

GONAD MATURATION AND SEXUALITY IN THE WEST AFRICAN BLOODY COCKLE, *ANADARA SENILIS* (L.)

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ABSTRACT

Gonad maturation and sexuality in Ghanaian populations of *Anadara senilis* were studied by means of macroscopic examination of the visceral mass, microscopic examination of smears of sexual products and by histological methods. Sexual differentiation into either distinct male or female, begins at a shell length of 10-12mm. Only 1% or less have both ovary and testis. First spawnings take place at shell size 18-20mm. Although in tidal lagoons the sex ratio is 1:1, in the adverse conditions of closed lagoons, the male to female ratio is about 1:2. Unbalanced sex ratios recorded in a population in Lagos, Nigeria, led a previous worker to the conclusion that the species is a protandric hermaphrodite. It is argued here that hermaphroditism is an insignificant feature in *A. senilis* and that the species is basically gonochoristic (dioecious).

"Sex in molluscs has been studied by scientists since the end of the 17th century" (Quayle, 1943), and the reproductive biology of the major groups is well known. Among the class Bivalvia may be found every grade of sexual differentiation, and even species of the same genus may differ in sexuality (Coe, 1943). Approximately 96% of those bivalves whose sexes are known are reported as being gonochoristic (= dioecious) (Coe, 1943; Morton, 1958). These authors report that the remaining 4% of bivalves exhibit one or more of the following grades of hermaphroditism: 1. Simultaneous hermaphroditic (= ambisexual) bivalves in which: (a) sperms and ova are formed in different regions of the same gonad, e.g. most Pectinidae, some Tridacnidae and many species of *Anodonta*; and (b) distinct testis and ovary open separately on either side, e.g. in members of the order Anomalodesmata. 2. Sequentially hermaphroditic bivalves having one sex change in the life history (generally protandric), e.g. *Venus mercenaria* L. (Loosanoff, 1937), *Xylophaga dorsalis* (Turton) (Purchon, 1941) and *Astarte sulcata* da Costa (Saleuddin, 1964).

Two further sorts of sequential hermaphroditism are recognized in the oyster family: (i) rhythmically consecutive sexuality:— in which individuals are protandric, but show a series of alternating male and female phases throughout their life, e.g. the larviparous oysters (*Ostrea edulis* L., *O. lurida* L.). (ii) Alternative sexuality:— in which the sexual phase cannot be predicted for the next season since an individual can either be functionally male or female for two or more successive seasons. This is shown by the oviparous oysters (*Crassostrea virginica* (Gmelin), *C. angulata* (Lamarck), *C. gigas* (Thunberg)).

The literature on the genus *Anadara* is rather scanty. Pathansali & Snoog (1958) mentioned briefly the sexes in *Anadara granosa* L., while Pathansali (1966) reported on the breeding season of the species in Malaya. Sullivan (1960) also referred to the sexuality of *A. trapezia* (Deshayes). In these studies the two species are referred to as dioecious (= gonochoristic).

Yoloye (1975) gave an account of the habits and functional anatomy of *A. senilis*. Some physiological studies on the species have also been conducted recently (see Djangmah, Gabbott & Wood, 1978; Djangmah, Shumway & Davenport, 1979, 1980). However, its reproductive biology has received little attention. In a paper on sexual phases of *A. senilis* from the Onijegi

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lagoon of the Lagos lagoon system (Nigeria), Yoloye (1974) described the species as a proandric hermaphrodite. It was claimed that all individuals started life as males, but by the end of their first year about 76% had changed into females. This, however, contradicts preliminary observations made on populations from coastal lagoons in Ghana (present author's personal records), which revealed that juveniles (up to 20mm shell length) were either distinctly male or female. The present investigation was therefore carried out to study in more detail the gonad maturation and sexuality of *A. senilis* and to find out whether it varies in different habitats.

MATERIALS AND METHODS

Specimens for this study were mainly collected from two contrasting lagoon systems situated 8km apart on the coast of Ghana located at approximately $5^{\circ} 05' N$, $1^{\circ} 20' W$.

(1) Benya Lagoon: this is an 'open' lagoon, in contact with the sea throughout the year and therefore partly under tidal influence. Its water level rise at spring tide is 1.0 m, compared to the spring tidal range of 2.5 m of the adjacent sea. The salinity is similar to that of the sea except during the rainy season (May to July) when it is lowered as a result of dilution from three streams (Udu, Anwin and Anomadua) and direct rainfall. Temperature and salinity ranges recorded during the study were 24 to 32°C and 10 to 40‰ respectively. The substratum shows a gradation from coarse sand at the mouth through sandy mud to flocculent mud at the head. The 'cockles' occur mainly subtidally on the sandy-mud substratum but isolated patches of the cockle bed become exposed at low tide. The main bed is located in the middle section of the lagoon approximately 500 m from the sea.

(2) Brenu Lagoon: This is a 'closed' lagoon which gets cut off from the adjacent sea by a sand bar (about 40 m wide) for the greater part of the year. Contact with the sea is re-established during the rainy season either naturally, or artificially to prevent flooding of nearby farms and villages. The temperature and salinity ranges recorded were 27 to 34°C and 27 to 70‰ respectively. Dilution is mainly from direct rainfall and small creeks, whereas the hyperhaline condition results from evaporation during the dry season. The substratum is predominantly sandy mud, and the cockles remain covered by water except during the dry season when the exposed banks reveal shells of dead cockles and other bivalves, including *Tivela tripla* (L.).

Specimens were also obtained from six other lagoons all on the coast of Ghana (see Table 1).

Cockles were collected with a steel framed bottom sampler (24 x 22 cm) of 4 mm wire mesh, fitted with a handle 2-m long. In the open (tidal) lagoons sampling was done only at low tide when the water level became conveniently low.

The developmental state of the gonads of young cockles measuring between 5 mm and 20 mm in shell length (S.L.) and not more than 6 months old, was determined by histological methods. The external appearance of the gonads was also noted. Four stages of gonadal development were recognized namely:—

(i) undifferentiated: in which no trace of gonad material is visible; usually in individuals less than 10 mm S.L.;

(ii) recently differentiated: showing initiation of follicle development above the pedal musculature; occurs in the size range 10-12 mm;

(iii) mature: showing follicle proliferation, and identifiable but unripe sex cells. Cockles between 13 mm and 17 mm S.L. are mostly at this stage;

(iv) ripe: presence of rounded ova and tailed spermatozoa. Dorsally, portions of the visceral mass containing gonad material extend into the umbonal cavity. Most individuals above 18 mm S.L. are ripe.

For determination of the sex ratio in the adult populations 50 randomly selected adult cockles (S.L. > 20 mm) were sexed fortnightly from October 1975 to December 1976. This was done concurrently in Benya and Brenu lagoons. The sex was determined by microscopic examination of fresh smears of the gonad. In mature specimens the gonad is extremely profuse and imparts a characteristic colour on the visceral mass depending on the sex. In the female, the ovary is soft and appears light yellow to orange colour. The sex products flow easily when the gonadal wall is punctured with a needle. The testis of the male on the other hand, is firmer and whitish or cream in colour.

Adult specimens whose sexes could not be established by the above procedures were processed for histological examination together with the young specimens. Material for histological studies were fixed in Bouin's fluid, dehydrated and embedded in paraffin wax. These were sectioned at 6-8 μ m and stained with Ehrlich's haematoxylin counterstained with eosin.

RESULTS

Gonad maturation

The proportions of individuals at various stages of gonadal development (differentiation) are plotted in Fig. 1. It is seen that 17% of 154 juveniles (up to 20 mm S.L.) collected from Benya (open) lagoon were sexually undifferentiated; 7% were differentiated but their sexes could not be identified; 37% and 39% were distinct males and females respectively. All specimens of shell

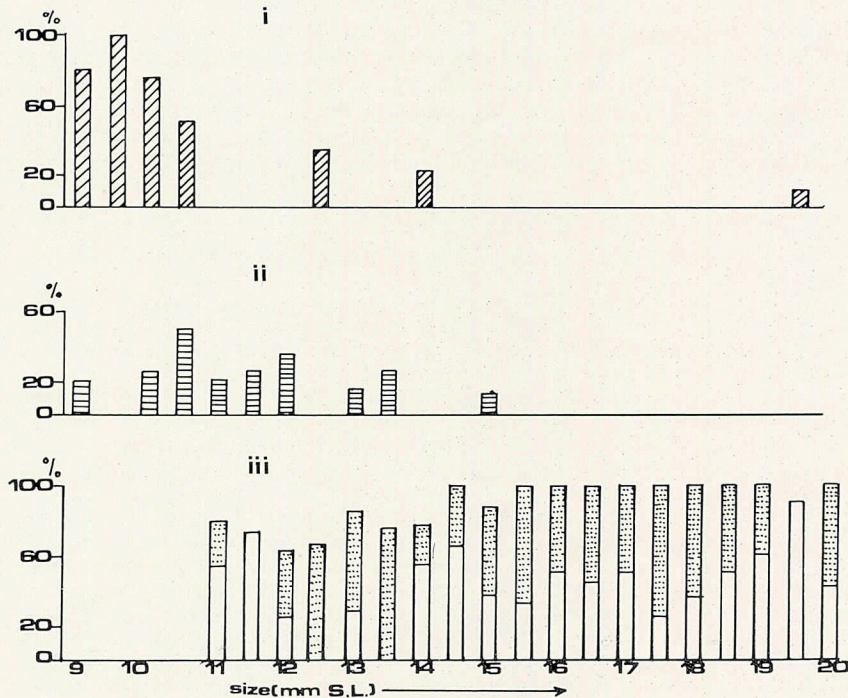


Fig. 1. Sexual differentiation in juvenile *A. senilis* (up to 20 mm S.L.) from Benya (open) lagoon. (i) Undifferentiated; (ii) recently differentiated (unidentified sex); (iii) mature, and ripe (stippled – females; unstippled – males).

length less than 9.0 mm were undifferentiated and it was therefore quite impossible to determine their sex by any method. A sample of 93 juvenile cockles (measuring 14 – 20 mm S.L.) collected from Brenu (closed) lagoon were all found to be sexually differentiated into either males or females when examined microscopically.

It is also seen from Fig. 1 that the gonad begins to differentiate after the cockles have attained a shell length of 10 mm, except for a few precocious individuals. From 12 mm onwards, a majority have undergone sexual differentiation and can be identified either as male or female. Only one hermaphrodite (of 19 mm S.L.) was encountered among the 154 juvenile cockles from the open lagoon and none in the closed lagoon. Among the adults, 4 out of 1 294 specimens from the open lagoon, and 3 out of 220 from the closed lagoon, were found to be hermaphrodite. These eight hermaphrodites (simultaneous) all had male and female tissue in the same gonad rather than separate testis and ovary with their own ducts.

In the sexually-undifferentiated state, the muscular wall of the visceral mass is separated from the gut and the digestive gland by only a thin layer of connective tissue (Fig. 2A). Prior to sexual differentiation, the connective tissue above the pedal musculature becomes more prominent due to the presence of deeply-stained cells. There is ramification of follicles with primary germ cells occurring on the follicle walls (Fig. 2B). The spermatogonia are numerous, smaller in size and deeply stained, whereas the ovogonia are fewer, larger and less intensely stained.

The stages of spermatogenesis and oogenesis identified are similar to the usual pattern in bivalves, but no follicle (nutritive) cells of the sort seen in *Paphia staminea*, *Macoma balthica* (L.) etc. (see Quayle, 1943; Caddy, 1967 respectively), were observed in either sex. The primary gonad of *A. senilis* thus seems to fit the type described by Ansell (1961) as consisting

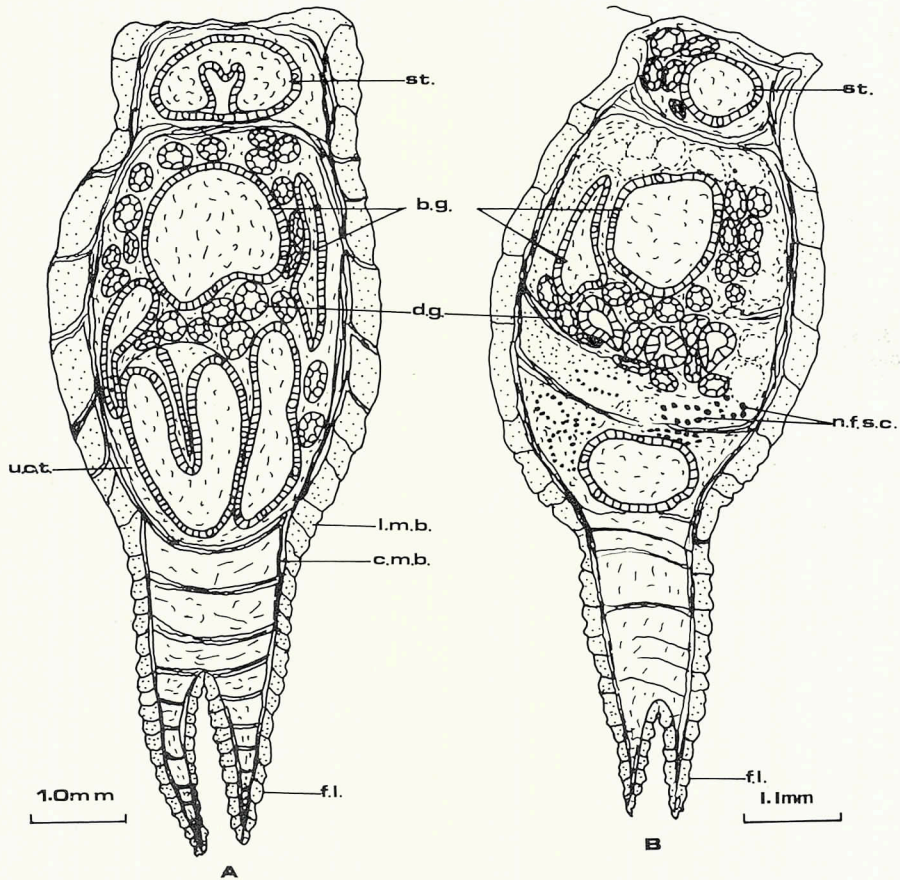


Fig. 2. Diagrammatic representation of T.S. of: A, sexually undifferentiated and B, recently differentiated *A. senilis*. b.g., branches of gut; c.m.b., circular muscle band; dg., digestive gland; f.l., foot lobe; l.m.b., longitudinal muscle block; n.f.s.c., newly formed sex cells; st., stomach; u.c.t., undifferentiated connective tissue.

of profusely-branched follicles in which the gametogenic cells are nourished by the surrounding mesenchyme or vesicular connective tissue.

Sex ratios

Fig. 3 shows the distribution of sexes in juvenile and adult populations of *A. senilis* from Benya (open) and Brenu (closed) lagoons. It is seen that in the open lagoon approximately 52% of both juveniles and adults are female. In the closed lagoon, on the other hand, the sex ratio is biased in favour of females which comprise 70% of the juveniles and 66% of the adults.

The sex ratios of the adult populations of *A. senilis* recorded monthly from the two lagoons are shown in Fig. 4. It is apparent that the ratio remained approximately 1:1 throughout the year in the open lagoon. In the closed lagoon, however, a ratio of approximately one male to two females was maintained until the population was exterminated in March 1976, presumably as a result of the hyperhaline conditions (salinity $>50\text{‰}$) and high temperatures ($32\text{--}34^{\circ}\text{C}$) which prevailed in the lagoon at that time. The population however, regenerated after the rainy season (late May to July), when the lagoon-sea contact was re-established. It is believed that the re-stocking was achieved through the dispersal of larvae by the sea, from the nearby open lagoon (Benya) where breeding of *A. senilis* was found to be continuous throughout the year.

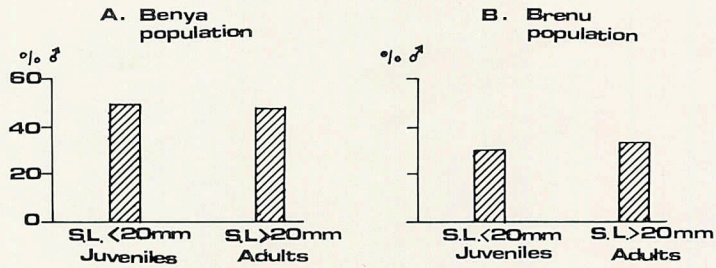


Fig. 3. Sex ratios among juvenile and adult *A. senilis* from A, Benya (open) and B, Brenu (closed) lagoons.

Table 1 shows the sex ratios of *A. senilis* from some coastal lagoons in Ghana. It is evident that the results of the detailed study of Benya and Brenu lagoons are mirrored in the six other lagoons — depicting approximately 1:1 sex ratio in the open (tidal) lagoons while in the closed (non-tidal) lagoons the ratio favours the females significantly.

Table 1
Sex ratio of *Anadara senilis* in some coastal lagoons in Ghana (X^2 value at 5% level of significance = 3.84 for a departure from 1:1 sex ratio)

Name of Lagoon	Type	Sample Size (N)	% Females	X^2 Value
Amisa	open	119	45	1.02
Benya	"	1 290	52	1.31
Nyanya	"	128	45	1.02
Brenu	closed	220	66	23.60
Ehone	"	214	83	94.22
Keta	"	101	63	7.22
Muni	"	121	65	11.32
Sakumo	"	107	61	4.94

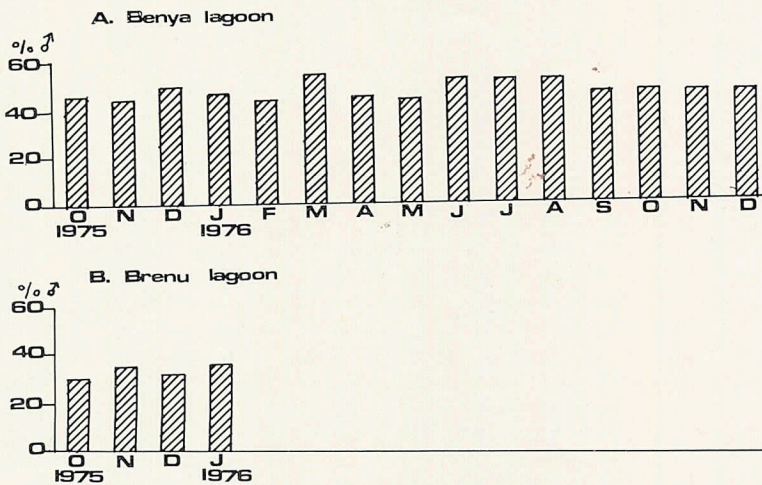


Fig. 4. Monthly sex ratios in adult *A. senilis* from A, Benya (open) and B, Brenu (closed) lagoons.

DISCUSSION

The results of the present study need to be interpreted with care in view of their conflict with the results of Yoloye mentioned earlier. The small number of hermaphrodite individuals observed in the present work suggests that they can be regarded as unimportant, abnormal occurrences representing only 0.35% and 0.96% respectively in the two contrasting lagoons (open and closed). They could not be interpreted as transitional phases of a protandrous sequential hermaphrodite since there was no evidence of a change in sex ratio with age. Nor was there evidence, in the hermaphrodites, of follicles of one sex being in regression. Furthermore, their numbers were insufficient to satisfy a hypothesis of occasional random sex change by many or all members of the species. Lucas (1975) showed that the percentage of hermaphrodites amongst juvenile bivalves, for instance, may be quite high, quoting 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 even though it is not known if they survive to be adults. Hermaphroditism in *A. senilis* on the other hand should be interpreted as an unimportant phenomenon and not representing transitional forms. Such occurrences are common in many gonochoristic bivalves (see Coe, 1943, 1945; Purchon, 1968).

The occurrence of different sex ratios in open and closed lagoons is apparently a result of the different environmental factors in the two lagoon systems. However, it is not easy to explain the preponderance of females in the closed lagoons where food is relatively less abundant (see Kwei, 1977). According to Morton (1958), in the bivalves and molluscs at large, the genetic rhythm is finely balanced and the determination of sex so labile that nutritive conditions can tip it either way. But since the development as a female is thought to require considerably more energy than male development (M.M. Helm, personal communication), in the poorer closed lagoons a tip in the genetic rhythm would be expected to favour males. Sex change in already sexually-differentiated individuals cannot provide the answer either, since it would imply higher proportions of females in the adult populations than in the juveniles. All the samples taken from the closed lagoons showed a preponderance of females in both juveniles and adults. Differential mortality against the would-be male larvae and spat caused by factors other than food could possibly explain the skewed sex ratios in the non-tidal lagoons.

Turning to the work of Yoloye (1974), it is seen that he too observed a very small number (3) of juvenile hermaphrodites (sample size not indicated). The total absence of hermaphrodites from his adult population may be merely a consequence of his sample size (not stated) being insufficient to reveal a very low percentage of such individuals. But the more crucial evidence for his interpretation of *A. senilis* as a protandrous hermaphrodite was that all specimens up to 19 mm in shell length (below 6 months old) were males, whereas 76% of the adults were females. Taking the latter point first, it can now be seen that the high percentage of females would be expected from the Ghanaian closed lagoons (see results) since the lagoon where Yoloye worked was of the closed type. But the total absence of juvenile females in his samples is completely at variance with the results of the present work.

Yoloye's reports of individuals measuring between 5 and 9 mm in shell length containing spermatozoa is surprising in the light of the evidence of Ghanaian populations in which animals of such size are all immature and do not have differentiated gonads at all. It is conceivable, however, that populations from different locations might achieve sexual maturity at different shell sizes. If his observations were correct then an explanation must be sought for the exhibition of the two sorts of sexuality, namely protandric hermaphroditism and dioecism, by the species. Purchon (1968) invoked changes in the gene complex or environment to explain the attainment of either simultaneous hermaphroditism or protandric hermaphroditism at entirely different geographical locations, in some bivalves which are basically dioecious (e.g. *Tivela stultorum* L. and *Crassostrea virginica*). But it seems unlikely that the pattern of sexuality in *A. senilis* should be so different at comparable habitats on the West African Coast.

It must be concluded that the results of the present study provide strong evidence that *A. senilis*, like *A. granosa* and *A. trapezia*, is basically gonochoristic (dioecious). Previous reports of protandric hermaphroditism in *A. senilis* therefore, need to be confirmed before the species is considered as capable of exhibiting entirely different patterns of sexuality in different localities.

SUMMARY

The primary gonads of *Anadara senilis* begin to differentiate into testis or ovary when the animals are between 10-12 mm in shell length, and by the time they are 20 mm long they have begun spawning for the first time.

The primary gonad of *A. senilis* is profusely branched and gametogenic cells derive nourishment from the surrounding vesicular connective tissue. No nutritive cells have been observed.

In one particular lagoon system on the Ghana coast (Benya lagoon) where inundation by the sea takes place at every tide (an 'open' lagoon), the juvenile population consisted of 48.4% males and 51.6% females and the adults of 48% and 52% females. In another lagoon (Brenu) which is non-tidal ('closed' lagoon), 30% males and 70% females made up the juvenile population, while the adults consisted of 33.6% males and 66.4% females. Similar ratios were observed in other lagoons and from these data, sex ratios of approximately 1:1 in open lagoons and 1:2 in closed lagoons were inferred.

There was no evidence of protandry. Individuals were found to differentiate sexually, as either distinct males or females in the same ratio as exists in the adults in a particular habitat. Hence *A. senilis* occurring in Ghanaian coastal lagoons is normally gonochoristic (dioecious).

Simultaneous hermaphroditism occurred, but only rarely. In an open lagoon 5 out of 1 448 cockles were found to be hermaphrodites, and in a closed lagoon 3 out of 313 contained follicles of both sexes.

Reports of one month old cockles (5 mm in shell length) containing spermatozoa; absence of juvenile females and hence protandric hermaphroditism in *A. senilis* by Yoloye (1974) are in conflict with the results of the present study and therefore open to criticism and alternative explanation.

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