Embrionary and larval development of the marine clam *Tivela mactroides* (Bivalvia: Veneridae) in Zulia State, Venezuela

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Abstract: The marine clam, *Tivela mactroides*, from Caño Sagua beach, Venezuela, was spawned and reared under laboratory conditions to monitor its early development. Spawning was spontaneous but in some cases it had to be induced by the additon of eggs and sperm. After fertilization, the embryonic development occurred at 5 hr approximately. Trochophore larvae were observed between eight and ten hours later. Straight-hinged veliger stage appeared 15 hr after fertilization. Transition from veliger stage to the umbo stage occurred about eight days after fertilization. Pediveliger stage was observed 22 days after fertilization. Metamorphosis of *T. mactroides* was not successful under our laboratory conditions; probably the bacterial contamination and subsequent mortalities were important factors constraining the final phase of the larval cycle. However, in a few cases young individuals were observed. We suspect that this was due to unfavorable conditions (*e.g.*: bacterial contamination, unsuitable food availability, etc.) and the broad variation in developmental times, suggesting that these stages might be particularly sensitive to environmental changes. These results may not necessarily reflect what happens under natural conditions. Rev. Biol. Trop. 52(4): 903-909. Epub 2005 Jun 24.

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Tivela mactroides, commonly known as "Guacuco", is a clam from the Veneridae family, frequently used as a source of human food. It ranges from the West Indies to Brazil (Warmke and Abbott 1961), with particularly high abundances on the coasts of Puerto Rico, Jamaica (Rehder 1981), and some parts of Venezuela (Etchevers 1976, Tatá and Prieto 1991). Studies on *T. mactroides* have been concentrated on population dynamics (Etchevers 1976, Brito 1984, Marcano 1993, Severeyn *et al*. 1996, Godoy 1997), ecology (Prieto 1977, 1980, 1983, 1987, Delgado 1997), gonadal development (Prieto 1977), microbiology (Andrade 2000) and simulation models (García de Severeyn *et al.* 2000).

Until 1993, *T. mactroides* used to be a resource exploited by fisherman of Caño Sagua beach, Zulia State, to defray the demand of local markets. The increase of fishery, and the installation of an industrial plant to sue clam, made that the fishery of *T. mactroides* in the zone drained, and it was replaced for the estuarine clam *Polymesoda solida* (Philippi 1946), a bivalve of high abundance in the Lago of Maracaibo, Venezuela (García de Severeyn *et al.* 1994).

In the face of this situation, our goal was to study the embrionary and larval development of the marine clam *T. mactroides* under laboratory conditions, and so contribute to design a future technology package that would include the necessary tools for captive breeding and subsequent restocking of wild populations.

MATERIALS AND METHODS

The specimens of *T. mactroides* used in this study were collected between 1995 and 1997, from Caño Sagua beach, on the southwestern coast of the Venezuelan Gulf (71°56'21.5" W; 11°21'8.5" N). Specimens were collected manually removing the sediment up to 5 cm deep. Clams between 30 and 40 mm long were brought to the laboratory in plastic containers, filled with water from the capture site, to avoid stress, because there is evidence that *T. mactroides* may initiate spawning in response to changes in temperature, salinity or other conditions (Morales *et al.* 1996, Reverol 1997).

In the laboratory, the clams were placed in 9 l glass aquaria containing filtered and UV-radiated water. These closed systems were aerated, maintaining the salinity at 29 ‰ and temperature at 25 ± 1°C. The period of acclimatization of the animals was between 24 and 48 h. In the majority of the cases, *T. mactroides* spawned spontaneously, however, sometimes it was necessary to use the addition of eggs or sperm (Galtsoff 1940).

The rearing of embryos and larval stages followed the standard techniques of Loosanoff and Davis (1963). Embryos and larval stages were kept in 250 ml glass aquaria, containing filtered and UV-radiated water and placed into environmentally controlled cabinets (Percival, Model I-35LL). Temperature was maintained at 25 ± 1°C, and salinity at 29 ‰. A cycle of 12 hr of darkness and 12 hr of light simulated a natural daily cycle.

Axenic algal cultures of *Isochrysis galbana* were used as a source of food for larval stages. They were obtained from Rosenstiel School of Marine and Atmospheric Sciences in Coral Gables, Florida. This algal clone is part of the permanent collection of the Laboratorio de Microalgas, Facultad Experimental de Ciencias, La Universidad del Zulia in Maracaibo, Venezuela. Ten ml of algae solution in exponential phase, from a 2 l mass culture, were added every three days to the aquarium containing 200 trochophore larvae. Before each addition of algae concentrate, water and detritus were removed and replaced with sterilized water of the same salinity and temperature, filtered and radiated with UV light.

RESULTS

In most cases the spawning took place during the period of acclimatization, without the use of physical or chemical agents. However in some cases, it was necessary to stimulate spawning through the addition of eggs and sperm as described by Galtsoff (1940) and Loosanoff and Davis (1963).

Spermatozoa of *T. mactroides* have a hook-shapep head, with a mean length of 5.29 mm (Fig. 1a). They own a flagellum about 53 mm long. Eggs are completely spherical at spawning with a mean diameter of 60.69 mm (Fig. 1b). Obtaining of fertilized eggs was no problem; nearly 100% of the eggs were fertilized using the techniques suggested by Loosanoff and Davis (1963). In each experiment, unfertilized eggs were removed to decrease the chance of bacterial contamination.

Tables 1 and 2 show mean developmental times for all stages and its morphometry. Fertilization was recognized by the appearance of a thick fertilization membrane (Fig. 1c) at 5 min. After 30 min, a polar body appeared as a protuberance at the egg animal pole (Fig. 1d).

The first cleavage occurred 1.05 hr after fertilization (Fig. 2a). The second and third cleavages occurred 1.22 and 1.39 hr after fertilization, respectively (Fig. 2b,c). The fourth cleavage occurred 1.62 hr after fertilization (Fig. 2d). Two to five hours after fertilization, most embryos

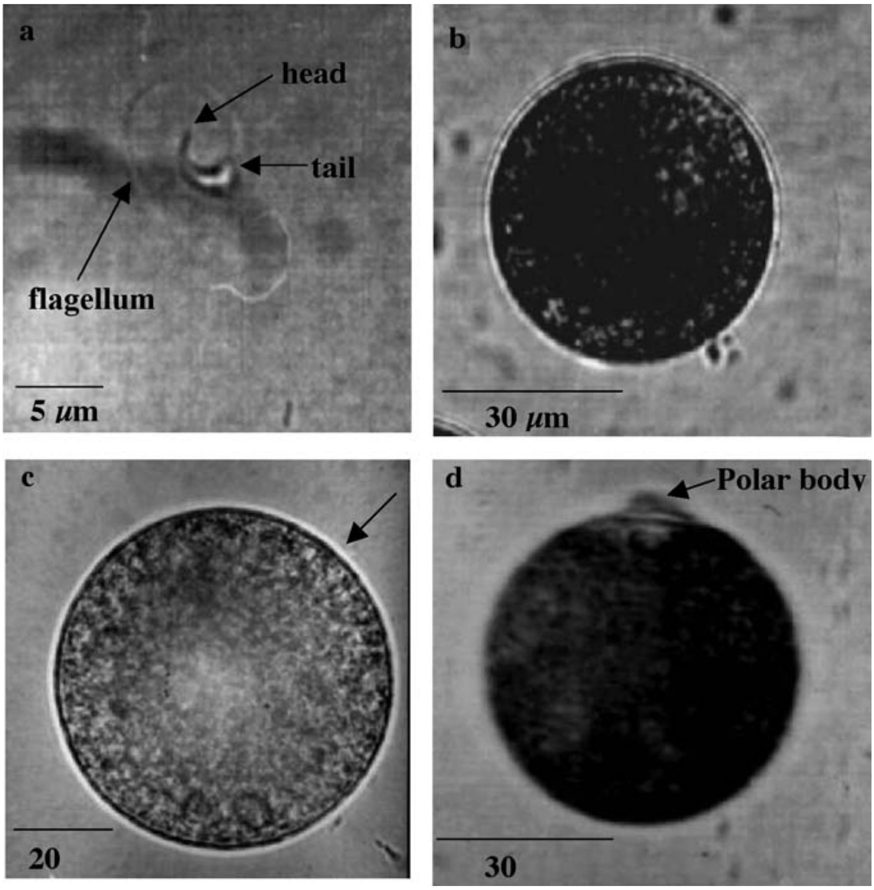

Fig. 1. Early embrionic stages of *Tivela mactroides* a) Spermatozoa showing the head, tail y flagellum. b) Egg non fertilizied. c) Recently eggs showing the fertilization membrane. d) Polar body.

were in blastula (Fig. 2e) and gastrula or mobile blastula stages, respectively (Fig. 2f).

Trochophore larvae (Fig. 3a) were observed approximately 10 hr after fertilization, with mean length and width of 60.69 mm and 57.12 mm respectively. Straight-hinged veliger (Fig. 3b) appear approximately 15 hr after fertilization, with a mean lenght of 92.82 mm and 67.83 mm of width. The veliger development to the umbo stage occurred about eight days after fertilization, with a mean length of 157.08 mm and a mean width of 128.52 mm (Fig. 3c). Pediveliger larvae (Fig. 3d) were observed 22 days after fertilization, with a mean length of 185.69 mm and a mean width of 157.08 mm (Table 2).

TABLE 1

Development time of embryonic and larval stages after fertilization of Tivela mactroides *under laboratory conditions*

Stage	Mean Time
Fertilization membrane	4.2 min
Polar body	28.8 min
Cleavage first	1.05 hr
Cleavage second	1.22 hr
Cleavage third	1.39 hr
Cleavage fourth	1.62 hr
Blastula	2.77 hr
Gastrula or Blastula movil	5.01 hr
Trochophore	10.39 hr
Straight-hinged veliger	14.54 hr
Veliger (umbo)	7.72 d
Pediveliger	22 d

TABLE 2

Morphometric measures of the larval stages of Tivela mactroides

Stage	Mean (mm) ± SD
Egg	D: 60.69 ± 0.97
Spermatozoa	L: 5.29 ± 0.19
Trochophore	L: 60.69; W: 57.12 ± 6.18 x 1.68
Straight-hinged veliger	L: 92.82; W: 67.83 ± 4.11x 5.67
Umbo	L: 157.08; W: 128.52 ± 6.92 x 36.40
Pediveliger	L: 185.69; W: 157.08 ± 7.18 x 5.26

L = lengh, W = width, D = diameter.

DISCUSSION

Under natural conditions, two spawning peaks have been registered for *T. mactroides*, one in May and the other in November, with ripe gonads observed all year around. (Severeyn *et al.* 1996, Reverol *et al.* 1998). In this sense, our success in getting mature gametes is in agreement with Chanley (1981) and García *et al.* (1994), who indicated that gonadal stage is a key factor controlling the possibility of spawning and production of viable gametes under laboratory conditions.

T. mactroides from Caño Sagua beach has been described to show separated sexes (Severeyn *et al.* 1996, Reverol 1997). However, Prieto (1980) described the presence of hermaphrodites in his study at Guiria Beach, Venezuela. Our analysis of gonads in Caño Sagua beach confirmate this bivalve has separate and independent sexes. We do not have explanations for this dual behavior.

It is important to mention that it is normal that the eggs of *T. mactroides* do not have a hyaline capsule. However, after an oil spill in February 1997 in the Gulf of Venezuela, pilot sampling demostrated the presence of a hyaline capsule in all eggs analyzed. Probably this modification was a product of the high stress caused by the spill, and the capsule may function as a protective structure (Reverol *et al.* 1998). The role of the capsule is controversial. Severeyn (1993) argued that it could play an important role in protecting the eggs and

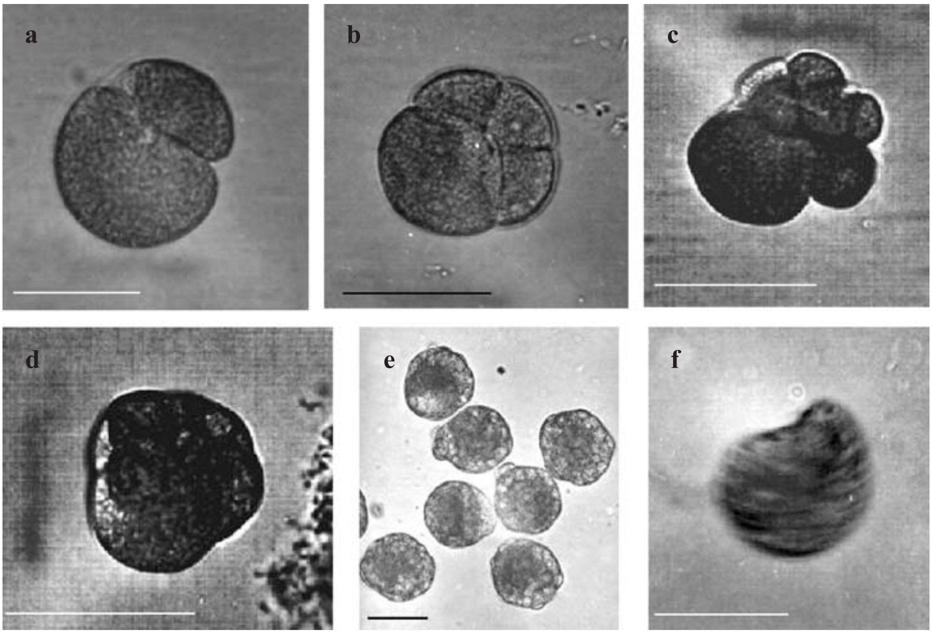

Fig. 2. Early embrionic stages of *Tivela matroides*. a) First cleavage (two-celled embryo). b) Second cleavage (four-celled embryo). c) Third cleavage (eight-celled embryo). d) Fourth cleavage (sixteen-celled embryo) e) Blastula. f) Gastrula or Movil Blastula. Scale = 30 µm.

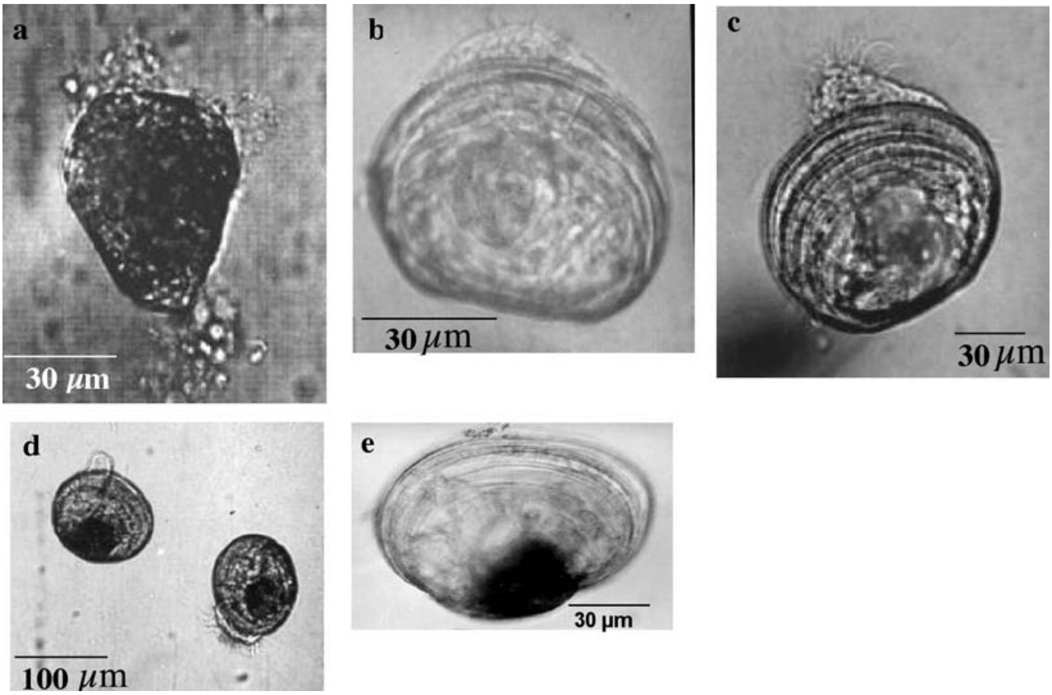

Fig. 3. Larval stages of *Tivela mactroides*. a) Trochophore. b) Straight-hinged veliger. c) Veliger (umbo) larvae. d) Pediveliger larvae. e) Juvenile.

improving their ability to disperse within and between estuarine areas. García *et al*. (1994) hypothesized that the capsule may protect advanced larval stages as long as unfavorable environmental conditions persist. However, further research is needed to clarify this issue and how the oil spill may have stimulated hyaline capsule formation in *T. mactroides*.

Results suggest that *T. mactroides* has a larval planktonic cycle that is shorter in comparison with other bivalves (Vélez and Martínez 1967, Vélez *et al*. 1985, Fitt and Trech 1981, Alargarswani *et al.* 1989, Sundberg 1991, García de Severeyn *et al.* 1994). The embryological development of *T. mactroides* is completed in five hours, and the larval cycle in 20-22 days (Reverol 1997, Reverol *et al*. 1998).

Metamorphosis of *T. mactroides* was numerically not successful under our laboratory conditions because only in few cases juveniles were observed (Fig. 3e). We believe that bacterial contamination and subsequent mortalities were important factors constraining the final phase of the larval cycle. We suspect that this was due to unfavorable conditions (*e.g.:* unsuitable bacterial contamination, available food, etc.) in the laboratory.

These results are based upon laboratory experiments and may not necessarily reflect what happens under natural conditions. The broad variation in developmental times suggests that these stages may be particularly sensitive to environmental changes. The mean developmental times observed in *T. mactroides* (Table 1) differ from those reported for *Polymesoda solida* (García de Severeyn *et al.* 1994), *Perna perna* (Vélez and Matínez 1967), *Tridacna squamosa* (Fitt and Trench 1981), and *Rangia cuneata* (Sundberg 1991). The dissimilarities may be due to taxonomic differences and ecological factors (García de Severeyn *et al*. 1994).

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RESUMEN

La almeja *Tivela mactroides*, de la playa Caño Sagua, Venezuela, fue desovada y cultivada bajo condiciones de laboratorio, monitoreando su desarrollo embrionario y larvario. El desove fue espontáneo, sin embargo, en algunos casos se indujo adicionando óvulos y espermatozoides. El desarrollo embrionario se produjo en cinco horas, aproximadamente. La larva trocófora fue observada a las diez horas, mientras que la prodisoconcha aparece a las 15 horas después de la fertilización. La larva disoconcha aparece ocho días después de la fecundación y la veliconcha, aproximadamente a los 20 días. La metamorfosis de *T. mactroides* no fue satisfactoria bajo estas condiciones, ya que la contaminación bacterial fue uno de los principales factores que provocó la mortalidad de las larvas durante la fase final del ciclo, sin embargo, en algunos casos, se observaron juveniles. Las condiciones no favorables (contaminación bacteriana, disponibilidad de alimentos, etc.) y la variación en los tiempos de desarrollo, sugieren que estos estadios son muy sensibles a los cambios ambientales. Estos resultados no necesariamente reflejan lo que ocurre en el medio natural.

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