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Growth and development of the veliger larvae and juveniles of *Polinices pulchellus* (Gastropoda: Naticidae)

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Adult *Polinices pulchellus* were collected from the field and held in aquaria under ambient conditions. Egg collars laid by *P. pulchellus* were cultured at 14°C and 20°C and larval development after hatching was documented photographically. Planktotrophic *Polinices pulchellus* veligers hatched from egg collars cultured at 20°C after nine to ten days and after 14 to 15 days at 14°C. Veligers spent most of their time close to the water surface and began feeding within one hour of hatching. Repeated attempts to raise larvae to metamorphic competency at 14°C were unsuccessful. Morphological changes, most notably in the colour and size of the velum and foot, were observed in larvae raised at 20°C. During the first 25 days of larval development the velum broadened and bifurcated into four velar arms, the distal regions of which acquired a deep red coloration. By day 40 the foot had increased considerably in size and the degree of black pigmentation. By day 45 pediveligers were competent to metamorphose to the juvenile stage. Exposure to sediment from the adult habitat induced metamorphosis, larvae lost their vela and became benthic juveniles. Within three days of metamorphosis, juvenile snails drilled the bivalve *Lasaea adansoni* (~2 mm), later drilled *Cerastoderma edule* (~4 mm), and displayed cannibalistic behaviour. Larvae survived for ~6 months in the absence of a suitable settlement cue.

INTRODUCTION

The larval biology of the Naticidae is poorly described, with the notable exception of the recent publication by Pedersen & Page (2000). Ambiguity exists within the limited literature available for this taxon regarding the mode of larval development attributable to a given species. Poecilogony, the phenomenon of a single species exhibiting different modes of larval development, has been suspected for several naticid or 'moon snail' species, including *Polinices triseriata*, *Euspira catena*, and *Polinices lewisii* (Thorson, 1950; Giglioli, 1949; Bernard, 1967). Bouchet (1989) dismissed its occurrence within the Naticidae, while Pedersen & Page (2000) considered the occurrence of cryptic sibling species as a possible explanation for poecilogony.

The present study documents for the first time the development and growth of veliger larvae of *Polinices pulchellus* (Risso), an important predator of bivalves, that occurs commonly around the coast of Britain on sandy substrata in shallow inshore waters (Kingsley-Smith, 2002). The authors examine the existence of poecilogony in this species and present observations of the predatory behaviour of newly settled juveniles.

MATERIALS AND METHODS

Adult *Polinices pulchellus* were collected by beam trawl sampling in Red Wharf Bay, Anglesey, UK (April–November 2000), and maintained in groups of five in 3.5-l aquaria. Egg collars produced by *P. pulchellus* (see Kingsley-Smith et al., 2003) were placed individually in 2-l beakers containing 1.8 l of 0.2-μm filtered and UV-irradiated filtered seawater (FSW), and aerated. Beakers were placed in thermostatically controlled water baths held at 14°C and 20°C and inspected daily for the presence of larvae. Temperatures simulated early (June) and late (August) summer temperatures (data from Kingsley-Smith, 2002). Upon the completion of hatching, water containing the larvae was decanted into a sterilized 2-l culture jar. The total volume of FSW was made up to 1.8 l. Larvae were raised on a mixed diet of 10 cells per μl *Rhinomonas reticulata* and 40 cells per μl *Pavlova lutheri* and were transferred to fresh FSW containing a replenished algal mixture on alternate days. Veligers were raised under a 18:6 light:dark cycle in culture jars placed in thermostatically controlled cabinets maintained at 14°C and 20°C. At weekly intervals after hatching, at least 15 larvae were transferred to a cavity slide using a sterile glass pipette and sacrificed by the addition of 1 ml of 70% industrial methylated spirit. Shell length measurements (see inset of Figure 1) were recorded to the nearest 5 μm using an inverted microscope with a calibrated eyepiece graticule. Only larvae with bright red guts, indicating the recent consumption of *Rhinomonas reticulata* cells were measured.

Larvae were photographed on the day of hatching and at weekly intervals thereafter. Up until day 45 post-hatching larvae were transferred to the 15 mm cavity of a Perspex slide (75×35×6 mm) containing a small volume of seawater and photographed under a compound microscope. Pediveligers (45 days post-hatching), newly-settled

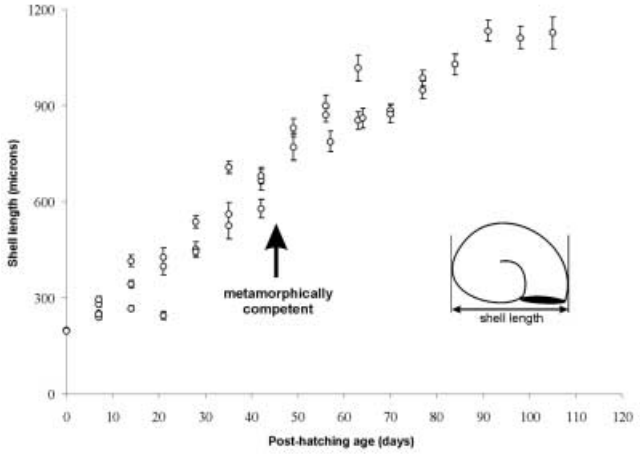

Figure 1. Growth of *Polinices pulchellus* veligers raised at 20°C; values are mean shell lengths (N=15) ±95% confidence intervals. Arrow denotes the point at which larvae become competent to settle. Inset: diagram of the larval shell showing the axis of measurement.

moon snails, and juveniles (up to 1 month post-metamorphosis) were photographed in a glass Petri dish (50 mm diameter×10 mm depth) using a stereomicroscope. A video camera connected to a time-lapse video cassette recorder was fitted to either microscope and still images captured from VHS footage using Adobe Première software.

Individual larvae were examined daily for acquisition of metamorphic competence, based on their ability to crawl across a glass Petri dish, whilst still possessing vela.

Samples of sediment were collected from an area of Red Wharf Bay where *P. pulchellus* occurs (Kingsley-Smith, 2002) using a hand-operated grab. Sediment samples were stored overnight in a cold room at 5°C prior to washing through a 500-μm stainless steel mesh sieve to remove macrofauna. A layer of sediment (1–2 mm depth) was placed on the bottom of a sterilized 2-l beaker, 1.8 l of FSW and algal mixture added, and time allowed for the sediment to settle. Approximately 50 pediveligers were then transferred to the 2-l beaker and left overnight. Between 12 and 24 hours after the introduction of pediveligers the overlying water was decanted and the sediment carefully spread over the bottom of a white plastic tray to a depth of 1 mm. Snails that produced trails in the sediment were examined under a stereomicroscope to confirm the loss of the velum and the projection of a pair of cephalic tentacles beyond the front lip of the shell during crawling, indicating the post-metamorphic condition. A single larval culture was maintained with changes of water and algae on alternate days, until no healthy larvae remained, to investigate the duration of delayed metamorphosis.

Newly settled snails (~1 mm) were held individually in glass dishes (50 mm in diameter×10 mm depth) filled with FSW and covered with glass lids. Each snail was offered two bivalves and maintained at 20°C in a thermostatically controlled cabinet under constant illumination. Initially the small bivalve *Lasaea adansoni* (~2 mm) was offered as prey. From 14 days post-metamorphosis onwards, cockles, *Cerastoderma edule* (~4 mm), were offered as prey. Dishes were checked daily and prey items that had been drilled or had died were replaced with animals of similar size. *Lasaea adansoni* and *C. edule* were chosen as prey items based on their suitable size for effective manipulation by *P. pulchellus* and their local availability in high abundance.

RESULTS

Veligers hatched from egg collars after 9–10 days when held at 20°C and after 14–15 days at 14°C. During swimming, the veliger shell of *Polinices pulchellus* was generally directed downwards, with the aperture and expanded velum oriented upwards. Upon hatching, larvae were not positively phototropic in response to a focused light source, although they spent most of their time close to the water surface. Young larvae frequently displayed discontinuous movements as a result of the interruption of feeding activity on reaching the water surface. At such times the velar cilia stopped beating, the velum became folded, and larvae sank short distances before swimming was resumed. Larvae spent less time at the water surface as they increased in age and size, and on approaching metamorphic competence were commonly observed close to the bottom of the culture jar. The presence of *Rhinomonas reticulata* cells in the guts of veligers within one hour of transfer to the culture jars indicated that they were actively feeding.

Repeated attempts (N=6) to raise *P. pulchellus* veligers at 14°C were unsuccessful. Figure 1 shows the mean shell length of larvae raised at 20°C. Shell growth was approximately linear up until day 90 post-hatching.

Figure 2 illustrates the changes in appearance of the veligers after hatching (Figure 2A,B), with increasing age (Figure 2C–E), at metamorphic competence (Figure 2F,G), and following settlement as juveniles (Figure 2H); where variation in the duration of developmental phases occurred, events are expressed in relation to median days, i.e.'6 to 7 weeks' is expressed as 'day 45'. The most notable changes with increasing age occurred in the appearance of the velum and foot. Newly hatched veligers had a bi-lobed velum, a pair of eye spots (Figure 2B), tentacles (Figure 2F), and statoliths that were visible through the shell (Figure 2A). During the first seven days of larval development, the velum broadened and began to develop pigmentation at its four corners. By day 14 this pigmentation was fully developed (Figure 2D). At 25 days the velum had become bifurcated into four velar arms. After 40 days considerable increases in both the size and degree of black pigmentation of the foot had occurred; by day 45 larvae (pediveligers) were able to crawl (Figure 2G). The pediveliger foot was clearly differentiated into the anterior propodium and the metapodium, with the operculum attached posteriorly.

Pediveligers spent most of their time exploring the bottom of the culture jar and retained the ability to swim up until metamorphosis (Figure 2F). The velum of the pediveliger stage was visible through the shell when the animal was withdrawn, was observed fully extended during swimming, or was seen protruding at the anterior of the shell during crawling (Figure 2G). The gill was visible in late-stage veligers as a longitudinal fold of the mantle skirt (Figure 2G). Competent pediveligers lost their vela and metamorphosed within 12 to 24 hours of exposure to sediment collected from the adult habitat. Metamorphosis was never observed in the absence of sedi-

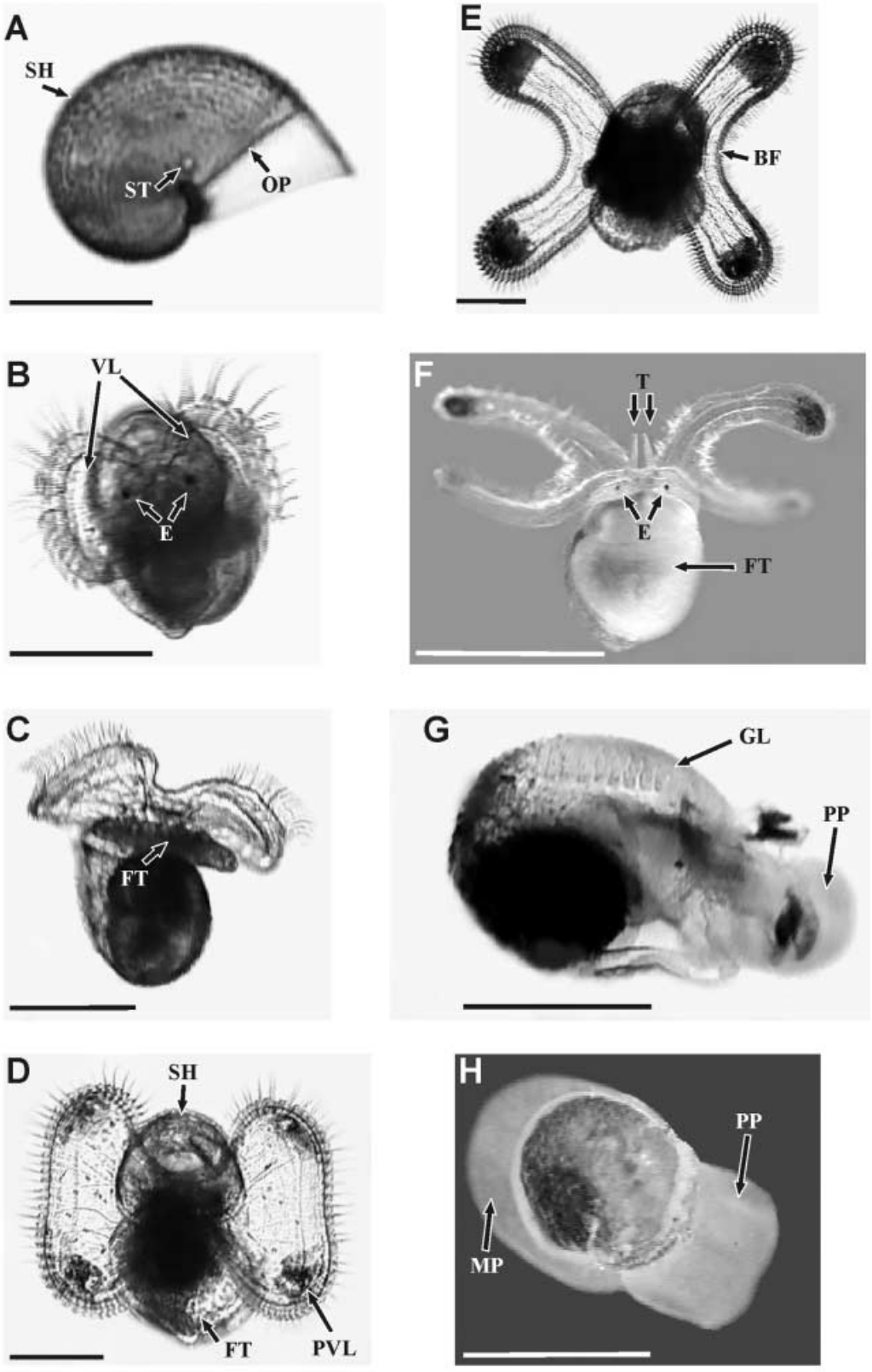

Figure 2. Larval development of *Polinices pulchellus*. (A) Lateral view of a newly hatched veliger (day 0) retracted into its shell (SH) with the shell aperture closed by the operculum (OP). One of the pair of statoliths (ST) is visible through the transparent shell; (B) view from above of a day 0 veliger swimming with its bi-lobed velum (VL) fully expanded. Pair of eyes (E) present that are directed upwards; (C) lateral view of a 7-day old swimming veliger. Little enlargement of the foot (FT) at this stage; (D) 14-day old veliger showing broadened and pigmented velar lobes (PVL); (E) 28-day old veliger in which the velar lobes have undergone bifurcation (BF) resulting in four pigmented velar arms; (F) 45-day old pediveliger with well-developed tentacles (T) and an enlarged foot; (G) 45-day old pediveliger capable of crawling and metamorphically competent. Gill (GL) lying close to the inner surface of the shell. Anterior propodium (PP) well developed; (H) post-metamorphic larva without velum. Foot differentiated into posterior metapodium (MP) and anterior propodium (PP). Scale bars: A, 100 μm; B, 100 μm; C, 150 μm; D, 150 μm; E, 300 μm; F, 800 μm; G, 750 μm; H, 1 mm.

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ment; under these conditions *P. pulchellus* pediveligers changed little in appearance and survived for ~6 months. Within three days of metamorphosis juvenile *P. pulchellus* drilled and consumed *Lasaea adansoni* (~2 mm), and by day 14 post-metamorphosis preyed upon *Cerastoderma edule* (3–4 mm). Juvenile moon snails were also cannibalistic.

DISCUSSION

Polinices pulchellus hatched as planktotrophic veligers in all cultures reared in this study and remained planktonic for a number of weeks. No other modes of development were observed (i.e. lecithotrophic, planktonic development, or direct development, in which juveniles hatched from egg collars). We therefore discount the existence of poecilogony in this species, at least within the population investigated in Red Wharf Bay. This phenomenon may occur among distinct populations of *P. pulchellus*, but such a conclusion requires further study by investigating the reproduction of individuals collected from different sites.

The finding that *Polinices pulchellus* completed its larval development and metamorphosed to the juvenile stage when cultured at 20°C but not at 14°C is perplexing. The seawater temperature for Red Wharf Bay in June 2001, a time of year when *P. pulchellus* is reproductively active and presumably veligers are present in the plankton, ranged from 12.6°C to 16.2°C (Kingsley-Smith, 2002). The failure of pediveligers to reach metamorphic competency at 14°C may therefore be an artefact of rearing in the laboratory. Pedersen & Page (2000) demonstrated that *P. lewisii* larvae raised at 20°C to 22°C were competent to settle after 4–5 weeks compared with those raised at 12°C which failed to settle after 3.5 months. This is surprising given that 12°C falls within the range of seawater temperatures around southern Vancouver Island where *P. lewisii* occurs. Pedersen & Page (2000) speculated that *P. lewisii* larvae are probably retained within the warmer waters of shallow protected embayments in which their development and successful metamorphosis takes place. The planktonic life of prosobranch gastropod veligers is generally short and as a general rule larvae of temperate-water species are planktonic for between two and six weeks. *Polinices duplicatus* veligers hatch from egg collars after ten to 12 days when reared at 18°C to 20°C, are planktonic for 25 days develop a foot, and metamorphose approximately five days later (Hanks, 1960). Metamorphosis typically occurs rapidly, which is advantageous given the vulnerability of larvae to predation and nutritional stress during this transition.

Newly settled *P. pulchellus* drilled and consumed *Lasaea adansoni* within three days of metamorphosis, and *Cerastoderma edule* after 14 days. Bernard (1967) reported that *Polinices lewisii* up to 5–6 mm feed on *Ulva* and diatoms; yet Pedersen & Page (2000) found that this species readily drills bivalves and ostracods within three to five days of metamorphosis. Ostracods have been identified as an important food source for juvenile naticids (Kabat, 1990). Cannibalism by *P. pulchellus* was observed in the present study and widely reported among the Naticidae (e.g. Dietl & Alexander, 1995).

Sediment from the adult habitat induced metamorphosis in *P. pulchellus* (although the precise nature of the cue has yet to be determined. Pedersen & Page (2000) demonstrated the equivalent response in *P. lewisii* and found that metamorphosis did not occur when autoclaved sediment was used. In the absence of a suitable metamorphosis-inducing cue, *P. pulchellus* larvae survived for ~6 months. If delayed metamorphosis occurs in the field, predator avoidance in the plankton may be less critical than finding a suitable substratum for juvenile survival and growth. Despite an evolutionary trend towards their loss (Pechenik, 1999), planktonic larval stages, such as those described here for *P. pulchellus*, remain common components of benthic invertebrate life histories (Levin & Bridges, 1995).

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