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Development of the planktotrophic veligers and plantigrades of *Strombus pugilis* (Gastropoda)

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ABSTRACT

The organogenesis, histogenesis and growth of larvae of the fighting conch *Strombus pugilis* (Linné, 1758) were studied over a period of 30 d after hatching in laboratory culture. Early development of *S. pugilis* was examined by light and scanning electron microscopy. Rearing was conducted at 27 ± 1 °C. Veligers were reared at 200 larvae l^{-1} in 4-l containers. Larvae were fed with the microalgae *Isochrysis galbana* and *Nanochloropsis oculata* at a concentration of 1,000 cell l^{-1}. The protoconch at hatching measured 212 ± 12.14 μm in length and the shell reached 1,100 ± 29.11 μm 29 d after hatching. Development characteristics are described from hatching to settlement. Newly hatched veligers possess two velar lobes, a larval shell consisting of 1.5 whorls, eyespots and a single right tentacle. Late veligers (5-d old larvae) have four velar lobes and two shell whorls and the left tentacle appears. Pediveligers show a functional adult heart at 11 d. Crawling behaviour and settlement were observed from 27 to 31 d. Plantigrades were observed after 29 d, when a functioning proboscis is observed and the velar lobes are lost. This study will facilitate the identification of gastropod larval shells in the plankton and of juveniles in the meiobenthos and will aid aquaculture of *Strombus* species.

INTRODUCTION

The fighting conch, *Strombus pugilis* (Linné, 1758), is one of the most important benthic resources in the Caribbean region. This marine gastropod is one of six species of conchs distributed throughout the Caribbean (Berg, 1976; Brownell & Stevely, 1981; Berg, ORR & Mitton, 1983). In the Yucatan Peninsula, Mexico, the marine gastropod fishery is an important social and economic resource for inhabitants of the region. In 1983, the total whelk and conch production for the Yucatan Peninsula reached a maximum of 1,250 metric tons, which generated a direct income of US $200,000 to the fishermen. At present, the stock is overexploited. In Campeche State, the fishery of five gastropods species represents a mean production of 700 tons per year with *S. pugilis* representing 6% of the total. As a herbivore this species is an important member of the food web. Even though five Caribbean strombid species are commercially exploited, research has mainly concerned *S. gigas*.

We conducted a literature search on strombids (BiOne, Web of Science from 1965 to 2013) and found 183 papers. Of these, 25 papers focussed on larval development, biology and aquaculture of 11 *Strombus* species. Only two of these papers (Brito-Manzano, Aldana-Aranda & Baqueiro-Cardenas, 1998, 1999) described growth and survival of larvae of *S. pugilis*. These authors described the morphological characteristics of the main stages of development as observed by light microscopy, but without giving any illustrations. On the other hand, three studies have used scanning electron microscopy (SEM): (1) identification of *Strombus* larvae from the plankton (Blumer, 1995); (2) shell biomineralization in *S. maculatus* (Hickman, 2013) and (3) shell development and growth of *S. raninus* (de Jesus Navarrete, Davis & Shawl, 2007). Histological investigations of planktotrophic veligers are important not only for providing morphological information to help in determining physiological and behavioural mechanisms of the larvae, but also to elucidate how the rudiments of benthic stage structures develop within the veligers without impeding the processes of swimming and feeding. No previous study has investigated the larval development and ultrastructure of larvae of *S. pugilis* using SEM.

The aims of this study were (1) to describe, using light microscopy and SEM, developmental patterns of organogenesis, growth and mortality of *S. pugilis* larvae; (2) to investigate the ultrastructure of shell and soft tissues in order to facilitate the identification of gastropods in the plankton and in the meiobenthos and (3) to perform larval rearing in support of aquaculture for *Strombus* species.

MATERIAL AND METHODS

Egg masses and reared larvae

Egg masses were collected by hand in shallow water in Celestun, Yucatan, Mexico and transported in a closed insulated container with seawater to the laboratory. Each spawn was maintained in 25-l containers until hatching. Development was carried out at room temperature (27 °C), in seawater at a salinity of 35 PSU, which is typical of the natural habitat. Seawater used for rearing was filtered through a 5-μm Millipore cartridge filter. Larvae were reared according to the methods described by Davis & Hesse (1983) for *Lobatus gigas*, since culture of *S. pugilis* has been poorly documented. Three experimental cultures were set up in 6-l containers with a density of 200 larvae l^{-1}. Larvae were fed daily with unicellular algae (*Isochrysis galbana* and *Nannochloropsis oculata*) at a concentration of 1,000 cell ml^{-1}.

Organogenesis

Developmental characteristics of larvae were analysed by light and SEM, paying particular attention to the velum (number of lobes), number of shell whorls, tentacles and presence of proboscis, foot, propodium and foot pigmentation, eye stalks, operculum claw, swimming and crawling behaviour and settlement. Developmental characteristics were classified chronologically for early veligers, late veligers, early pediveligers, late pediveligers and plantigrades. Each day, 10 larvae were randomly collected from the culture containers for further analysis. Shell growth was measured using a light microscope with a calibrated ocular micrometer to the nearest 0.10 μm. Mortality was established for each stage of development.

For the structural analysis, each day 10 larvae with normal behaviour (swimming veligers and pediveligers; crawling plantigrades) were randomly collected from the batches and placed in small baskets for safe handling during relaxation and fixation. The specimens were anaesthetized for 5 min using a solution of 3 mM $MgCl_2$ in sea water before fixation for SEM and for semithin investigations.

For SEM, larvae were fixed at 4 °C for 1 h in 2.5% glutaraldehyde in 0.1 mol l^{-1} cacodylate buffer, adjusted to pH 7.2. After rinsing in the same cacodylate buffer, larvae were dehydrated in an ascending series of acetone dilutions, and critical-point dried using CO_2 as transitional fluid.

For semithin sections, larvae were prefixed for 1 h at 4 °C following the same procedure as described for SEM and stored in the same cacodylate buffer until they were brought to Guadeloupe, after 1 month. They were then fixed for 45 min at room temperature in 1% osmium tetroxide in the same buffer and rinsed in distilled water. No decalcification process was undertaken. Each sample was dehydrated through a graded ethanol series and embedded in Epon-araldite according to Mollenhauer (in Glauert, 1975). Semithin sections (0.5 μm thick) were cut using an Ultracut E Leica ultramicrotome (Leica, Reuil-Malmaison, France) and mounted onto clean slide on a hot plate (90 °C), stained with 0.5% toluidine blue in 1% borax buffer (Richardson, Jarett & Finke, 1960), washed under distilled water and imaged on a Nikon eclipse 80i light microscope.

RESULTS

All the 10 individuals collected per day were observed even if only the most representative pictures are presented in this study.

Organogenesis observed by light microscopy and SEM

The developmental characteristics of *Strombus pugilis* larval organogenesis observed by light microscopy are shown in Table 1. Sensory organs (ocular tentacles) appeared in early veligers (4 d). The organs allowing benthic feeding appeared after 29 d of larval development. Transformation from pelagic to a primarily benthic behaviour occurred at 25 d and plantigrades were observed at 30 d.

Cumulative mortality was 80% at the end of the study; the main peaks of mortality were observed in the early pediveliger (28%) and late pediveliger stages (20%) (Table 1).

Early veliger development (from hatching to 4-d larvae)

After hatching, larvae showed a strong positive phototactic response. The veligers developed a pair of velar lobes, with a band of long preoral cilia and a band of shorter postoral cilia that allow larvae to swim through the water column, and with purplish pigmented cells along the margin (not shown). The eyes looked like black spots (Fig. 1A). Only one tentacle was present, as a blunt projection above the right eye (Fig. 1D).

The protoconch was transparent and not fully calcified, as suggested by the irregular aspect of the shell. It was cup-shaped, with only one rounded whorl at hatching (Fig. 1A, B). At the end of the first day of development, the shell had 1.5 whorls, with a beak projecting on its outer lip (Fig. 1C), between the dorsal velar lobes. The umbilicus and the beak, which was unsculptured and smooth, were visible in ventral view. The shell length at hatching was 212 (±12.14) μm (Table 1). The buccal mass was positioned between left and right velar lobes (Fig. 1D). The heart and the digestive gland were visible through the transparent shell. Transverse semithin sections of larvae (0.5 μm thick) did not show cellular differentiation in the digestive gland (Fig. 1E). The protoconch I was smooth, without sculpture or striae (Fig. 1C, F). The mortality observed among early veliger larvae was 5%.

Late veligers (5–9-d larvae)

At this stage, the velar lobes were divided and the second pair of lobes had formed. Twenty-four hours after this first velar

Table 1. Shell length, developmental characteristics and mortality of *Strombus pugilis* larvae reared in laboratory.

Stage	Age of larvae (d)	Shell length (μm) ± SD	Developmental characteristics in % of larvae						Mortality (%)/cumulative mortality
			V	%	S	%	T and E	%	
Early veliger	0–4	212 (±12.14)–288 (±40.03)	2	100	1.5	100	Right tentacle	100	5/5
Late veliger	5–9	362 (±27.58)–488 (±38.63)	4	100	2	100	Left tentacle	98	15/20
Early pediveliger	10–19	532 (±40.89)–798 (±66.11)	6	54	3	100	Eyestalk/eyes migration	56	28/48
Late pediveliger	20–29	864 (±33.90)–1,100 (±29.11)	6	93	3.5	66	Pigmented tentacles	74	20/68
Plantigrade	30–35	1,360 (±36.07)–	0	80	4.5	97	Eyestalk with eyes at termini	97	12/80

Abbreviations: V, number of velar lobes; S, number of whorls of shell; T and E, ocular tentacles and eyes.

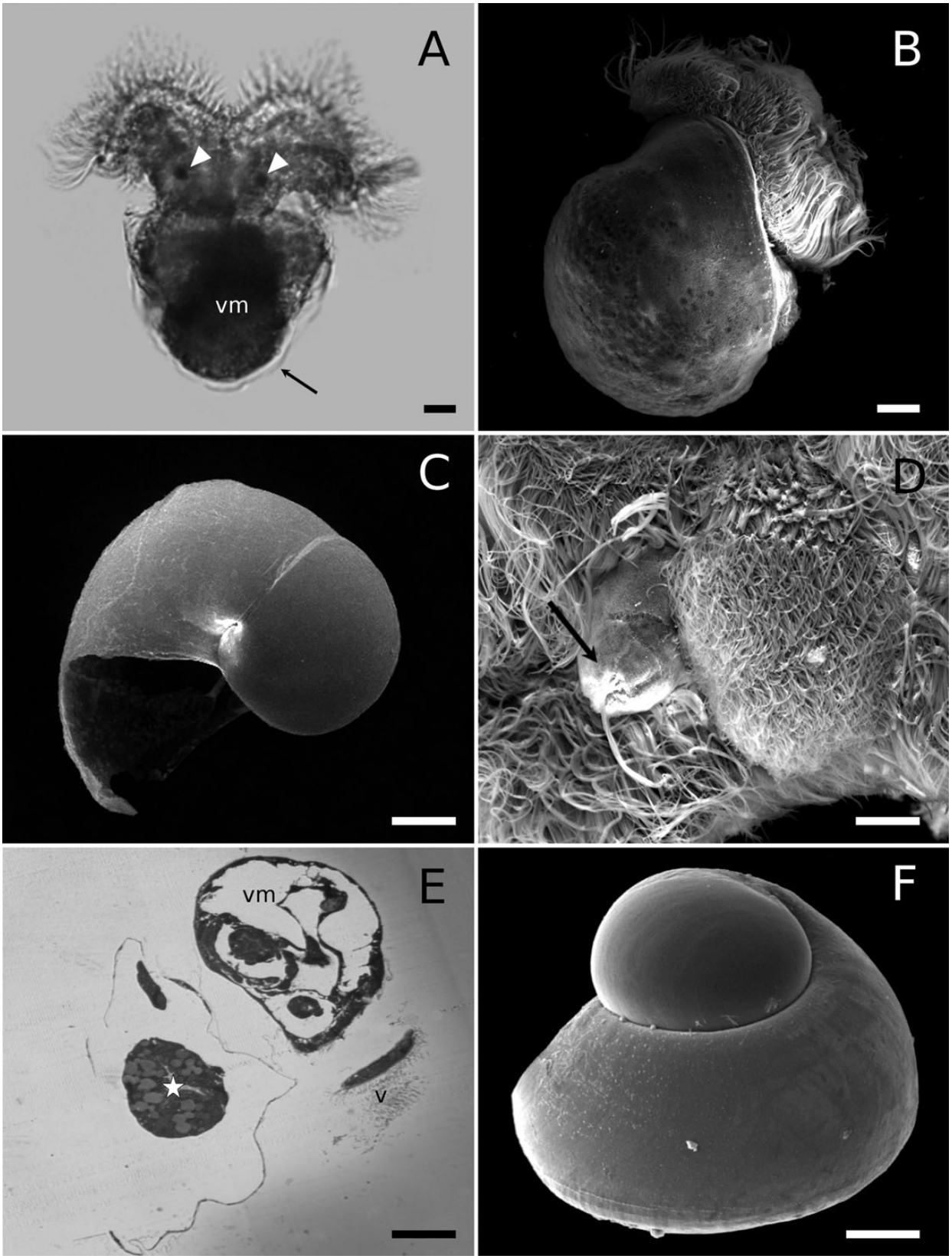

Figure 1. Early veliger larvae of *Strombus pugilis*. **A.** Light micrograph of newly hatched veliger showing ciliated two-lobed velum. Arrow, translucent shell; arrowheads, eye spots; vm, visceral mass. **B.** SEM of newly-hatched larva. Most of the soft body remains inside protoconch. **C.** A few hours later the shell is more calcified and of 1.5 whorls. **D.** SEM detail of central region of velar disk, showing the organ characterized by a principal tuft composed of long single cilium (arrow) surrounded by numerous short cilia belonging to the velar crowns. **E.** Semithin section showing digestive gland (star) composed of a single tube with only one cell type, containing large vesicles. Visceral mass (vm) appears more complex with large, apparently empty cells. V, section of velum. **F.** SEM of 4-d larval shell. Scale bars: **A–C, E–F** = 50 μm; **D** = 5 μm.

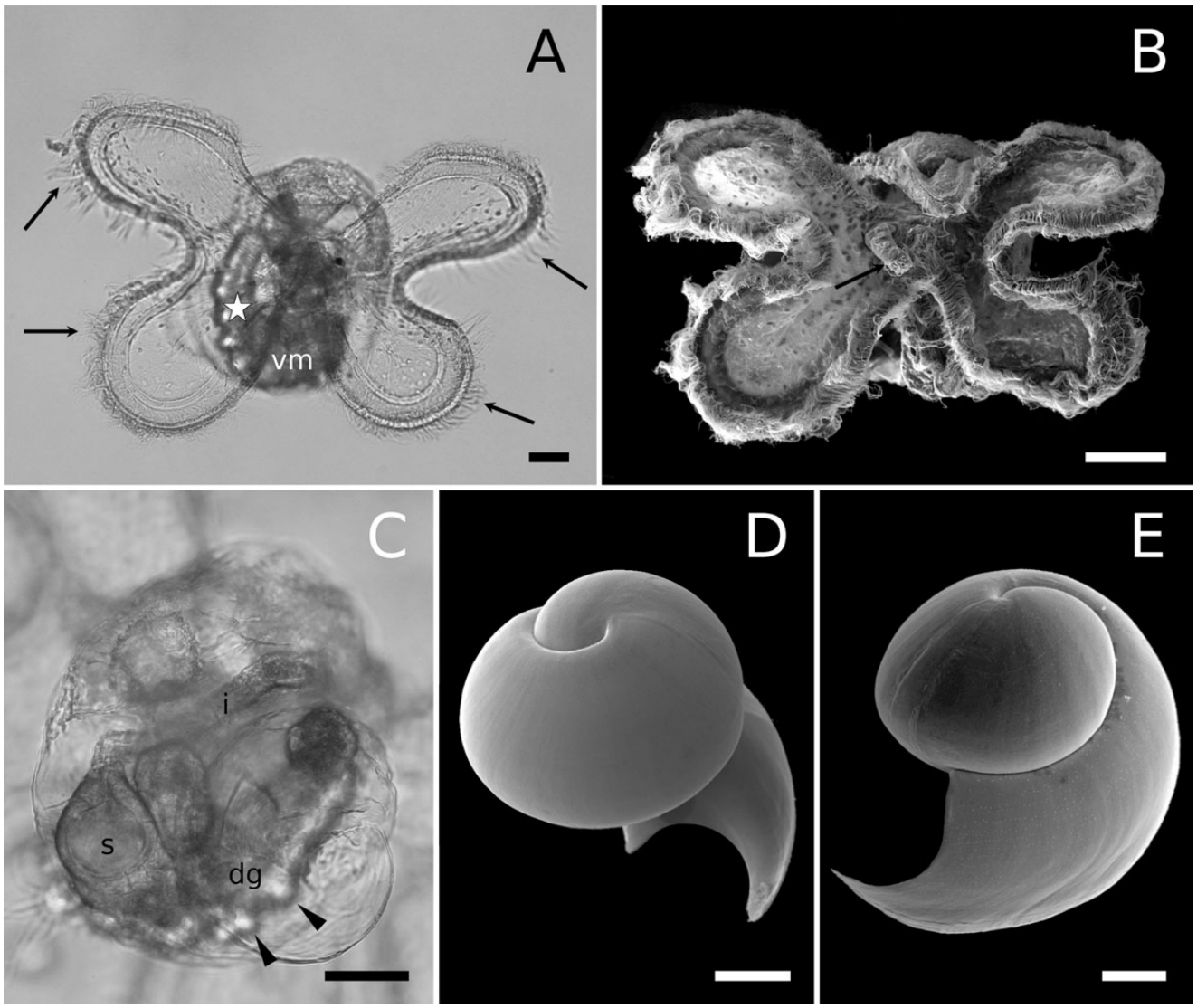

Figure 2. Late veliger larvae of *Strombus pugilis*. **A.** Light micrograph of 9 d larva. The velum has four lobes and cilia of preoral band are active at periphery (arrows). The digestive tract (star) and visceral mass (vm) are visible through shell. **B.** SEM view of four-lobed velum of 10-d larva. Long cilia responsible for propelling veliger are located at periphery of velum. **C.** Light micrograph of visceral mass as observed in a living 10-d veliger through its calcified shell. Arrow heads, digestive gland lobes; dg, digestive gland; i, intestine; s, stomach. **D–E.** SEM views of two-whorled shell of 9–10-d veliger. Scale bars = 50 μm.

division, the lobes were similar in size (Fig. 2A). The long cilia responsible for swimming were located on the periphery of the velum (Fig. 2A, B). A second tentacle developed above the left eye, while the right tentacle doubled in size and now appeared as an elongate knob. At this stage, the two ocular tentacles did not have the same size, suggesting asynchronous development of these organs. Both ocular tentacles developed a number of tactile cilia (Fig. 2B, C). The eyes were located just below each tentacle, like cup-shaped vesicles. The shell completed its second whorl (protoconch II) and remained transparent (Fig. 2D, E). The larval and adult heart and the digestive gland could be seen (Fig. 2B, C). The stomach looked like a straight tube connected to the digestive gland, which had few distinguishable lobes. The digestive gland appeared darker due to the fact that larvae had already ingested unicellular algae (Fig. 2C). On the beak, the sculpture was of five spiral dotted lines (striae), forming a single narrow band (Fig. 2E). The shell at the end of this stage was 488 (±38.63) μm in length (Table 1). The siphonal canal developed on the left side of the shell. At this stage there was 15% mortality of larvae.

Early pediveligers (11–19-d larvae)

The early pediveligers had elongated velar lobes and division of the dorsal lobes started. Although larger, the organization of the velum appeared similar to that in younger larvae, with long cilia responsible for swimming. At this stage (15 d), larvae had formed the third pair of velar lobes; the two pairs of dorsal lobes were more elongated while the ventral lobes were rounded (Fig. 3A, B). The velum showed a multibranched nervous network (Fig. 3C). The ocular tentacles were still not the same size, although they both showed the same functional structures. The eyes migrated onto tentacular stalks (Fig. 3B). The shell measured 798 (±66.11) μm in length at 19 d and was still transparent (Table 1). The sculpture of the shell showed a uniform band of striations on the periphery at midwhorl. The foot was

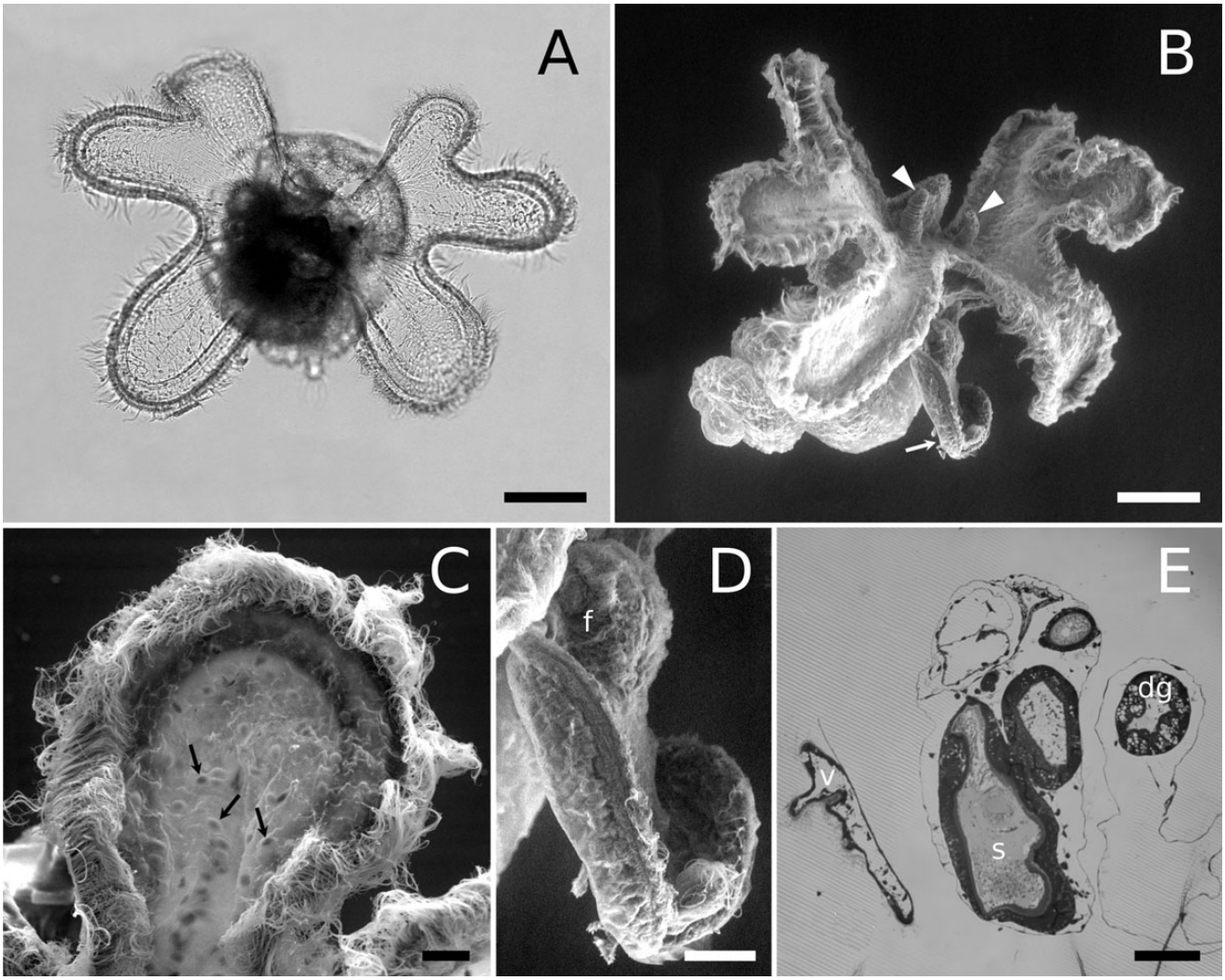

Figure 3. Pediveligers of *Strombus pugilis*. **A.** Light micrograph of a young swimming pediveliger showing a velum with six lobes. Scale bar: 100 μm. **B.** SEM view of the six-lobed velum from 10-d larva. The foot is covered by an operculum (arrow). In the centre of the large velum the ocular tentacles (arrows heads) are obvious. **C.** SEM detail of one lobe of velum showing numerous long cilia at periphery. Small nuclei of non-ciliated cells in central part of velum are visible (arrows). **D.** SEM micrograph of operculum (o) attached to foot (f). Its wrinkled surface is probably due to its uncalcified state. **E.** Semithin midgut transverse section of 14 d pediveliger. Dg, digestive gland; s, stomach; v, velum. Scale bars: **A** = 100 μm; **B** = 70 μm; **C** = 10 μm; **D** = 20 μm; **E** = 50 μm.

divided into two distinct sections, the propodium and metapodium (anterior and posterior sections). At this stage, a number of orange-pigmented cells appeared over the foot. The foot had expanded and had cells with numerous purplish brown pigment granules. The operculum had a translucent white colour (Fig. 3B, D). Semithin transverse sections of the midgut of 14-d larvae revealed a stomach and a digestive gland. The digestive gland was now composed of two of the three cell types observed in the adult digestive gland. The highest mortality (28%) was observed during the pediveliger stage.

Late pediveligers (20–29-d larvae)

Twenty days after hatching, the pediveligers had changed; the three pairs of velar lobes had an elongated appearance (Fig. 4A–C). The eye stalks at the bases of the tentacles were motile and showed white spots (Fig. 4C). The ocular tentacles still had differential development; the more developed right tentacle measured about 230 μm and the left one 110 μm (Fig. 4D). The shell had developed 3.5 whorls and was less transparent, measuring 1,100 (±29.11) μm at 29 d (Table 1); at the end of this stage the beak ceased development. The operculum widened and the terminal 'claw' extended beyond the posterior margin of the foot. The operculum showed a reddish-brown coloration with numerous small red dots. The propodium appeared well developed on the ventral side, suggesting that its main function is to push away the sediment when the animal crawls (Fig. 4C, D). The anterior tip of the propodium had a T-shaped groove with heavily ciliated borders. After 24 d, the velar lobes had shrunk, the jumping action of the foot had become synchronized, so that the late pediveligers pushed the shell along the substrate and started crawling behaviour. The larvae remained on the substrate most of the time.

The late pediveligers (21 d) had a digestive gland with the three characteristic cell types described in adults; the most abundant were vacuolated and digestive cells which were characterized by numerous empty vesicles inside their cytoplasm. The third cell type was noticeably dark and could represent

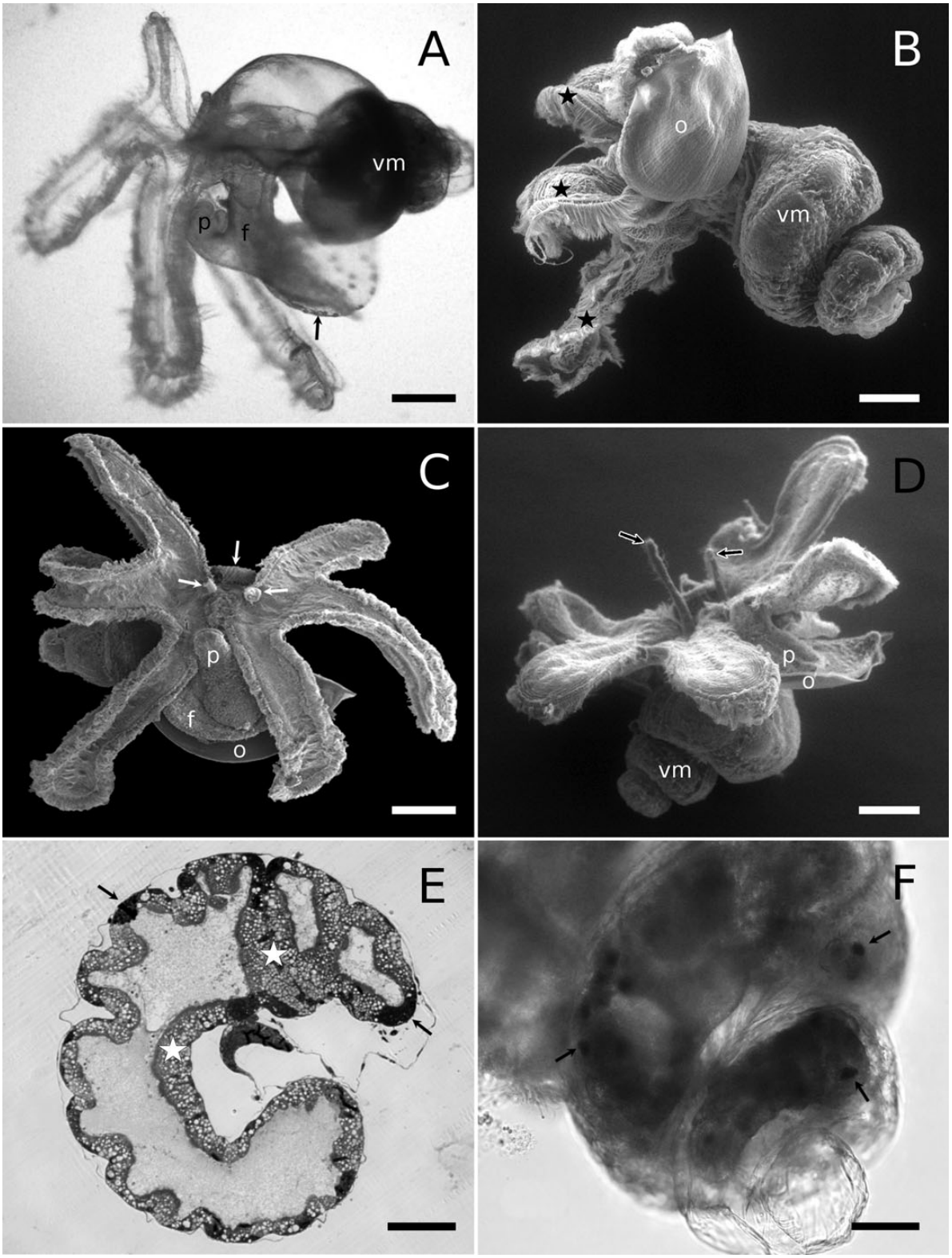

Figure 4. Late pediveligers of *Strombus pugilis*. **A.** Light micrograph of early pediveliger showing well-developed velum with six lobes and visceral mass (vm). When swimming, foot (f) and propodium (p) are outside shell. Arrow, operculum. **B.** SEM view of six-lobed velum from 10-d larva. Each lobe (stars) of velum still possesses large functional cilia allowing swimming. Operculum (o) covers the foot. Shell was dissolved during critical-point drying process, so whorls of visceral mass (vm) are visible. **C.** SEM view of six-lobed velum of pediveliger. The two ocular tentacles (white arrows) are visible close to beak of shell (white arrow). f, foot; o, operculum; p, propodium. **D.** SEM micrograph showing asynchronous development of tentacles (arrows). o, operculum; p, propodium; vm, visceral mass. **E.** Semithin section of digestive gland of 21-d larva, showing groups of vacuolated and digestive cells (stars) and putative crypt cells (arrows). **F.** Light micrograph of digestive gland of late pediveliger. Different whorls of digestive gland can be distinguished through shell. Black dots (black arrows) in cells could be the intracellular parasites already described in all adult individuals of *S. pugilis* examined to date. Scale bars: **A** = 65 μm; **B** = 80 μm; **C** = 10 μm; **D** = 80 μm; **E** = 50 μm; **F** = 40 μm.

the crypt cells (Fig. 4E). The digestive gland diverticulae had numerous black dots inside different cells; these could represent the Apicomplexa-like, intracellular parasites already described in adult individuals of various strombid species including *S. pugilis* (Gros, Frenkiel & Aldana-Aranda, 2009; Volland *et al.*, 2010) (Fig. 4F). Pediveligers reached plantigrade competence at 27–29 d. At this stage, the propodium had spots of a dark green colour. The larvae began to crawl with their propodium and the old pediveligers were now competent to metamorphose. Settlement occurred naturally.

Plantigrades (30–35 d)

The plantigrades (30 d and more) did not have any trace of velar lobes (Fig. 5A, C). Observations of living plantigrades under the light microscope showed that the spiral striae of the shell were smooth and reddish on the inner surface and beige on the outer one. The shell was still translucent with the characteristic band located at the periphery of the body whorl, allowing discrimination of *S. pugilis* larvae from other species (Fig. 5D). A new kind of multiple striations began to develop, covering the body whorl of the teleoconch (Fig. 5E). The eyestalks were well developed with big and bulging eyes at their tips; tentacles developed beyond the eyes (Fig. 5B). At this stage, both left and right ocular tentacles had the same length (Fig. 5C). The operculum and the foot were strongly pigmented. The proboscis was well developed and characterized by dark green pigmentation along its length. No ctenidium was observed through the translucent shell in plantigrades, even though the heart activity was visible. The pedal groove was present at the tip of the propodium, which provided the crawling surface of the foot (Fig. 5C). The mortality observed in the plantigrade stage was 12%.

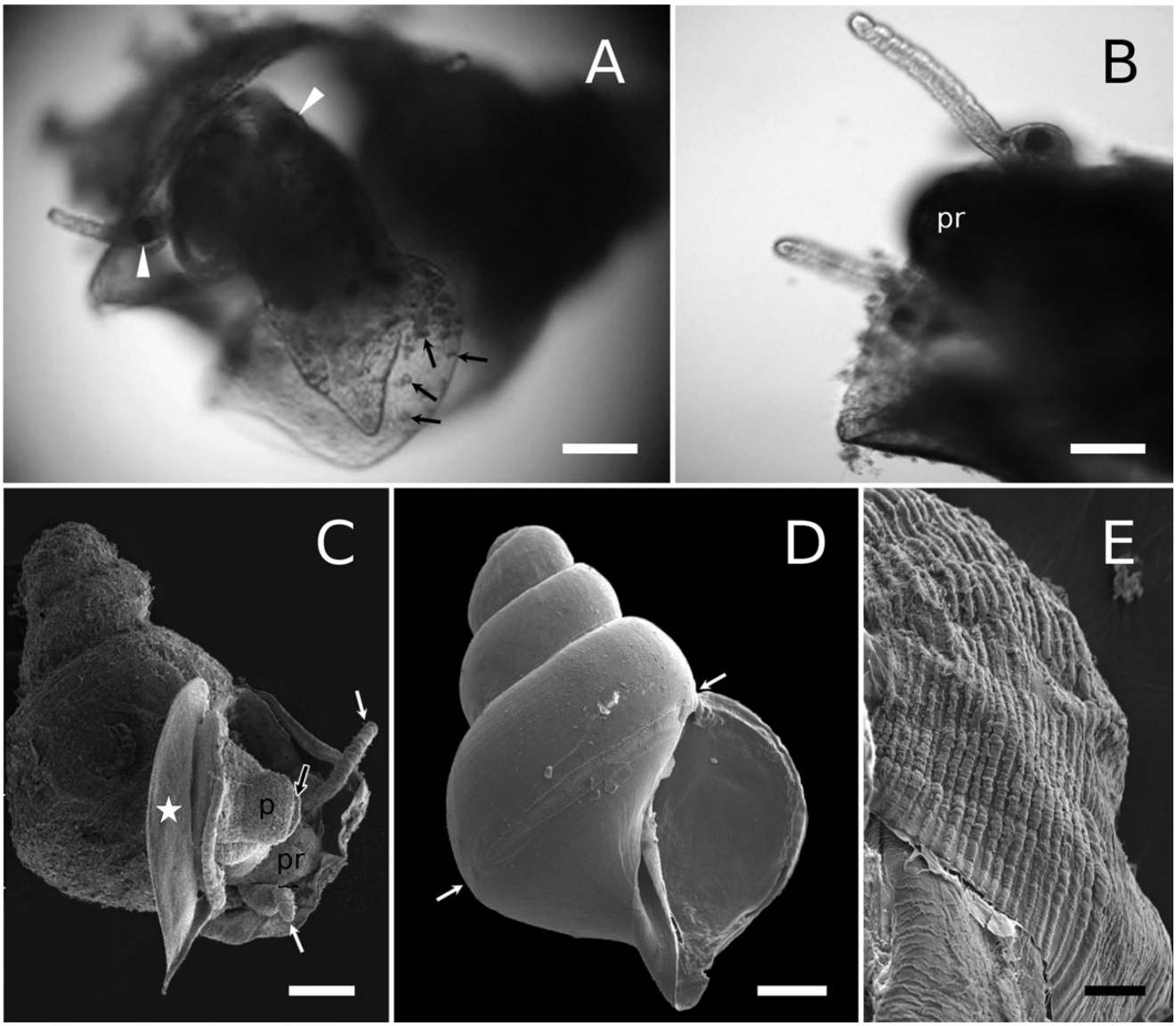

Figure 5. Plantigrades of *Strombus pugilis*. **A.** Light micrograph of plantigrade with eyes (white arrowheads) and tentacles. Operculum and foot possess several pigmented dots (arrows). **B.** Showing two eyes located at the bases of tentacles. The proboscis (pr) is outside shell. **C.** Proboscis (pr) and two ocular tentacles (arrows) are well developed. Shell appears dirty, covered with biofilm. Operculum (star) is attached to extended foot, with furrow (black arrow) in the propodium (p). **D.** At 35 d shell has 4.5 whorls; on body whorl the spiral sculpture band (arrows) is typical of juveniles of the genus *Strombus*. **E.** Shell of plantigrades is characterized by numerous striae that are typical of adult shell. Scale bars: **A** = 100 μm; **B** = 70 μm; **C** = 100 μm; **D** = 125 μm; **E** = 40 μm.

DISCUSSION

The family Strombidae includes at least 70 species, mainly distributed in the Western Atlantic and Indo-Pacific Oceans. According to morphological characteristics this family was divided into five genera (*Strombus*, *Lambis*, *Terebellum*, *Tibia* and *Rimella*) by Abbott (1960). Bandel (2007) suggested the existence of a single genus *Strombus*, subdivided into subgenera (Kronenberg, 2009). A recent molecular study based on mitochondrial (cytochrome oxidase I) and nuclear (histone 3) gene sequence analyses supported division into 11 genera (Latiolais *et al*., 2006). This study confirmed the monophyly of all Recent American strombids. Within this clade, the main commercial species in the Caribbean belong to two genera, *Lobatus* (*L. costatus*, *L. gigas*) and *Strombus* (*S. pugilis*). Both genera may have originated from *Persististrombus* (Kronenberg & Lee, 2007).

The number and types of structures that appear in strombid development during the veliger stage, such as the eyespots, the increase of velar lobes (from 2 to 6), heart and propodium are similar among the genera *Laevistrombus* from the Indo-Pacific and *Strombus* and *Lobatus* from the Atlantic (D'Asaro, 1965; Davis, Bolton & Stoner, 1993; Brito-Manzano *et al*., 1999; Brito-Manzano & Aldana-Aranda, 2004; Cob *et al*., 2009a, b).

Newly hatched *S. pugilis* larvae usually have 1.5 whorls, as do *Lobatus costatus*, *L. gigas*, *L. raninus* and *Laevistrombus canarium* (Davis & Hesse, 1983; Davis *et al*., 1993; Brito-Manzano & Aldana-Aranda, 2004; Cob *et al*., 2009b). The larval shell morphology has been extensively studied for gastropods from various environments (e.g. Fretter & Pilkington, 1971; Fretter & Graham, 1978; Young, 2002; Kowalke, 2006). Usually, planktotrophic larvae show shells with a division into protoconch I and protoconch II; the former develops within the egg capsule and is frequently without sculpture or ornamentation, while the latter forms after hatching and is often sculptured. Davis *et al*. (1993) showed that larvae of *L. costatus*, *L. gigas* and *L. raninus* all have long beaks on the larval shell; however, the sculpture of lines (bands) on the beaks differs among these. *Lobatus gigas* has four raised parallel lines; *L. raninus* has a single faint line that resembles a shaded band; *L. costatus* has sculpture with a C-shaped pattern on the ridge. In this study, we observed early veligers with a smooth unsculptured shell (protoconch I) of 1.5 whorls while, by the late veliger to late pediveliger stage, the protoconch II showed five parallel striae in a single narrow band on the beak. Further spiral striae develop uniformly all over the shell in the plantigrade stage (30 d). SEM characterization of lines on the body whorl of veligers and pediveligers therefore allows species of the two genera *Lobatus* and *Strombus* to be differentiated. Thus, SEM analysis of the larval shell represents an important tool that can potentially be used for ecological studies.

In the Gastropoda, the velum is usually characterized by lobe-shaped outgrowths dorsolateral to the mouth (Fretter, 1967): six lobes are common in caenogastropods with a long planktotrophic phase or holoplanktonic veligers, as in the Strombidae (Thiriot-Quiévreux, 1975). In this study, *S. pugilis* was found to increase the number of velar lobes during development. Two lobes are present at hatching, four lobes are observed between 5 and 15 d of larval development, and six are present 15 d after hatching until settlement. The three strombid species *L. costatus*, *L. gigas* and *L. raninus* also have larvae with six lobes between 15 and 18 d after hatching until settlement (D'Asaro, 1965; Davis & Hesse, 1983; Brito-Manzano & Aldana-Aranda, 2004). Only *Laevistrombus canarium* shows a larva with 6 lobes at 13 d (Cob *et al*., 2009b).

In our study, the organs and structures related to metamorphosis such as the proboscis appeared 30 d after hatching and became functional 3 d after their appearance. The report of the proboscis appearing between 17 and 20 d by Brito-Manzano *et al*. (1999) was a misunderstanding, due to confusion between the propodium and the proboscis. Brownell (1977) reported that the proboscis does not appear before day 25 of larval development and begins to function for grazing on algae within 2 d in *L. gigas* and *L. costatus*, while Cob *et al*. (2009a) observed this phenomenon 16 d after hatching in *Laevistrombus canarium*. The organs responsible for crawling behaviour of *S. pugilis* (the propodium and foot) became distinguishable 10 d after hatching. The propodium was well developed and highly mobile at 15 d, giving the foot an appropriate shape for crawling. Cob *et al*. (2009a) observed in *L. canarium* that crawling could be effected by the densely packed cilia on the pedal sole. While we also observed densely packed cilia on the foot of *S. pugilis*, crawling was the result of kicking movements of the foot, and of extension of the propodium into the sand while the shell and foot served as a penetration anchor, preventing the animal from being pushed

Table 2. Age and shell length at plantigrade of various species of Strombidae reared at different temperatures.

Species	Temperature (°C)	Shell length of plantigrade (μm)	Plantigrade age (d)	References
Strombus pugilis	27 ± 1	1,100	27–29	This study
Strpmbus pugilis	26	–	28	Brownell (1977)
Strombus pugilis	28 ± 1	997	26–28	Brito-Manzano *et al*. (1999)
Lobatus costatus	26	–	36	Brownell (1977)
Lobatus costatus	24	–	35	Aldana-Aranda *et al*. (1989)
Lobatus costatus	26	–	28	Aldana-Aranda *et al*. (1989)
Lobatus costatus	28	–	26	Aldana-Aranda *et al*. (1989)
Lobatus gigas	26	–	27	Brownell (1977)
Lobatus gigas	26–30	–	18–21	Laughlin & Weil (1983)
Lobatus gigas	27	–	27 ± 2	Boidron-Metairon (1992)
Lobatus gigas	28 ± 1	–	27–30	Brito-Manzano & Aldana-Aranda (2004)
Lobatus gigas	28 ± 1	1,900	28	Siddal (1981)
Lobatus gigas	29	1,900	14–35	Davis & Hesse (1983)
Lobatus gigas	27–30	–	21	Davis *et al*. (1993)
Lobatus gigas	28–30	1,100	18–21	Davis & Stoner (1994)
Lobatus gigas	23–25	952–1,258	18–23	Davis (1994)
Lobatus raninus	29	–	–	Davis & Hesse (1983)
Laevistrombus canarium	29 ± 1	–	18	Cob *et al*. (2009a, b)

backwards by the forward thrust, as described by Brown & Trueman (1982) and Trueman & Brown (1992).

Larval development varies between strombid species depending on rearing temperatures (Table 2). Aldana Aranda, Baqueiro Cardenas & Patiño (2001) showed a strong correlation between age at metamorphosis and rearing temperature. In addition, Brito-Manzano & Aldana-Aranda (2004), working during three reproductive seasons, described differences in the larval development of *L. gigas* according to the biochemical contents of eggs and larvae. These authors observed that larvae had two lobes at hatching in March to June, while those obtained in July to August showed four lobes at hatching and settled earlier under the same rearing conditions (temperature, salinity, food, etc.). Similarly, Cob *et al.* (2009b) showed a difference in shell length between larvae obtained in the wet and dry seasons, although it is difficult to compare these data because culture conditions were different.

Most authors have considered that larvae become competent to metamorphose when the velar lobes are resorbed, but have not specified when the ctenidium of adults appears. Only D'Asaro (1965) has reported the first filaments of the ctenidium in a 46-d juvenile using light microscopy, although no ctenidial filaments were observed in histological sections. Could it be possible that strombid larvae become plantigrades before metamorphosis has been completely achieved? In the lucinid bivalve *Codakia orbicularis*, Gros, Frenkiel & Mouëza (1997) have shown that newly settled larvae, while not possessing a velum (i.e. plantigrade stage), did not have a ctenidium. Thus, in this case, metamorphosis is completed only with the differentiation of gills a few weeks after settlement. Thus, settlement does not mean complete metamorphosis.

Mortality was highest in the early and late pediveliger stages. This suggests that factors that contribute to mortality could be related to developmental processes such as formation of the adult heart, settlement or crawling behaviour. In a review Pechenik (1999) noted that some authors have suggested that most developmental mortality occurs at the time of substrate exploration before metamorphosis.

Further ultrastructural studies are needed to reveal the cellular organization of some organs like the larval digestive gland and ctenidium, in order to characterize organogenesis at the cellular level and the cellular changes that occur during metamorphosis.

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