



## Gametogenesis in the mangrove mussel *Mytella guyanensis* from northern Brazil

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**Abstract.** Gametogenesis was investigated using histological methods, in *Mytella guyanensis* from the Caeté mangrove estuary, northern Brazil. The sexes could not be distinguished from macroscopic observations of color. Histology and cellular organization was similar to that previously described for this and other species.

**Keywords:** Mytilidae, reproduction, histology, tropical estuary

**Resumo.** Gametogênese no mexilhão do mangue *Mytella guyanensis* do norte do Brasil. Gametogênese foi investigado usando métodos histológicos, em *Mytella guyanensis* no mangue do estuário do rio Caeté, Norte do Brasil. Os sexos não podiam ser distinguidos pelas observações macroscópicas da cor. A histologia e organização celular foi similar às que foram descritas anteriormente para esta e outras espécies.

**Palavras-chave:** Mytilidae, reprodução, histologia, estuário tropical

*Mytella guyanensis* (Lamarck, 1819) is a mangrove mussel with a wide distribution in Brazil (Klappenbach 1965, Rios 1994) and has both economic and ecological importance (Nishida & Leonel 1995, Mora & Alpizar 1998, Pereira et al. 2003, Nishida et al. 2006, Pereira et al. 2007). *M. guyanensis* is generally dioecious with a sex ratio of 1:1 (Cruz & Villalobos 1993, Carpes-Paternoster 2003) although Sibaja (1986), using both squash preparations and macroscopic observations of gonad color, reported sex-ratios biased towards females. Hermaphrodites are rare, representing only 0.2% of the population (Carpes-Paternoster 2003). Information on reproductive activity in mussels is important for their management (Narchi 1976; Fernandes & Castro 1982) and culture (Marques 1987, Sibaja 1988, Rajagopal et al. 1998) and the present study aims to describe gametogenetic activity in *Mytella guyanensis* from northern Brazil.

The study area, near the Furo do Meio tidal channel (00°52'14.6''S, 46°38'57.7''W), was located in the Caeté mangrove estuary, along the northeastern coast of the State of Pará. The mussel bed (120 m<sup>2</sup>) occurs in typical fine muddy mangrove sediment with aerial roots and is regularly flooded during high tide as it borders a secondary channel linked to the Furo do Meio. The mean density of the bed was 6 mussels m<sup>-2</sup>, somewhat lower than that of another bed in the same region (11.9 mussels m<sup>-2</sup>, Beasley et al. 2005) but similar to the mean density of *M. guyanensis* (5.2 mussels m<sup>-2</sup>) in Paraíba (Nishida & Leonel 1995).

Between January 2004 and January 2005, mussels were collected at low tide during the new moon phase, to standardize the timing of sampling. In order to obtain sexually mature individuals, mussels with an anterior-posterior shell length of at least 20 mm were selected. Mussels were obtained at

randomly chosen coordinates within the bed. An initial sample size of 20 individuals was used in the first two months of the study but from March 2004 onwards, the number of specimens collected was reduced to 10 to minimize any possible impact of sampling, such as trampling and/or a reduction in population size due to the removal of individuals.

A pair of gonads occurs in the dorsal part of the visceral mass from which ventrally ramified tubules unite to eventually open into the mantle cavity to release gametes into the sea water (Cox 1969, Gosling 2003). The gonads in mytilids develop extensively into the mantle tissue and both visceral mass and mantle tissue were sampled. The color of both the gonad and mantle tissue was noted for each individual. Both visceral mass and mantle tissue were fixed in Davidson's solution for 24 h before being stored in 70% alcohol. The material was dehydrated using a series of alcohol concentrations, cleared using xylol and embedded in paraffin wax. Thin sections (5  $\mu\text{m}$ ) were obtained using a microtome, which were subsequently stained using Haematoxylin and Eosin and mounted on glass slides for microscopic examination.

Qualitative evaluations of gametogenesis in both the visceral mass and mantle tissue were carried out using the criteria of Nascimento (1968) for reproductive development in the closely related *Mytella falcata* (*M. charruana* (d'Orbigny, 1842)). Up to 5 consecutive developmental stages were identified by Nascimento (1968): I (Immature), II (Maturing), III (Pre-spawning), IV (Total or partial spawning) and V (Recovery).

From the 150 individuals collected, 72 were male and 77 female with a single hermaphrodite, with mostly male reproductive tissue, found in September. There was no significant difference in the sex ratio during the study period ( $\chi^2=10.3$ , d.f.=12, n.s.). Male reproductive tissue in the mantle tended to be darker (light yellow to orange yellow) than that of females (ivory to light yellow). However, the degree of overlap in color precluded conclusive determination of sex in 50% of cases by macroscopic observation alone.

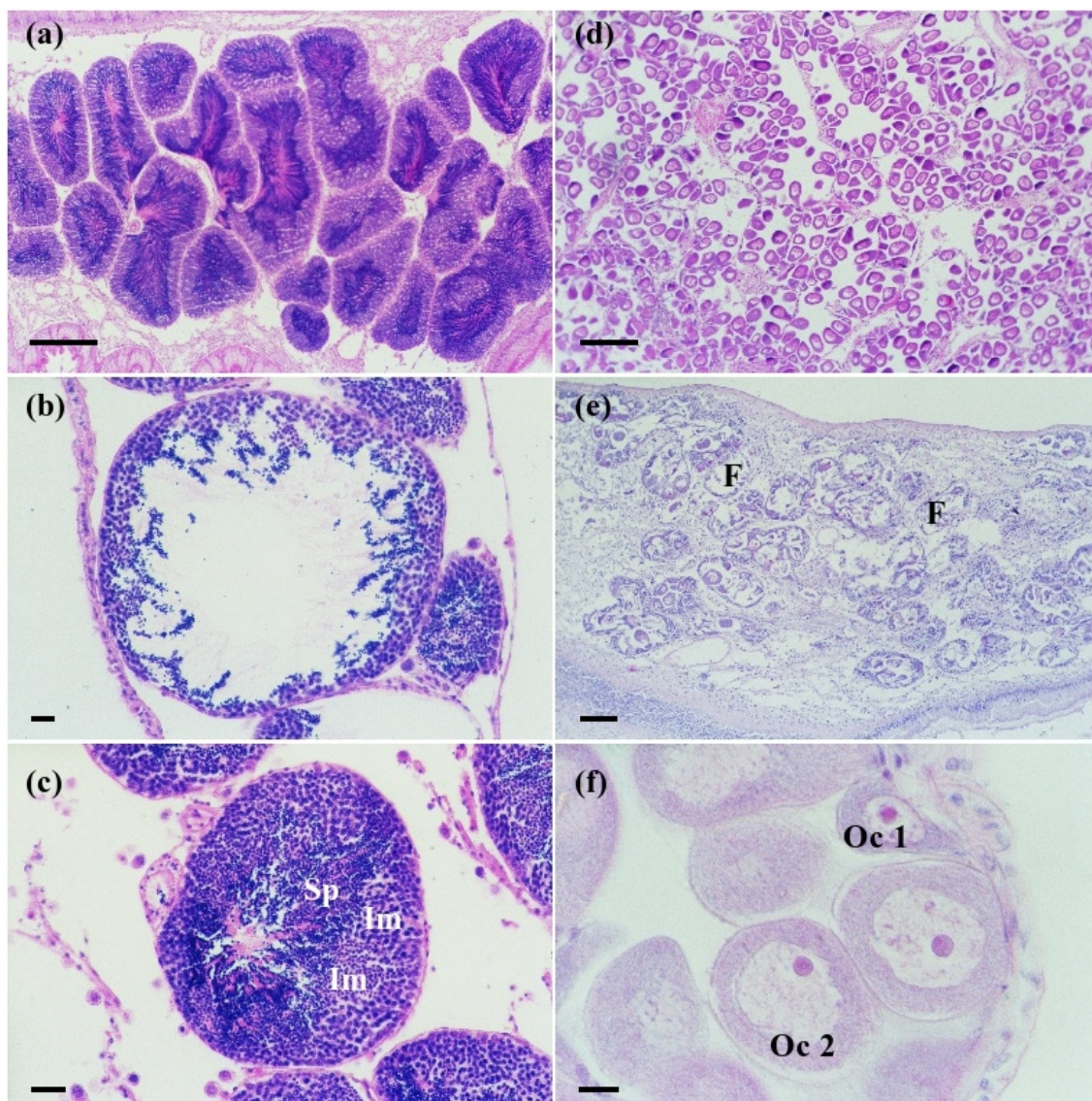
All individuals examined were sexually mature and belonged to reproductive stages III to V; there were no individuals at stages I and II. Pre-spawning (Stage III) males were characterized by long tubules with some immature cells close to the tubule wall and greater numbers of darkly stained mature spermatozoa in the lumen forming dense groups of cells (Figure 1a). During spawning (Stage IV) males are characterized by a reduction

in the number of spermatozoa and the presence of small numbers of immature cells around the tubule walls and residual spermatozoa in the lumen (Figure 1b). In males, tubules in mantle tissue were almost completely empty in contrast to tubules in the visceral mass where residual spermatozoa were common. During recovery (Stage V), the tubules were packed with immature cells but also contained lower numbers of spermatozoa (Figure 1c).

Pre-spawning (Stage III) females showed well developed oval follicles containing a large quantity of mature oocytes (round in shape and free in the lumen). Some oocytes were still attached to the follicle wall by a stalk (Figure 1d). Spawning (Stage IV) females were characterized by poorly developed follicles without a definite shape and containing occasional mature oocytes. Some degenerating oocytes that had not been released during spawning were also present (Figure 1e). In females, follicles were completely empty in the visceral mass but not in the mantle tissue. Recovering (Stage V) females contained many immature oocytes close to the follicle wall (Figure 1f) but these did not have the oval shape characteristic of the pre-spawning stage.

The sexes in *Mytella guyanensis* could not be accurately distinguished through macroscopic observation due to wide variation in the colour of the reproductive tissues, which were darker in males and lighter in females. This contrasts with other mytilids where the gonads are usually lighter in colour in males and darker in females (Nascimento 1968, Arrieche et al. 2002, Gosling 2003). However, our results agree with those of Cruz & Villalobos (1993) whose macroscopic description of gonad colour in *M. guyanensis* from Costa Rica varies from light to dark yellow in females and from light to dark brown in males.

The histology and cellular organization of the reproductive tissues of *Mytella guyanensis* is similar to what has been previously reported for this species (Cruz & Villalobos 1993, Carpes-Paternoster 2003), for the closely related *Mytella falcata* (Nascimento 1968), and, for other bivalves in general (Gosling 2003). Mean oocyte size of *M. guyanensis* ranged from 26 to 42  $\mu\text{m}$  (maximum individual observation was 62  $\mu\text{m}$ ) in northern Brazil, similar to the 30-45  $\mu\text{m}$  range described for a population in Costa Rica (Sibaja 1986). By comparison, maximum oocyte size is 63.3  $\mu\text{m}$  in *M. falcata* (Nascimento 1968) and 70  $\mu\text{m}$  in *Mytilus edulis* (Gosling 2003).



**Figure 1.** Photo-microscopy of thin sections of reproductive tissue from male and female *Mytella guyanensis*. (a) Male, Stage III, pre-spawning, (b) male, Stage IV, spawning and (c) male, Stage V, recovery. (d) Female, Stage III, pre-spawning, (e) female, Stage IV, spawning and (f) female, Stage V, recovery. Sp Spermatozoa, Im Immature cells, F Follicle, Oc 1 primary oocytes, Oc 2 secondary oocytes. Bar is 100  $\mu$ m.

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