development and could also be participating in the embryo-egg capsule dynamic metabolism.

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## Sexual differentiation of *Crassostrea tulipa* in two contrasting brackishwater environments

Kobina Yankson

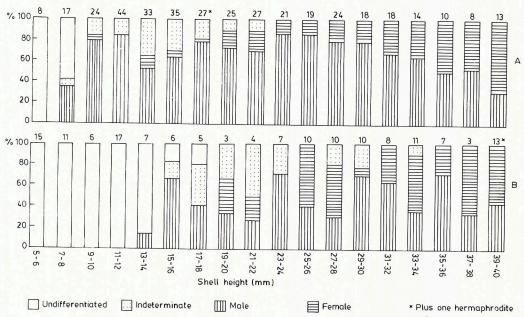
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The West African mangrove oyster, Crassostrea tulipa (Lamarck, 1819) provides a cheap source of protein for many coastal communities in the region. 1,2,3 Other benefits derived from the species include the use of the shell in poultry and building industries and in traditional medicine.1 Breeding of the species has been found to be continuous in open lagoons but seasonal in estuaries. 4.5.6 The Lagos Harbour population of the species (referred to as Gryphaea gasar Adanson) has been reported to differentiate sexually through 'male', 'intermediate' and 'female' phases.' Preliminary observations on samples from Ghanaian waters, however, showed a deviation from this pattern. Since the only available report (to my knowledge) on the sexual differentiation of the species is that of Sandison," it was deemed necessary to undertake a histological analysis of the gonads of juvenile oysters as part of an extended study on the biology of the species in Ghanaian

Specimens were collected in May 1992 from two brackishwater bodies (Benya Lagoon and Pra Estuary) located between latitudes 5° and 5° 30'N and longitudes 1° and 2°W. The lagoon is of the 'open' type and hence subjected to the daily tidal influence

of the adjacent sea, while the estuary is a 'positive' one formed by a moderately large river (Pra). The period of sample collection coincided with the beginning of the wet season (May-July) which also marked the end of oyster spat settlement in the estuary.4 Oysters measuring between 5 and 40 mm in shell height (from the umbo to the ventral shell margin) and not more than 4 months old were fixed in Bouin's fluid within 24 hours of collection. They were subsequently processed by standard histological techniques before sectioning (6–8 µm) and staining with Ehrlich's haematoxylin and eosin. Four 'serial' sections were made from each oyster and examined microscopically at magnifications ranging from 50 to 400 to determine their sex and level of gonadal development. Slides from 386 and 154 juvenile oysters from the lagoon and estuary, respectively, were analysed.

Oysters with no traces of gonadal material in the visceral mass were classified as 'undifferentiated'. The smallest identifiable male oysters measured 8 mm and 14 mm in the lagoon and estuary respectively, while their female counterparts were 10 mm and 19 mm, again respectively (Fig. 1). The proportion of females increased with size in both popula-



**Figure 1.** Sexual differentiation in juvenile *Crassostrea tulipa* (5–40 mm shell height) occurring in A, Benya Lagoon and B, Pra Estuary.

tions till the sex ratio approximated 1:1 in oysters measuring ≥31 mm in the lagoon and ≥25 mm in the estuary. The sex ratios of adult oysters(>40 mm) at the time of sampling (sexed by microscopic examination of fresh gonadal smears of 100 individuals from each habitat) were not significantly different from 1 male:1 female (P>0.05) in both habitats. The smallest spawning male and female in the lagoon measured 12 mm and 15 mm, respectively, but their respective counterparts in the estuary were 17 mm and 25 mm (Table 1). Gonads in which sex cells were unidentifiable as either male or female were designated as 'indeterminate'. In the lagoon sample, the size range of oysters in this group was 8-21 mm while in the estuary they ranged between 15 and 34 mm. The gonads of 85% and 76.9% of such oysters in the lagoon and estuary, respectively, contained collapsed follicles indicating previous spawning.

These results suggest that the majority of oysters in both habitats differentiated sexually and spawned first as males. Some of these apparently re-differentiated as females suggesting a protandric mode of development typical of most oviparious oysters. This pattern of protandry is at variance with that described for the Lagos Harbour population in which 'male', 'intermediate' and 'female' phases were linked sequentially to specific size/age groups among 4-month old oysters. Sample sizes and the method of gonadal examination in that work were not stated and hence, the reported pattern of sexual differentiation does not lend itself easily to plausible explanation. A possible recourse may, however, be

found in the existence of opportunistic sexual strategies in many oysters. 9

The few individuals with ambisexual gonads (<0.7%) observed in each of the Ghanaian populations, as noted in Figure 1, may be interpreted as transitional phases of a protandric sequential hermaphrodite due to (i), the occurrence of mature spermatozoa at the follicular centres with younger female cells attached to the walls; (ii), evidence of a change in sex ratio with age (Fig. 1) and (iii), evidence of monthly changes in adult sex ratios. Their

**Table 1.** Least sizes (mm) at which the various gonadal developmental stages were detected in *C. tulipa* (I = Early gametogenesis, II = Developing, III = Ripe-spawning, IV = Spent).

Sex	Developmental stage	Benya Lagoon	Pra Estuary
Male		8.0	14.0
		10.0	17.0
	-    -	12.0	17.0
	IV	10.0	-
Female		10.0	19.0
	11	14.0	22.0
		15.0	25.0
	IV	20.0	

small numbers, however, suggest that such a transitional phase is probably of a short duration.

The following differences in the sexual strategies adopted by the two populations are worthy of note: (i), Earlier differentiation and spawning in the lagoon and (ii), faster increases in the proportion of females in the estuary. These may be attributed to environmental differences. Salinity has been identified as the main environmental factor influencing the reproductive biology of tropical bivalves.  $^{2.5,10.11}$  Annual salinity ranges of 29.5-40% and 0-29%have been recorded in the Benya Lagoon and Pra Estuary, respectively.<sup>4</sup> Since the breeding of this oyster is enhanced in high salinity regimes, <sup>2,5</sup> the apparently more rapid sexual differentiation, maturation and spawning of the lagoon oysters may be attributed to the higher salinity of this habitat. Like the Lagos Harbour population, the Pra Estuary ovsters suffer approximately 100% annual mortality after the wet season when salinities and transparencies are low.4 The presence of more females in the population prior to the onset of unfavourable conditions (named above) appears to be a strategy to improve breeding success and hence ensure survival into the next year, since a few males can produce sperm to fertilize eggs from a large number of females. The faster increases in the proportion of females in the estuarine juvenile oysters could therefore be an adaptive feature against imminent environmentally imposed mortalities. The absence of spent individuals among such oysters (Table 1) further strengthens the proposition of an efficient reproductive strategy for survival in the estuary.

It may be concluded that the two investigated Ghanaian populations of *Crassostrea tulipa*, exhibited protandric sexual development like other oviparous oysters but different from that reported

previously for the species. The lagoon population initiated sexual differentiation at a smaller size than their estuarine counterparts, but the rate of differentiation into females appeared to be faster in the latter habitat resulting in an earlier attainment of a 1:1 sex ratio.

I am grateful to Mr P. Aubyn for technical assistance.

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### Corrigendum

In Journal of Molluscan Studies Volume 60 part 4, published in November 1995, we inadvertently printed the Research Note by A.E. Yaseen without the table and figures. With apologies to the author and our subscribers, we reprint the entire article below.

J. Moll. Stud. (1996), 62, 137-141

# The chromosomes of the Egyptian freshwater snail *Melanoides tuberculata* (Gastropoda: Prosobranchia)

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The species of *Melanoides* are widely distributed in eastern Pacific islands, southern Asia and Africa. Some inhabit somewhat brackish water as well as fresh water. Of about 30 species known in Africa, the most widespread is *M. tuberculata* (Müller)<sup>1</sup>. The female of this species has a brood pouch separate

from the uterus and must be commonly parthenogenetic, as males are rare or apparently lacking in many populations. Males have been reported in varying proportions of some populations of *M. tuberculata* in Israel<sup>2,3,4</sup>.

Few of the many species of molluscs are known