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**FIELD EVIDENCE FOR DELAYED METAMORPHOSIS OF LARVAL
GASTROPODS: *CREPIDULA PLANA* Say, *C. FORNICATA* (L.), AND
BITTIUM ALTERNATUM (Say)**

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Abstract: The ability of marine invertebrate larvae to delay metamorphosis to adult form and habitat is known mostly from laboratory studies. However, larvae of *Bittium alternatum* (Say), *Crepidula fornicata* (L.), and *C. plana* Say that are delaying metamorphosis in the plankton should be recognizable since the larvae of these species continue to increase their length and biomass after becoming competent to metamorphose and, in the two *Crepidula* species, also show altered shell morphology. Delaying larvae of *C. fornicata* and *C. plana* made up $\approx 5\text{--}20\%$ of the *Crepidula* spp. larvae captured in the plankton in June, 1974. Larvae of *Bittium alternatum* that could be induced to metamorphose within 8–10 h of their collection from the plankton were $\approx 25\text{--}50\%$ heavier than individuals induced to metamorphose upon first becoming competent to do so, suggesting that the larvae of this species also delay metamorphosis in the field under at least some circumstances.

Key words: larvae; metamorphosis; gastropods; *Crepidula* spp.; *Bittium alternatum*

INTRODUCTION

In the laboratory, the free-swimming larvae of benthic marine invertebrates must generally develop for a period of days or weeks before becoming competent to metamorphose to the sedentary, or sessile, adult form (Thorson, 1950; Crisp, 1974; Chia, 1978). Competent larvae can generally be triggered to metamorphose through exposure to chemical and physical cues typically associated with some component of the adult habitat (e.g., Wilson, 1948; Knight-Jones, 1951, 1953; Scheltema, 1961; Crisp & Meadows, 1963; Williams, 1964; Doyle, 1975; Hadfield, 1977; Sebens, 1983; Benayahu & Loya, 1984; Burke, 1984; Olson, 1985). In the absence of such cues, metamorphosis can be delayed; the duration of the delay period varies with species and culture conditions (Pechenik, 1980, 1984a,b, 1985; Jackson & Strathmann, 1981; Lima & Pechenik, 1985).

Evidence that molluscan larvae delay metamorphosis in the field is limited to a study on mussel larvae (*Mytilus edulis*) (Bayne, 1965). Laboratory observations indicated that in *M. edulis*, the velum, a uniquely larval organ for swimming, food collection, and gas exchange, degenerates during delayed metamorphosis; thus, mussel larvae that are delaying metamorphosis in the field should be identifiable by examining the velum. Up

to 28% of the mussel pediveligers collected by Bayne (1965) off the coast of Denmark showed this degenerative condition.

Evidence that larvae of three gastropods – *Bittium alternatum* (Say), *Crepidula fornicata* (L.), and *C. plana* Say – also delay their metamorphosis in the field is presented here. The larvae of all three species have been previously described (Werner, 1955; Thiriot-Quévieux & Scheltema, 1982). In the laboratory, larvae of *C. fornicata* become competent to metamorphose at $\approx 700\text{--}800\ \mu\text{m}$ (longest shell length) (Werner, 1955; Pechenik, 1980; Thiriot-Quévieux & Scheltema, 1982); competent larvae of *C. fornicata* generally display a conspicuous flat brim around the entire edge of the earlier spiral shell (Pechenik, 1980). Larvae of *C. plana* also typically form such a shell brim once they become competent to metamorphose, at $\approx 640\text{--}700\ \mu\text{m}$ (Thiriot-Quévieux & Scheltema, 1982 – see their Fig. 4; Lima & Pechenik, 1985). The larvae of both *Crepidula* species continue to grow after the onset of competence for metamorphosis, and eventually metamorphose spontaneously at shell lengths ranging between ≈ 1.2 and $2.0\ \text{mm}$ when reared at $18\text{--}25\ ^\circ\text{C}$ (Pechenik, 1980, 1984a; Pechenik & Lima, 1984; Lima & Pechenik, 1985). Thus, larvae of *C. fornicata* and *C. plana* that are delaying metamorphosis should be recognizable in the plankton on the basis of shell morphology and size.

Laboratory-reared larvae of *Bittium alternatum* first became competent to metamorphose in response to red filamentous algae (*Ceramium* sp., *Neoagardiella* sp.) at a shell length of $\approx 325\ \mu\text{m}$ (2.8–3.0 shell whorls), and a total dry weight (including shell) of $\approx 4.2\ \mu\text{g}/\text{individual}$; loss of the velum was completed within 2 h of exposing larvae to the algae (Pechenik, 1980). Competent larvae of this species continued to swim and grow for at least several more weeks in culture at room temperature; average total dry weight after a delay period of ≈ 30 days was $5.35\ \mu\text{g}/\text{larva}$ (Pechenik, 1980). Competent larvae of *B. alternatum* can therefore be identified by shell size and total dry weight.

MATERIALS AND METHODS

Larvae were obtained by sampling from a bridge at the mouth of Little Pond, E. Falmouth, Mass. (*Bittium alternatum*) or by plankton towing in Buzzard's Bay, Mass. (*Crepidula fornicata* and *C. plana*). A mesh of $150\ \mu\text{m}$ diameter was used for all plankton collections, and all samples were obtained during summer 1974.

To determine whether any larvae of *Bittium alternatum* were delaying metamorphosis at the time of collection in the field, all larvae of this species were removed from freshly collected plankton samples and exposed to filaments of the alga *Ceramium* sp. All individuals that metamorphosed within the next 8–10 h were measured, rinsed in distilled water to remove adhering salts, dried, and weighed as a group to the nearest $0.1\ \mu\text{g}$ using a Cahn electrobalance. Desiccant (indicating CaSO_4) was placed in the weighing chamber of the balance before weight determinations were made.

The unmetamorphosed larvae of *B. alternatum* were transferred to a clean dish of freshly filtered sea water, more *Ceramium* filaments were added, and another series of

measurements were made on metamorphosed individuals after an additional 8–10 h. This process was repeated daily until all larvae had lost the velum through metamorphosis. Two such experiments were conducted about 1 month apart. The sizes and weights of these field-collected individuals at metamorphosis were compared with those of juveniles previously reared in the laboratory and induced to metamorphose at the onset of competence (Pechenik, 1980).

On two occasions, discrete depth samples (near surface and near bottom – ≈ 10 m) were taken in Buzzard's Bay to assess the depth distribution of *Crepidula* spp. larvae possessing shell brims; in the laboratory, shell brims form in these two species only after larvae become competent to metamorphose (Pechenik, 1980; Pechenik & Lima, 1984; Lima & Pechenik, 1985). Volume of water filtered by the plankton net during each tow was determined using a calibrated flow meter mounted across the mouth of the net; at least 20 m³ of sea water was filtered for each sample. Samples were sorted within 2 h of collection; all brimmed larvae of *Crepidula* spp. were removed from the samples and counted. Twenty-five of these competent larvae from each sample were held in glass dishes and fed the naked flagellate *Isochrysis galbana* Parke at $\approx 18 \times 10^4$ cells/ml until spontaneous metamorphosis took place. Juveniles were subsequently reared for 5–7 days, until the identity of each individual (*Crepidula fornicata* or *C. plana*) could be determined; the larvae of the two species are virtually indistinguishable for most of their development.

RESULTS

In both studies, the larvae of *Bittium alternatum* that metamorphosed within ≈ 10 –48 h after their collection from the plankton were substantially heavier than larvae

TABLE I

Weight at induced metamorphosis for field-collected larvae of *Bittium alternatum*: the larvae used in an experiment were obtained from a single plankton tow and exposed to inducing substratum daily; Day 1 is the first day after collection of larvae from the plankton.

Day of metamorphosis	No. metamorphosed	Average total dry wt ($\mu\text{g}/\text{larva}$)
Expt. 1		
1	12	5.4
2	2	5.5
3	0	–
4	4	4.2
Expt. 2		
1	15	5.7
2	7	6.1
3	0	–
4	0	–
5	3	4.5
6	0	–
7	4	4.1

from the same collection metamorphosing several days later (Table I). These heavier larvae, metamorphosing soon after their collection from the plankton, were apparently delaying metamorphosis at the time of their capture. In contrast, the average total dry weight of individuals metamorphosing 4–7 days after collection was similar to that of individuals reared in the laboratory and induced to metamorphose upon becoming competent to do so (Pechenik, 1980); the lighter field-collected individuals apparently became competent to metamorphose only after their capture.

Although most of the field-collected larvae of *B. alternatum* that were induced to metamorphose in the laboratory had about three shell whorls, occasionally larvae were collected with up to 3.7–3.8 shell whorls, again suggesting a period of delayed metamorphosis for these individuals.

Plankton tows taken on 26 June 1974 in Buzzard's Bay contained a substantial proportion of large (shell lengths $> 800 \mu\text{m}$), brimmed larvae of *Crepidula* spp. (Table II). Many of these brimmed larvae, taken from both surface and deep tows, had shells > 1 mm in length. Of the two groups of 25 brimmed, competent larvae that were held to spontaneous metamorphosis, 33 and 47% were identified as *C. fornicata* and 53 and 67% were identified as *C. plana*. On 4 July of the same year, only one obviously competent (brimmed) larva of either *Crepidula* species – out of 1444 *Crepidula* larvae collected – was captured in the same area of Buzzard's Bay.

TABLE II

Occurrence of brimmed larvae of *Crepidula* spp. in plankton samples taken on the evening of 26 June 1974 in Buzzard's Bay, MA: bottom depth varied between 8.5 and 10.7 m.

Sampling depth (m)	<i>Crepidula</i> spp. larvae/m ³	Total no. <i>Crepidula</i> larvae taken	% of larvae with shell brims
Upper 0.75	40.3	1792	6.7
Upper 0.75	158.6	3188	4.9
5.3	14.6	768	6.9
8.4	23.9	666	21.6

DISCUSSION

The ability of larvae to delay metamorphosis may more than double the duration of the potential dispersal phase of marine benthic invertebrates (Scheltema, 1971; Pechenik, 1980; Jackson & Strathmann, 1981), probably contributing to species-specific differences in recolonization ability, genetic exchange between geographically segregated adult populations, and rates of extinction and speciation (Crisp, 1974; Shuto, 1974; Strathmann, 1974, 1978, 1980; Scheltema, 1977; Hansen, 1978, 1983; Jablonski & Lutz, 1983). Despite its potential ecological and evolutionary significance, delayed metamorphosis is a phenomenon known primarily from the laboratory.

To demonstrate that a species delays metamorphosis in the field, one can work with a species that continues to grow or change morphologically as a competent larva,

determine the size or stage of development at which the larvae of that species first become competent to metamorphose, and then document the occurrence of such large or morphologically advanced larvae in the plankton. These approaches have been applied to larvae of *Mytilus edulis* (Bayne, 1965), *Bittium alternatum*, *Crepidula fornicata*, and *C. plana* (this study), all of which seem to delay metamorphosis in the field under at least some circumstances. The potential ecological or evolutionary significance of these findings depends in part on a demonstration that delaying larvae of these species do not lose the capacity for successful metamorphosis; delaying larvae of the three gastropod species collected from the field in this study clearly retained this capacity, since all tested larvae eventually underwent successful spontaneous or induced metamorphosis.

An alternative approach in demonstrating delayed metamorphosis in the field has been described by Hadfield (1978), working with tornaria larvae of the hemichordate *Ptychodera flava* in Hawaii. Eighty plankton samples were taken over several years; the 48 tornaria larvae captured were only found in samples collected from mid-February through early September. Significantly, most of the individuals that Hadfield collected seemed to be in the process of metamorphosis, which he suggests was triggered by the trauma of collection; this implies that these individuals were competent at the time of collection. Since spawning begins in mid-November and ends by the end of December, and assuming that the larvae are derived from local populations, Hadfield reasoned that the September larvae must be at least 8 months old. Field data suggest that the tornaria larvae of this species become competent to metamorphose within 4 months after spawning, so that the September-collected larvae had apparently been delaying metamorphosis in the plankton for at least 4 months (i.e., 8-4 months).

The conditions under which metamorphosis is delayed in the field remain to be determined. Incidence of delayed metamorphosis is expected to vary with changes in the availability of suitable adult habitat (e.g., Crisp, 1974; Doyle, 1975; Obrebski, 1979; Pechenik, 1980), with delayed metamorphosis becoming more common as habitat suitable for juveniles and adults becomes rarer. In support of this relationship, Bayne (1965) reported a high incidence of mussel larvae (28% of all pediveligers collected) delaying metamorphosis over an area poor in filamentous substratum suitable for juvenile life, and a relatively low incidence (<5%) of larvae delaying metamorphosis over an area rich in suitable filamentous substratum. Comparable field studies can now be undertaken for the larvae of *Bittium alternatum*, *Crepidula fornicata*, and *C. plana*, exploring the relationships between habitat availability and incidence of delayed metamorphosis over the course of the reproductive season.

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