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SPAWN AND REPRODUCTION OF THE GASTROPOD *TROCHITA PILEUS* (LAMARCK, 1822) FROM THE SOUTHWESTERN ATLANTIC OCEAN

María Eugenia Torroglosa[1] & Juliana Giménez[1,2*]

ABSTRACT

Specimens and eggs masses of the gastropod *Trochita pileus* were collected during a research cruise by bottom trawling at depths between 82 m and 120 m. Adult *T. pileus* were found attached to such hard substrata as the shells of scallops and oysters. The egg mass of *T. pileus* is composed of 7–8 transparent, triangular-shaped egg capsules fixed to the substratum with stalks. Four egg masses collected from brooding females, with a total number of 241 embryos, were examined. *Trochita pileus* is a protandrous hermaphrodite, and histological studies of the male, transitional, and female gonads were performed. The testicular portion of the gonad in both male and transitional individuals and the ovary of the female were studied by histological techniques. Spermatogenesis was observed for males and transitional individuals.

Key words: Egg capsules, protandry, Calyptraeidae, reproduction, Caenogastropoda.

INTRODUCTION

All members of the caenogastropod family Calyptraeidae are believed to be protandrous hermaphrodites (Coe, 1938; Wyatt, 1960; Gallardo, 1976; Collin, 2000). Hoagland (1986) first published a review of the development and reproduction of 30 species and described the different types of development observed in the calyptraeids. This was followed with additional information by Collin (2003). Five modes of development are present in the family. Either small or large eggs can develop directly into juveniles; additionally, small eggs can develop into small planktotrophic larvae, and large eggs can develop into pediveligers that swim before settling. Or pediveligers can develop from small eggs. Planktotrophic and direct development are the two most common modes of development in the calyptraeids; the presence of nurse eggs is common for this group, as is lecithotrophic development with a short-lived pediveliger stage (Gallardo, 1976; Collin, 2003).

Trochita pileus (Lamarck, 1822) is a common species living subtidally on hard substrata with a conical shell and a reported maximum diameter of 30 mm. Radial ribs are normally absent, but when present are narrow, and the septum margin is sigmoid (Pastorino & Urteaga, 2012). It occurs in Argentina from Buenos Aires (38°S) to the Patagonian coast (54°S) (Pastorino & Urteaga, 2012). *Trochita pileus* is not an exploited resource, but is extremely vulnerable, because it is part of the bycatch with the *Zygochlamys patagonica* (King, 1832) fishery. *Zygochlamys patagonica* shells constitute hard substrata available for the settlement of sessile organisms, which contributes to increased benthic biodiversity in the continental soft-bottom areas where scallop beds are located (Schejter & Bremec, 2007).

We note herein a description of the spawn of *Trochita pileus,* including the gonad morphology at hatching stage of a population from Buenos Aires coast, Argentina, on the southwestern Atlantic Ocean. Other aspects of the biology of *T. pileus* are also described, including the histological characterization of the gonad portions of the female, the transitional stage, and the male of *T. pileus*.

MATERIALS AND METHODS

Adults specimens and egg masses of *T. pileus* were collected off Buenos Aires Province (37°33'S and 55°57'W) during a research cruise aboard of the Argentine B/I "Puerto Deseado". Samples were collected by bottom trawling using a research dredge (0.8 m mouth opening, 10 mm mesh size at the cod-end), at depths between 82 m and 120 m during June and July 2010. The bottom water temperature

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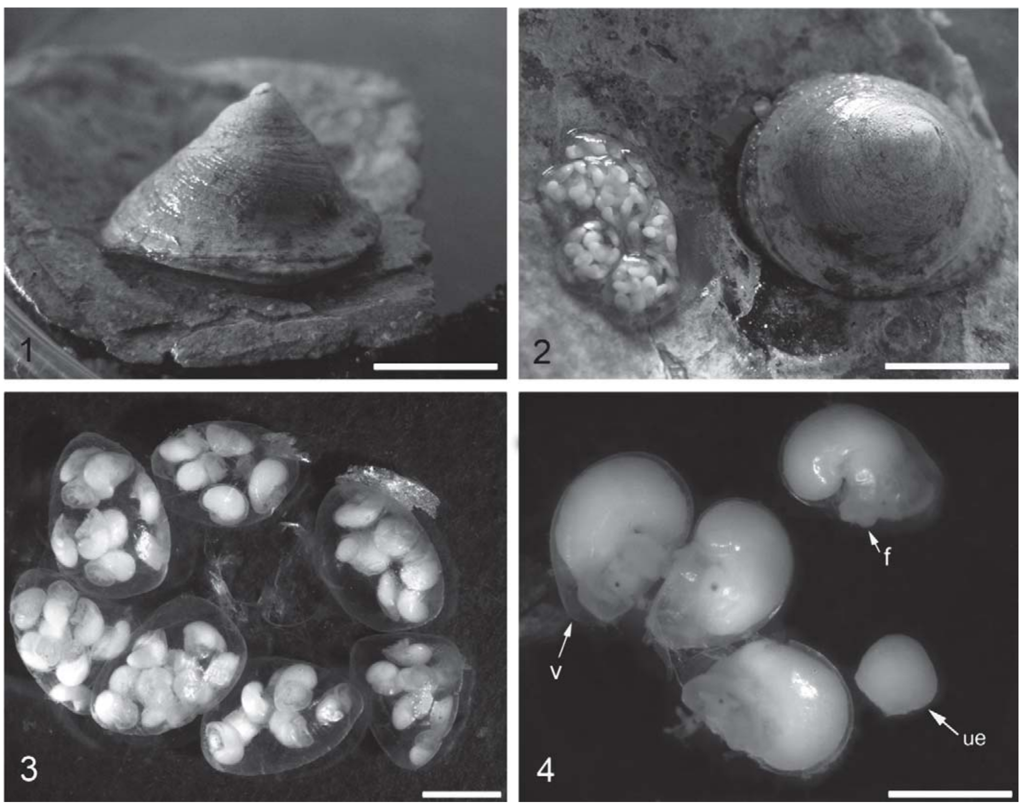
was 10.96°C, and conductivity was 33.48‰. Both were measured with a CTD Sea-Bird Electronics 911 Plus.

Trochita pileus were found attached to hard substrata, such as shells of scallops and oysters (Fig. 1). Immediately after collection, nine individuals were fixed in 5% formalin-seawater solution for 24 h, and stored in 70% ethanol. In the laboratory, *T. pileus* individuals were removed from the substrata and measured with a digital caliper at a precision of 0.01 mm. Nine individuals and four eggs masses were collected. For each capsule, the height (excluding the stalk), and the maximum width (apical region) were measured with a stereomicroscope. The egg masses are brooded under the shell, with the neck above the capsules and the substratum and propodium below them (Fig. 2).

The length of the eggs and the embryos were measured for each brood. The embryos in the broods were counted and photographed using a Zeiss Stemi 2000 C stereomicroscope.

For light microscopy, gonadal tissue was embedded in HistoResin using standard procedures. Serial sections (4 μm thick) were stained with a modified haematoxylin-eosin. Cell structures were determined by viewing and photographing tissue sections using a Zeiss Axiostar light microscope.

The reproductive state of individuals was characterized as immature when neither follicles nor sex cells were observed, as male when spermatozoa were observed, as a transitional individual when both an ovary and testis were found, and as female when only an ovary with gametes was observed.



FIGS. 1–4. Adults and egg masses of *Trochita pileus*. FIG. 1: *T. pileus* specimen attached to an oyster; FIG. 2: Egg mass and brooder adult; FIG. 3: Egg mass, composed by 7 ovicapsules; FIG. 4: Embryos at the early pediveliger stage; note presence of the velum (v) and incipient foot (f). unsegmented egg (ue). Scale bars: Figs. 1, 2 = 10 mm; Fig. 3 = 1 mm; Fig. 4 = 2 mm.

RESULTS

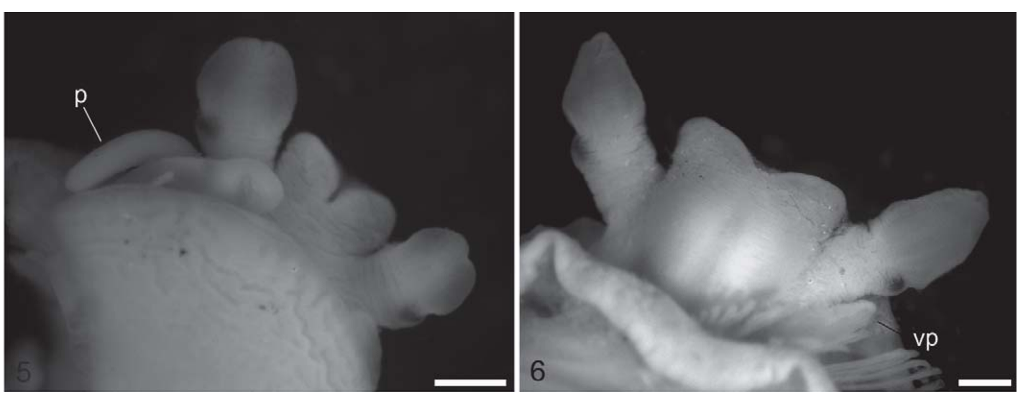
Specimens were attached to a hard substratum (Fig. 1). Egg capsules are brooded under the body of females (Fig. 2). The diameter of females ranged from 15.99–20.68 mm (X = 19.38 mm, n = 5, SD = 1.93). The three transitional individuals measured 7.11, 7.42 and 8.19 mm (X = 7.57mm, n = 3, SD = 0.55) in diameter, and have a vestigial penis. The male diameter was 6.2 mm and exhibited a well-developed penis. No immature individuals were found in this range of sizes. We examined four egg masses for a total number of 241 embryos. Each egg mass was composed of 7–8 egg capsules, and 30 capsules were analyzed. *Trochita pileus* has transparent, triangular-shaped egg capsules with an attachment stalk (Fig. 3). For the egg capsules, the average height was 3.31 mm (n = 28, SD = 0.55), and the average maximum width was 3.98 mm (n = 28, SD = 0.60). The average stalk length was 3.14 mm. Each capsule contained 5–14 embryos showing a velum and, in most cases, an incipient foot. These characters allowed us to classify these embryos as being in the early pediveliger stage. In some cases, we found uncleaved eggs. Development was synchronous within and among capsules. Shell length at the embryo stage was 0.72–1.6 mm (X = 1.26 mm, n = 241, SD = 0.14) (Fig. 4).

Transitional individuals still have a well-developed penis (Fig. 5). Females have a vestigial penis just posterior to right cephalic tentacle (Fig. 6). Histological studies of the gonads of males, transitional individuals, and females were performed.

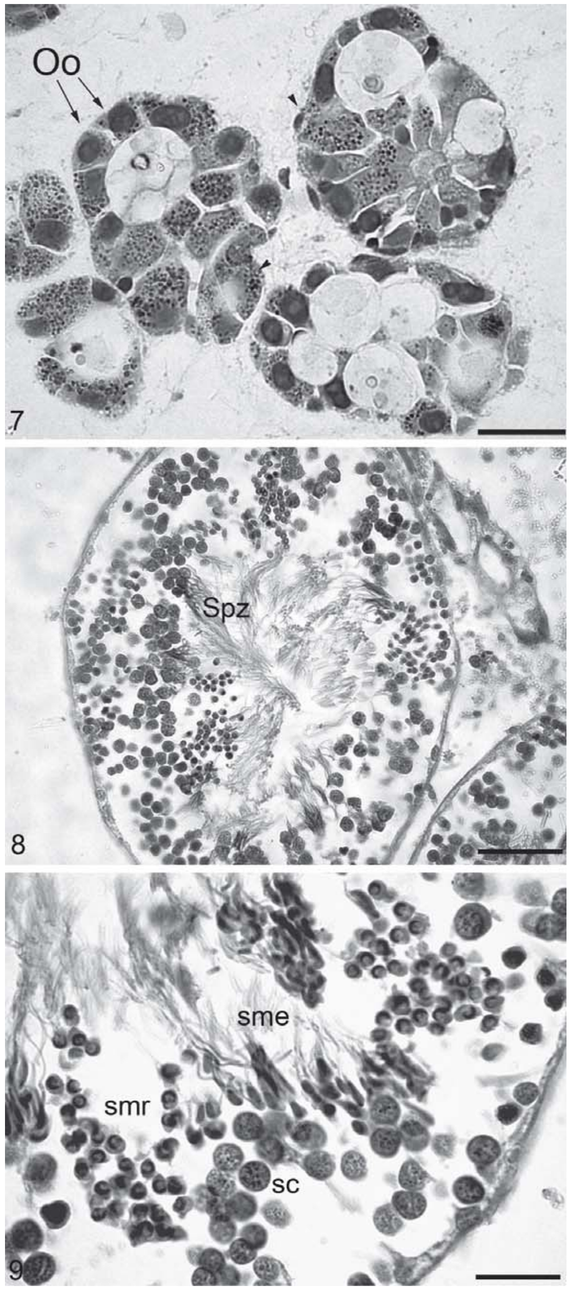
In individuals larger than 15.9 mm, we found only ovaries (Fig. 7). The histological analysis suggested that spawning in *T. pileus* is not complete, because we found oocytes remaining in the gonad after spawning (Fig. 7). For specimens less than 7 mm, we found only a testis, and we found a testis and an ovary in the individuals of the transitional phase, between 7 and 8 mm. The testis consisted of numerous spermatogenic tubules separated from each other by connective tissue (Fig. 8). Within each spermatogenic tubule, the spermatogenic cells were situated towards the interior side of the basal membrane. Spermatogenic cells, spermatogonia, spermatocites, ring-shaped, elongate spermatids were generally clustered in small groups in the same maturation phase and are distributed throughout the tubule. Mature spermatozoa generally filled the lumen of the tubule (Fig. 8). In transitional individuals, the spermatogenesis occurs similarly to male individuals; details show the different stages of spermatogenic cells (Fig. 9).

DISCUSSION

Protandric hermaphroditism is frequently found in gastropods (Coe, 1938). About 40% of mollusc genera are either simultaneous or sequential hermaphrodites (Heller, 1993). Calyptraeids have a reproduction cycle with



FIGS. 5, 6. Anterior view of *T. pileus.* FIG. 5: Ventral view of *T. pileus* male, detail of the penis (p) in male (1.3 mm); FIG. 6: Dorsal view of the female, detail of the vestigial penis (vp) (0.5 mm) in a female (19 mm SL). Scale bars: Fig. 5 = 1 mm; Fig. 6 = 0.5 mm.



FIGS. 7–9. Light micrographs of histological sections of *T. pileus.* FIG. 7: Ovary; note the presence of oocytes (oo) and the vitellogic oocytes (arrowhead). Scale bar = 0.5 mm; FIG. 8: Transversal section of the spermatogenic tubule in transitional individual, with different stages of spermatogenesis. Scale bar = 50 µm; FIG. 9: Detail of testis of a transitional individual showing sperm (spz) at the lumen, spermatocites (sc), and spermatids in the ring stage (smr) and elongated stage (sme). Scale bar = 20 µm.

a sequence of sexual phases (Heller, 1993; Collin, 2000; Chaparro et al., 2001; Cledón & Penchaszadeh, 2001; Simone, 2002; Chaparro et al., 2005; Collin et al., 2005). Wyatt (1960) described five stages or sexual phases for *Calyptraea chinensis* (Linnaeus, 1758): immature male, functional male, associated male (in species where individuals do not form chains and males associate with females only for copulation), immature females, and females. During the transition period, morphological changes from the male phase to the female phase were observed. The penis is absorbed and the reproductive system is reorganized; transitional individuals have a penis in retraction (Coe, 1938). Usually, there are several phases: undifferentiated juveniles, males, transitional individuals and females (Coe, 1938; Wyatt, 1960; Heller, 1993; Brown & Olivares, 1996; Gallardo, 1996; Collin, 2000, 2003). In the present study, male, transitional individuals and females were analyzed. The individuals at the male phase and the transitional phase have a well-developed penis. The female phase showed a vestigial penis. The successive stages in the development of the reproductive system in the two sexual phases are correlated with the development of secondary sexual characters. Males have a penis, and transitional individuals and females present a vestigial penis just posterior to right cephalic tentacle.

Sometimes the transition from the male to the female phase takes place rapidly, but often there is a prolonged period of apparent inactivity, during which the gonadal follicles contain few oogonia. The duration of the transitional period is related to the isolation degree for individuals (Coe, 1938).

Egg Capsules and Developmental Pattern

Caenogastropods show a considerable morphological variation in spawn, ranging from gelatinous masses attached to hard substrata at the bottom to pelagic capsules. Capsules may be complex, with delicate to tough walls, and their shape and size can vary within families and genera (Thorson, 1946; Fretter & Graham, 1962). There are many kinds of parental care in caenogastropods. Adults can defend their spawn from predators actively, whereas others brood capsules in the mantle cavity, oviduct, foot or even a special chamber in the head (Ponder et al., 2007). All calyptraeids show parental protection during brooding; it involves the physical care of an egg mass or cluster of capsules, which are attached to substratum under the female (Collin, 2003). Calyptraeids are characterized by the production of stalked egg capsules with a thin membrane (Gallardo, 1979; Hoagland, 1986; Chaparro et al., 1999; Cledón & Penchaszadeh, 2001). *Trochita pileus* has transparent, triangular-shaped egg capsules with a fixation stalk, and exhibits parental care.

The fixation stalk length is related to the position of the incubated egg mass. Brooding over the head requires a longer fixation stalk or peduncles compared to brooding between the head and the propodium (Hoagland, 1986). Egg capsule morphology is not a taxonomically useful character according to Hoagland (1977); however, for species in which this character has been described, differences in shape or size could have taxonomic value (Gallardo, 1977, 1979).

Calyptraeids present various reproductive strategies; some species have nurse eggs, whereas other do not. Also there is considerable variation in the morphology and consumption of nurse eggs (Collin, 2003).

We did not find enough evidence to affirm there are nurse eggs in *T. pileus*. We found only two unsegmented eggs from a total 241 embryos in the same development stage. Collin (2000) also described synchronous development for *Crepidula adunca* (G. B. Sowerby I, 1825) and *Crepidula lingulata* (Gould, 1846).

There are a few descriptions about the gonadal histology of these species (Chaparro et al., 1998). The testis is composed of numerous spermatogenic tubules where the spermatogenesis, which is responsible for the production of sperm, occurs. Our observations show that spermatogenesis occurs in male and transitional individuals.

Further studies are necessary to elucidate the spermatogenesis and sperm ultrastructure of these species. Studies employing transmission electron microscopy would expound upon these reproductive aspects.

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