kastoro, W. 1978. Reproduksi Kerang Bulu *Anadara antiquata* (Linnaeus), Suku Arcidae. Oseanol. Indones., 9: 51-59

Kayombo, N.A. & J.R. Mainoya, 1987. The Biology of the Bivalve *Anadara antiquata* from the Dar es Salaam Coast. Kenya J.Sci. (B) 8 (1-2): 105 – 119

Kim, J.D. & J.H. Koo, 1973 Studies on the Seedling Production of the Ark, *Anadara broughtoni* (Schrenck) in tank. (1) Bull. *Fish. Res. Develop. Agency. Pusan 11*: 71-78

Loosanoff, V.I., 1937. Development of the Primary gGonad and Sexual Phases in *Venus mercenaria* Linnaeus. Biol. Bull. 72: 389 – 405

Lucas, A., 1975. Sex Differentiation and Juvenile Sexuality in Bivalve Mollusks. Proc. VIII European Symp. on Marine Biology, Sorento, Naples, 1973. (Supplementary): 532 – 541

Morton, J.E., 1963. Molluscs, 2nd ed. Hutchinson University Library, London. 229 pp

Narasimham, K.A., 1988. Biology of the Blood Clam *Anadara granosa* (Linnaeus) in Kakinada Bay. J. *Mar. Biol. Ass. India* 30 (1 & 2): 137 – 150

ISSN : 1410 - 5217
Accredited : 23a / Dikti / Kep / 2004

Pathansali, D. 1964. Notes on the Biology of the Cockle, *Anadara granosa* L., Proc. Indo-Pacific Fish. Coun. 11 (II): 84-98

Pathansali, D. & M.K. Soong, 1958. Some Aspects of Cockle (*Anadara granosa* L.) Culture in Malaya. Proc. Indo-Pacific Fish. Coun. 8 (II): 26 – 31

Russell-Hunter, W.D. & R.F. McMahon, 1975. An Anomalous Sex Ratio in the Sublittoral Marine Snail *Lacuna vincta* Turton, from near Woods Hole. *Nautilus* 89: 14-16

Saleuddin, A.S.M. 1964. The Gonad and Reproductive Cycle in *Astarte sulcata* (da Costa) and Sexuality in *A. elliptica* (Brown). Proc. Malacol. Soc. Lond. 36: 141 - 148

Seed, R. & R.A. Brown, 1977. A Comparison of the Reproductive Cycles of *Modiolus modiolus* (L.) *Cerastoderma* (= *Cardium*) *edule* (L.) and *Mytilus edulis* (L.) in Strangford Lough, Northern Ireland. *Oecologia* 30: 173 – 188

Toral-Barza, L. & E.D. Gomez, 1985. Reproductive Cycle of the Cockle *Anadara antiquata* L. in Calatagan, Batangas, Philippines. *J. Coast. Res*. 1(3): 241 – 245

Wong, T.M., Lim, T.G., Ng, F.O. & Rai H.S., 1985: Induced Spawning and Larval Development in the Cockle, *Anadara granosa* (L.), The Asia Tech. 1985 Conference in Kuala Lumpur 4-7 December 1985

Yankson, K. 1982. Gonad Maturation and Sexuality in the West African Bloody Cockle, *Anadara senilis* (L.). J. *Molluscan Stud*. 48: 294 – 300

Yoloye, V.L. 1974. The Sexual Phases of the West African Bloody Cockle, *Anadara senilis* (L.). Proc. Malacol. Soc. Lond., 41: 25 - 27