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## Food limitation stimulates metamorphosis of competent larvae and alters postmetamorphic growth rate in the marine prosobranch gastropod *Crepidula fornicata*

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**Abstract** The effects of food limitation on growth rates and survival of marine invertebrate larvae have been studied for many years. Far less is known about how food limitation during the larval stage influences length of larval life or postmetamorphic performance. This paper documents the effects of food limitation during larval development (1) on how long the larvae of *Crepidula fornicata* (L.) can delay metamorphosis in the laboratory after they have become competent to metamorphose and (2) on postmetamorphic growth rate. To assess the magnitude of nutritional stress imposed by different food concentrations, we measured growth rates (as changes in shell length and ash-free dry weight) for larvae reared in either 0.45- $\mu\text{m}$  filtered seawater or at phytoplankton concentrations (*Isochrysis galbana*, clone T-ISO) of  $1 \times 10^3$ ,  $1 \times 10^4$ , or  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ . Larvae increased both shell length and biomass at  $1 \times 10^4$  cells  $\text{ml}^{-1}$ , although significantly more slowly than at the highest food concentration. Larvae did not significantly increase ( $p > 0.10$ ) mean shell length in filtered seawater or at a phytoplankton concentration of only  $1 \times 10^3$  cells  $\text{ml}^{-1}$ , and in fact lost weight under these conditions. To assess the influence of food limitation on the ability of competent individuals to postpone metamorphosis, larvae were first reared to metamorphic competence on a high food concentration of *I. galbana* ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ). When at least 80% of subsampled larvae were competent to metamorphose, as assessed by the numbers of individuals metamorphosing in response to elevated  $\text{K}^+$  concentration in seawater, remaining larvae were transferred either to 0.45- $\mu\text{m}$  filtered seawater or to suspensions of reduced phytoplankton concentration ( $1 \times 10^3$ ,  $1 \times 10^4$ , or  $5 \times 10^4$  cells  $\text{ml}^{-1}$ ), or were maintained at  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ . All larvae were monitored daily for metamorphosis. Individuals that metamorphosed in

each food treatment were transferred to high ration conditions ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ) for four additional days to monitor postmetamorphic growth. Competent larvae responded to all food-limiting conditions by metamorphosing precociously, typically 1 wk or more before larvae metamorphosed when maintained at the highest food ration. Surprisingly, juveniles reared at full ration grew more slowly if they had spent 2 or 3 d under food-limiting conditions as competent larvae. The data show that a rapid decline in phytoplankton concentration during the larval development of *C. fornicata* stimulates metamorphosis, foreshortening the larval dispersal period, and may also reduce the ability of postmetamorphic individuals to grow rapidly even when food concentrations increase.

### Introduction

Fenaux et al. (1994) presented five lines of evidence for the echinoid *Paracentrotus lividus* that rates of larval growth and morphological development are food limited at certain times of the year in temperate coastal waters, and generalized that "...growth and development of other larval forms... are often food limited in coastal waters". Various workers have studied the effects of food limitation on larval survival and rates of growth or, for crustaceans, rates of development through different larval stages (e.g., Perron and Turner 1977; Lucas 1982; Dawirs 1983, 1984; Olson 1985, 1987; Anger 1987, 1995; Fenaux et al. 1988; Wehrman 1991; His and Seaman 1992; Qian and Chia 1991, 1993; Allison 1994; Eckert 1995), and compensatory amplification of larval feeding structures has been demonstrated for certain echinoids (Boidron-Métairon 1988; Hart and Strathmann 1994) and for a bivalve (Strathmann et al. 1993). The potential effects of food limitation on the duration of larval dispersal periods and on postmetamorphic performance, however, have been little studied. The duration of dispersal periods will depend not on growth rates per se, but rather on rates of physiological development, spe-

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cifically on the duration of the precompetent period and the length of time that metamorphosis is delayed in the absence of the external cues that initiate metamorphosis (reviewed by Crisp 1974; Jackson and Strathmann 1981; Scheltema 1986; Pechenik 1990).

Although food limitation is generally assumed to prolong larval life (e.g. Thorson 1950; Vance 1973; Olson and Olson 1989; Morgan 1995), largely because of its known effects on larval growth rates (reviewed by Pechenik 1987), few studies have quantified the effects on duration of larval life, particularly for larvae already competent to metamorphose. Starving or severely reducing the amount of food available to precompetent larvae typically prevents them from attaining competence or significantly prolongs the precompetent period (e.g. Perron and Turner 1977; Lucas 1982; Fenaux et al. 1988; Qian and Chia 1993; Allison 1994; Eckert 1995). Elsewhere we have shown that larvae of *Crepidula fornicata* can attain metamorphic competence even while starving, in the absence of detectable growth (Pechenik et al. 1996a). Here we document the effect of various low food concentrations on larval growth rates for this species, and report the effect of food limitation on duration of the competent period. In addition, we report that starving or food limiting competent larvae of *C. fornicata* can affect postmetamorphic growth rates, a result previously found for larvae starved during precompetent development (Pechenik et al. 1996a).

## Materials and methods

### Obtaining and rearing larvae and juveniles

Adult *Crepidula fornicata* (L.) were obtained from Woods Hole, Massachusetts or Saunderstown, Rhode Island, USA in 1993 and 1994 and held at room temperature (ca. 23 to 25 °C) on a mixed diet of the naked flagellates *Isochrysis galbana* (clone T-ISO) and *Dunaliella tertiolecta* (clone DUN) until larvae were released. All larvae used in an experiment were released on the same day, but not necessarily from one female. At hatching, larvae were about 400 to 450 µm in mean shell length (in one experiment, mean shell length ± SD was 403.9 ± 23.6 µm,  $n = 17$ ; in another, mean shell length was 448.1 ± 21.7 µm,  $n = 25$ ). Larvae were reared in batch culture at approximately 1 larva ml<sup>-1</sup> on a diet of *I. galbana* (clone T-ISO) at approximately 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup> for 3 to 6 d before being used in any experiment; 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup> is hereafter referred to as "full ration." Larvae of *C. fornicata* survive well and grow quickly at this phytoplankton concentration (Pechenik 1980, 1984). Phytoplankton suspensions were prepared by adding an appropriate volume of stock phytoplankton cultures to well-aerated 0.45-µm filtered seawater.

In all experiments, larvae and juveniles were reared at 25 °C with a photoperiod of 12 h light:12 h dark, in 8.5-cm diameter glass dishes containing 45 ml of phytoplankton suspension or other medium. Individuals were changed to fresh, well-oxygenated medium at 25 °C in clean dishes daily; larvae experienced no temperature shocks at water changes. Periodically we determined the phytoplankton cell concentrations in several dishes from all treatment groups to determine the extent to which cell concentrations changed between water changes. In some experiments we also measured all larvae in all replicates on a number of occasions, to determine whether there were any significant differences in mean larval growth rates among replicates within treatments. Shell

lengths were measured at 50× using a dissecting microscope equipped with an ocular micrometer.

### Influence of food concentration on shell growth and biomass for precompetent larvae

Two experiments were conducted. In Experiment A we looked only at larval growth rates at different food concentrations. Adults were collected on 25 April 1994 and larvae were released on 4 May; these larvae were used in no other experiments. Within 24 h of release, at a mean shell length (± SD) of 432.8 ± 20.3 µm ( $n = 20$ ), larvae were transferred to one of three food treatments (1 × 10<sup>4</sup>, 5 × 10<sup>4</sup>, or 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup>), with 4 replicates per treatment and 10 larvae per replicate. Larvae reared at the two highest food concentrations were remeasured 8 d later, while those at the lowest food concentration were remeasured 7 d after that.

For Experiment B, effects on growth and biomass were determined. Adults were collected on 8 July 1994 and larvae were released on 11 July; these larvae were used in no other experiments. Larvae were reared at 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup> in batch culture for 2 d, at which time mean shell length (± SD) was 555.5 ± 25.0 µm, and then distributed among four treatments (5 replicates per treatment, 12 larvae per replicate). Treatments consisted of 0.45-µm filtered seawater or phytoplankton concentrations of 1 × 10<sup>3</sup>, 1 × 10<sup>4</sup>, or 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup> (controls). Precompetent larvae were used in these studies, to avoid complications from spontaneous metamorphosis occurring between measurements. Larval growth rates were assessed nondestructively by measuring changes in larval shell lengths over the next 4 d; larvae were returned to their containers unharmed after measurements were made (Pechenik 1984; Pechenik et al. 1996a).

Changes in larval biomass at the different phytoplankton concentrations were assessed by destructive sampling using larvae from the same batch. To determine initial biomass, well-fed precompetent larvae [12 to 15 larvae in each of three groups, mean shell length ± SD = 628.4 ± 34.2 µm ( $n = 24$ )] were rinsed quickly in distilled water, transferred individually at the tip of a pin to preweighed foil pans, dried at 60 °C for 24 h, weighed, and combusted in a muffle furnace for 5 h at 525 °C; biomass was estimated as ash-free dry weight (Pechenik 1980, 1984). Other larvae from the same batch culture were transferred to dishes containing phytoplankton suspension at either 1 × 10<sup>3</sup>, 1 × 10<sup>4</sup>, or 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup> (controls) (4 replicates per treatment, 15 larvae per replicate). Larvae were transferred to freshly mixed phytoplankton suspension in clean dishes daily for 4 d, and then weighed as already described. Weights were determined to the nearest microgram using a Cahn Model 21 electrobalance, with indicating Drierite placed in the weighing chamber to prevent specimen rehydration (Pechenik 1980, 1984).

### Effects of food limitation on duration of the competent period

Five experiments were conducted on five different dates in 1993 and 1994. In each experiment, larvae were transferred 3 to 6 d after release from batch culture to glass dishes containing 45 ml of phytoplankton suspension at 1.8 × 10<sup>5</sup> cells ml<sup>-1</sup>, with 12 larvae per dish, and maintained at 25 °C. All larvae in several dishes were measured nondestructively (Pechenik 1984; Pechenik et al. 1996a) immediately after this transfer and 3 to 4 d later, to assess larval growth rates.

In each experiment, we tested for metamorphic competence when larvae were about 1000 µm in mean shell length (7 to 11 d after emergence) by elevating the K<sup>+</sup> concentration of seawater by 20 mM and subjecting all larvae in three dishes to these conditions for 5 h (Pechenik and Heyman 1987). Larvae of *Crepidula fornicata* become responsive to natural cues and to elevated potassium concentrations at about the same age (Pechenik and Gee 1993).

When at least 80% of tested larvae were competent to metamorphose, we initiated experimental treatments. In the laboratory,

well-fed, competent larvae of *Crepidula fornicata* eventually metamorphose "spontaneously" in frequently cleaned glassware (Pechenik 1980, 1984; Pechenik and Lima 1984), so the effects of food limitation were quantified by measuring the time to "spontaneous" metamorphosis. In Experiments I and II, larvae were either transferred to dishes containing phytoplankton suspension at  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$  (controls) or to dishes containing 0.45- $\mu\text{m}$  filtered seawater. Larvae used in these experiments were released on 29 September 1993 and on 25 February 1994. In Experiment III, dishes of competent larvae were also transferred to two intermediate food concentrations ( $1 \times 10^3$  and  $1 \times 10^4$  cells  $\text{ml}^{-1}$ ), and in Experiment IV larvae were transferred either to full ration or to  $5 \times 10^4$  cells  $\text{ml}^{-1}$ . Larvae used in Experiment III were released 29 June 1994, and those used in Experiment IV were released 11 August 1994.

We routinely prepare our phytoplankton feeding suspensions by diluting phytoplankton stock cultures with filtered seawater; our feeding suspensions therefore contain diluted phytoplankton growth medium (*f/2*, Guillard and Ryther 1962) and diluted phytoplankton exudate in addition to cells. In Experiment V we therefore tested the possibility that phytoplankton growth medium or exudate might influence duration of the competent period. Competent larvae in some dishes were maintained at full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ), while competent larvae in other dishes were either transferred to phytoplankton that had been centrifuged and resuspended (to  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ) in filtered seawater or to a particle-free filtrate containing algal *f/2* medium and algal exudate; the filtrate was prepared by diluting a phytoplankton stock culture to  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$  with filtered seawater and then filtering the suspension through a 0.45- $\mu\text{m}$  filter.

#### Influence of food limitation on postmetamorphic growth rates

We reared nearly 100 individuals for 4 d following their spontaneous metamorphosis 1, 2, or 3 d after transferring them to low food concentrations in Experiment III, to determine whether food limitation of competent larvae affects their postmetamorphic growth rates. All juveniles were reared individually on *Isochrysis galbana* at  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$  in 45 ml of phytoplankton suspension, with food suspension renewed daily; they were measured at metamorphosis and remeasured after 4 d, at  $50 \times$ .

#### Data analyses

Data were analyzed by standard parametric statistical procedures unless assumptions about equal standard deviations among treatment groups failed Bartlett's test for homogeneity of variances. In those cases, data were analyzed by Kruskal-Wallis nonparametric analysis of variance (ANOVA).

## Results

### Larval survival and growth

No larvae died during these experiments, although a few individuals (never more than 5% in any experiment) were lost or damaged in handling and subsequently discarded.

In spot checks throughout the experiments one-way ANOVA revealed no significant differences ( $p > 0.10$ ) in mean larval shell lengths among replicates while all larvae were being fed full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ), and within each treatment group thereafter. Thus, all larvae were growing at comparable rates among dishes before being subjected to food limitation, and subse-

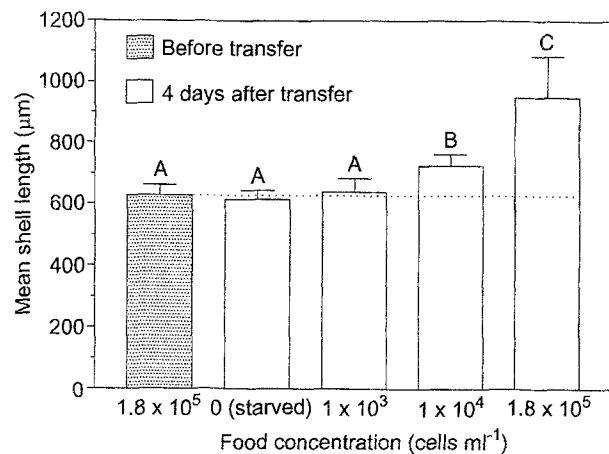
quently within each treatment group. Spot checks on phytoplankton cell concentrations indicated that cells  $\text{ml}^{-1}$  never changed more than 12% between water changes in any treatment.

### Influence of food limitation on shell growth and biomass of precompetent larvae

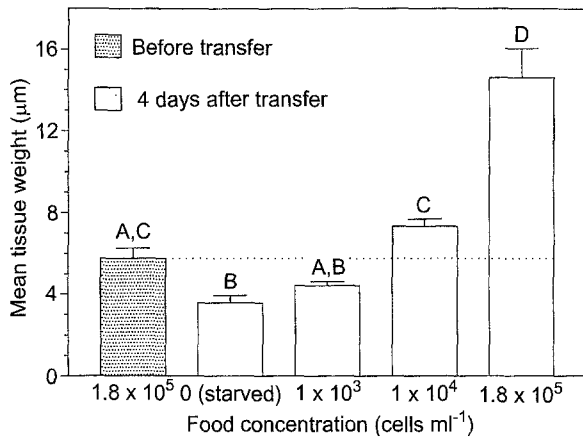
In Experiment A, larvae reared at full ration grew (mean  $\pm$  SD) at  $76.9 \pm 10.0 \mu\text{m d}^{-1}$ , at  $5 \times 10^4$  cells  $\text{ml}^{-1}$  they grew at  $26.5 \pm 9.1 \mu\text{m d}^{-1}$ , and at  $1 \times 10^4$  cells  $\text{ml}^{-1}$  they grew at  $4.3 \pm 1.0 \mu\text{m d}^{-1}$ . In Experiment B, larvae again increased significantly ( $p < 0.01$ ) in shell length following their transfer to  $1 \times 10^4$  cells  $\text{ml}^{-1}$ , although not nearly as much ( $p < 0.05$ ) as they did when maintained at  $1.8 \times 10^5$  cells  $\text{ml}^{-1}$  (Fig. 1). Larvae did not grow at all ( $p > 0.10$ ) following their transfer to filtered seawater or to a phytoplankton concentration of  $1 \times 10^3$  cells  $\text{ml}^{-1}$  (Fig. 1).

Larvae lost weight over 4 d after being transferred to filtered seawater or to phytoplankton at  $1 \times 10^3$  cells  $\text{ml}^{-1}$  in Experiment B (Fig. 2). In contrast, larvae gained weight when maintained at  $1 \times 10^4$  cells  $\text{ml}^{-1}$ , although not nearly as much as larvae maintained at full ration (Fig. 2); differences among mean larval weights were highly significant (one-way ANOVA,  $F = 148.1$ ,  $df = 4, 15$ ;  $p < 0.0001$ ).

Data from both experiments indicate that larvae of *Crepidula fornicata* continued to grow at food concentrations  $\geq 1 \times 10^4$  cells  $\text{ml}^{-1}$ .



**Fig. 1** *Crepidula fornicata*. Experiment B: influence of starvation or food limitation on shell growth at 25 °C. Larvae were reared for 3 d to a mean shell length ( $\pm$  SD) of  $628 \pm 34 \mu\text{m}$  ( $n = 24$ ) at full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ), at which time larvae were transferred to the food treatments indicated and remeasured 4 d later. Each bar represents the mean shell length ( $\pm$  SD) of 36 to 40 larvae pooled from 4 replicates. Means differing significantly from each other ( $p < 0.01$ , Dunn's multiple comparisons test following Kruskal-Wallis nonparametric ANOVA) are denoted by different letters above each bar. Ash-free dry weights of these larvae are reported in Fig. 2

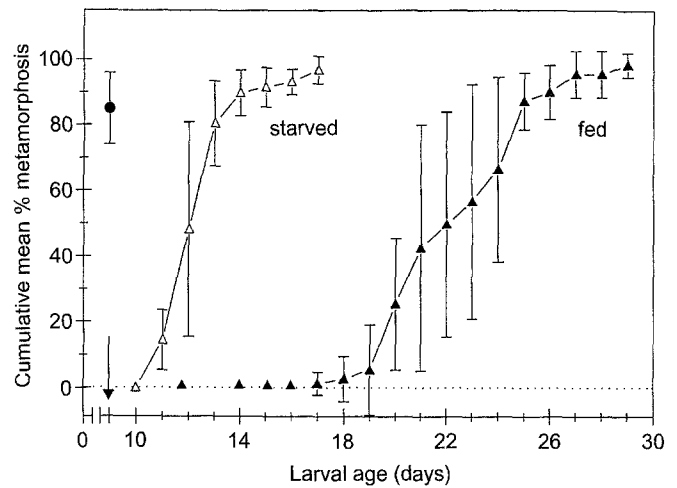


**Fig. 2** *Crepidula fornicata*. Experiment B: influence of starvation and food limitation on larval biomass at 25 °C. Larvae were reared for 3 d at full ration, transferred to the indicated conditions, and prepared for weighing (ash-free dry weight) after 4 d. Each bar represents the mean ash-free dry weight ( $\pm$  SD) of 4 replicates, with 10 to 12 larvae per replicate. Means that differ significantly from each other ( $p < 0.05$ , Dunn's multiple comparisons test following Kruskal-Wallis nonparametric ANOVA) are indicated by different letters above each bar. Mean shell lengths for these larvae are reported in Fig. 1

#### Influence of food limitation on duration of the competent period and size at spontaneous metamorphosis

In Experiment I, larvae were either maintained at full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ) or transferred to 0.45- $\mu\text{m}$  filtered seawater. About 85% of the larvae were competent to metamorphose when these transfers were made (Fig. 3). All larvae in both treatments eventually metamorphosed on glassware but, as illustrated, starved competent larvae of *Crepidula fornicata* metamorphosed 11 to 12 d sooner than larvae maintained at full ration (Fig. 3). Indeed, 50% of starved larvae metamorphosed by Day 12, while no fed larvae metamorphosed until 5 d later. Nearly identical results were obtained in Experiment II performed on a different batch of larvae obtained 4 mo later (data not shown). In Experiment II, 100% of the tested larvae were competent to metamorphose at the beginning of the starvation treatment on Day 11, and starved larvae metamorphosed, on average, 8 to 9 d earlier than fed larvae.

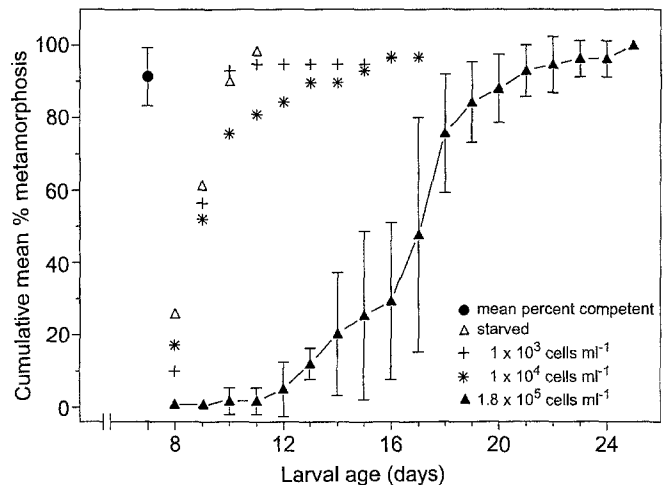
In Experiment III, competent larvae of *Crepidula fornicata* behaved similarly whether they were transferred to filtered seawater, to  $1 \times 10^3$  cells  $\text{ml}^{-1}$ , or to  $1 \times 10^4$  cells  $\text{ml}^{-1}$ ; in each case, they metamorphosed about 8 to 9 d sooner, on average, than larvae maintained at full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ) (Fig. 4). Moreover, food-limited competent larvae metamorphosed at significantly smaller mean shell lengths than did larvae that were fed at full ration throughout the experiment (Fig. 5) (Kruskal-Wallis statistic = -3179,  $n = 54$  to 58 points per treatment,  $p < 0.0001$ ). Mean shell length at metamorphosis did not differ significantly from mean initial shell length for larvae reared at any of



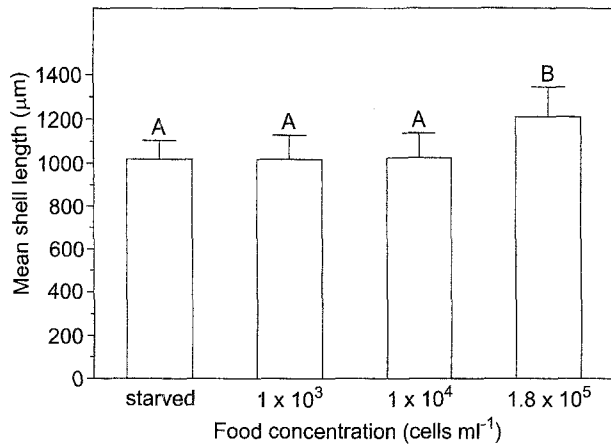
**Fig. 3** *Crepidula fornicata*. Experiment I: influence of starvation on ability to delay metamorphosis at 25 °C. Larvae emerged from egg masses on Day 0, at a mean shell length ( $\pm$  SD) of  $403.9 \pm 23.6$   $\mu\text{m}$  ( $n = 27$ ). Some dishes of larvae were tested for metamorphic competence on Day 9 (filled circle), at a mean shell length of  $1109.7 \pm 110.1$   $\mu\text{m}$  ( $n = 59$ ). Arrow indicates transfer of some larvae to 0.45- $\mu\text{m}$  filtered seawater (open triangles). Other larvae (filled triangles) were maintained at full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ ). Each data point is the mean ( $\pm$  SD) of 5 replicates with 12 larvae per replicate

the three food-limiting conditions (Dunn's multiple comparisons test,  $p > 0.10$ ).

Larvae transferred to moderately food-limiting conditions ( $5 \times 10^4$  cells  $\text{ml}^{-1}$ ) in Experiment IV also metamorphosed sooner than larvae maintained at full ration, although differences in the timing of spontaneous



**Fig. 4** *Crepidula fornicata*. Experiment III: influence of starvation and food limitation on ability to delay metamorphosis at 25 °C. Some dishes of larvae were tested for metamorphic competence on Day 7 (filled circle), at a mean shell length ( $\pm$  SD) of  $1004.7 \pm 106.6$   $\mu\text{m}$  ( $n = 33$ ). Also on Day 7, other larvae were transferred either to 0.45- $\mu\text{m}$  filtered seawater (open triangles),  $1 \times 10^3$  cells  $\text{ml}^{-1}$  (+),  $1 \times 10^4$  cells  $\text{ml}^{-1}$  (\*), or maintained at full ration ( $1.8 \times 10^5$  cells  $\text{ml}^{-1}$ , filled triangles). Each data point is the mean ( $\pm$  SD) of 5 replicates with 12 larvae per replicate. Standard deviation bars are omitted for intermediate food concentrations to improve clarity

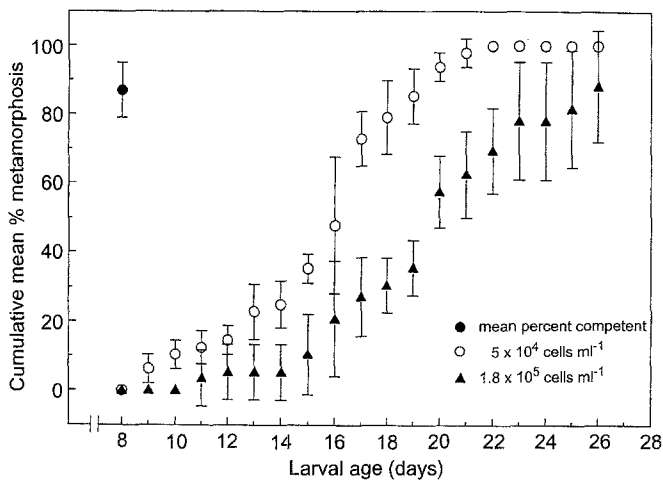


**Fig. 5** *Crepidula fornicata*. Experiment III: effect of food concentration on mean size at spontaneous metamorphosis. Each bar represents the mean shell length (+1 SD about the mean) of 54 to 58 individuals. Means that do not differ significantly ( $p > 0.10$ , Dunn's multiple comparisons test following nonparametric ANOVA) are indicated by the same letter above each bar

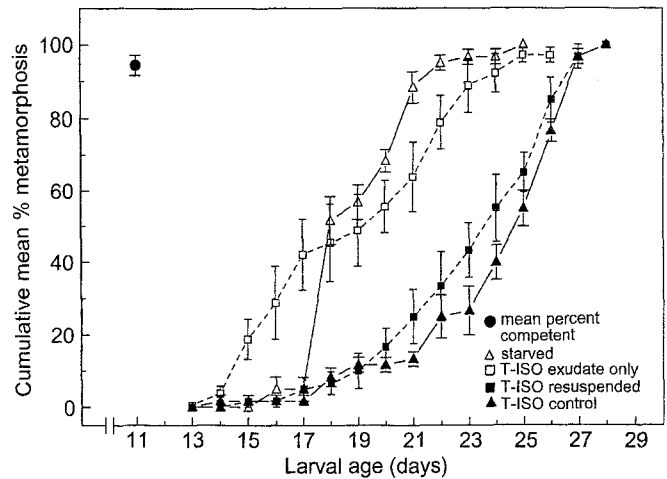
metamorphosis were not as pronounced as in other experiments (Fig. 6).

#### Influence of culture medium and algal exudates on timing of spontaneous metamorphosis

The precocious metamorphosis observed in response to reduced phytoplankton concentration was a response to food limitation rather than an artifactual response to reduced concentrations of potentially inhibitory *fl2* medium or algal exudate: spontaneous metamorphosis



**Fig. 6** *Crepidula fornicata*. Experiment IV: influence of mild food limitation on ability to delay metamorphosis at 25 °C. Some dishes of larvae were tested for metamorphic competence on Day 8 (filled circle), at a mean shell length ( $\pm$  SD) of  $950.0 \pm 71.3$   $\mu\text{m}$  ( $n = 41$ ), while other larvae were transferred to a low food concentration ( $5 \times 10^4$  cells ml<sup>-1</sup>, open circles) or maintained at full ration ( $1.8 \times 10^5$  cells ml<sup>-1</sup>, filled triangles). Each datapoint is the mean ( $\pm$  SD) of 5 replicates with 12 larvae per replicate



**Fig. 7** *Crepidula fornicata*. Experiment V: influence of phytoplankton exudate (filtrate of T-ISO suspension) or phytoplankton growth medium (*fl2*) on delayed metamorphosis. Some larvae were tested for metamorphic competence on Day 11 (filled circle), while remaining larvae were either transferred to filtered seawater (open triangles), filtered algal exudate (open squares), phytoplankton that had been centrifuged and resuspended at full ration ( $1.8 \times 10^5$  cells ml<sup>-1</sup>, filled squares), or phytoplankton at full ration accompanied by algal medium (control treatment, filled triangles). Each data point represents the mean percentage metamorphosis from 5 replicates, 12 larvae per replicate. Error bars show 1 SEM about the mean

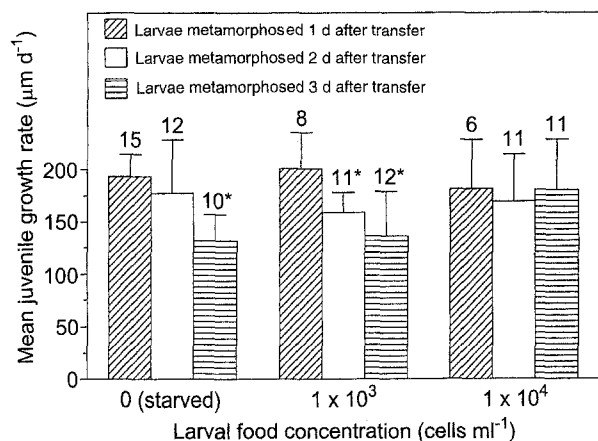
was not inhibited by *fl2* or algal exudate, and the timing of spontaneous metamorphosis was not altered appreciably when we resuspended phytoplankton after centrifugation (Fig. 7).

#### Influence of food limitation during larval life on postmetamorphic growth rate

Individuals metamorphosing 2 to 3 d after being transferred to filtered seawater or to  $1 \times 10^3$  cells ml<sup>-1</sup> in Experiment III grew significantly more slowly as juveniles than those metamorphosing 1 d after transfer (Fig. 8). This effect of larval food limitation on post-metamorphic growth rate was not seen for larvae metamorphosing at a phytoplankton concentration of  $1 \times 10^4$  cells ml<sup>-1</sup> (Fig. 8). The slopes of lines relating juvenile growth rates to number of days of treatment differ significantly from zero for individuals metamorphosing at the two lowest food concentrations ( $F = 12.81$  and  $36.7$  for starved and  $1 \times 10^3$  cells ml<sup>-1</sup> treatments, respectively;  $p < 0.01$ ), but not for individuals metamorphosing at  $1 \times 10^4$  cells ml<sup>-1</sup> ( $F = 0.007$ ,  $p > 0.10$ ).

## Discussion

We have previously shown that postmetamorphic growth rates of *Crepidula fornicata* are severely depressed when precompetent larvae are starved for more



**Fig. 8** *Crepidula fornicata*. Experiment III: influence of food limitation on postmetamorphic growth rates at 25 °C. Larvae were transferred to the food conditions indicated 7 d after hatching, when about 90% of larvae were competent to metamorphose (see Fig. 4). Individuals metamorphosing 1, 2, or 3 d later were measured, transferred to full ration ( $1.8 \times 10^5$  cells ml<sup>-1</sup>), and then remeasured 4 d later. Each bar represents the mean growth rate ( $\pm$  SD) of 6 to 15 juveniles as indicated above each bar. Asterisks indicate means that differ significantly from mean growth rates for individuals metamorphosing on Day 1 within the same treatment group ( $p < 0.05$ , Tukey–Kramer multiple comparisons test following one-way nonparametric ANOVA)

than 3 to 4 d at 25 °C (Pechenik et al. 1996a). The present study indicates that postmetamorphic growth rates may be similarly depressed when competent larvae are starved or food limited for only 2 to 3 d. These results extend earlier findings for larvae of other species from a number of different phyla that some larval experiences can impact postsettlement or postmetamorphic growth or survival (Highsmith and Emler 1986; Woolcott et al. 1989; Qian et al. 1990; Pechenik and Cerulli 1991; Miller 1993; Pechenik et al. 1993). The factors responsible for these reductions in early juvenile performance have not yet been assessed, although a reduced ability to collect or assimilate food seems likely; Wendt (1996), for example, has recently demonstrated that prolonging the larval period of the bryozoan *Bugula neritina* by as little as 8 h results in reduced surface area and volume of the juvenile food-collecting organ.

The extent to which such larval experiences compromise juvenile growth or survival in the field has not been widely considered. Jarrett and Pechenik (1996) report that juvenile barnacles (*Semibalanus balanoides*) recruiting early in the 1995 season tended to have higher growth potential than those recruiting later in the same season, a result that could reflect delayed metamorphosis by the nonfeeding cyprid stage or nutritional stress experienced by the preceding feeding naupliar stages.

Regarding the effects of food limitation on duration of the competent period, the only comparable data we have found are those of Miller (1993) and Pawlik and Mense (1994), for the opisthobranch gastropod *Phestilla sibogae* and the polychaete *Phragmatopoma lapidosa californica*, respectively. Competent larvae of *P. sibogae*

lost the ability to metamorphose successfully in response to natural cues after being starved for 2 wk. Competent polychaete larvae lost the ability to metamorphose several days after being transferred to filtered seawater, but regained competence within a few days after being returned to high ration; this response could be adaptive in increasing the time available for dispersal to more favorable habitats (Pawlik and Mense 1994). In marked contrast, competent larvae of *Crepidula fornicata* responded to food limitation in our experiments by metamorphosing precociously, at least 1 wk earlier (at 25 °C) than if they were well fed. The adaptive benefits of such a response are less clear. The response might limit exposure to planktonic predators (Young and Chia 1987; Rumrill 1990; Morgan 1995). However, Pechenik (1979) has argued that benthic predation rates are likely to be far greater than planktonic predation rates, in which case prolonging larval life and continuing to grow would seem more adaptive, allowing individuals to attain a greater size before metamorphosing and thereby reducing size-related risks of postmetamorphic predation. Under this scenario, the response to food limitation shown by *C. fornicata* larvae would seem maladaptive, since it promotes metamorphosis at smaller size; larvae of *C. fornicata* become competent to metamorphose in the laboratory at shell lengths as small as about 700  $\mu$ m (Pechenik and Heyman 1987; Zimmerman and Pechenik 1991; Pechenik et al. 1996a). On the other hand, rapid metamorphosis under food-limiting conditions could be adaptive if a prolonged period at low food concentration compromises average rates of juvenile growth, as our data suggest. Finally, one could argue that the response may be adaptive in promoting the transition from a mediocre larval lifestyle to a juvenile habitat and lifestyle in which the prospects for rapid growth and higher survival are better. This idea has been widely accepted for amphibian larvae, which tend to metamorphose earlier and at a smaller size when food becomes limiting (e.g., Wilbur and Collins 1973; Semlitsch et al. 1988; Newman 1989; Hensley 1993; Leips and Travis 1994). Unlike these amphibian species, however, *Crepidula fornicata* suspension-feeds in both the larval and adult stages, so that metamorphosis does not confer the marked change in lifestyle characteristic of amphibian development; an environment in which food is limiting for larvae of *C. fornicata* is likely to provide an equally poor situation for juveniles and adults. Although juvenile *C. fornicata* may supplement their diet by radular rasping, an option unavailable during the larval stage, growth rates of radular rasplers seem to be much lower than those of suspension-feeding individuals; in the laboratory, suspension-feeding juveniles of *C. fornicata* typically grow at 100 to 200  $\mu$ m d<sup>-1</sup> (Pechenik and Eyster 1989; Pechenik et al. 1996b), while those limited to rasping on microbially filmed glass slides grew at only about 20  $\mu$ m d<sup>-1</sup> (Pechenik, unpublished data based on about 15 individuals maintained at ~23 °C).

One intriguing alternative interpretation of our data is that the response to food limitation that we have

documented in the laboratory for larvae of *Crepidula fornicata* is in fact maladaptive, promoting the metamorphosis of individuals into unsuitable environments. The previously documented response (Pechenik et al. 1996a) in which starved precompetent larvae of this species apparently divert energy reserves into becoming competent rather than into growth seems to be equally maladaptive; the onset of competence during prolonged periods of starvation would again likely promote metamorphosis of individuals into food-poor environments, and at minimal shell sizes. Seeing these responses in the laboratory may simply suggest, then, that in the field, larvae of *C. fornicata* never experience food limitation or dramatic declines in food concentration of the magnitude studied in our experiments; species can not be expected to have evolved adaptive responses to stresses that they have never experienced. Although echinoderm larvae of some species may well be food limited in the field (Olson and Olson 1989; Fenaux et al. 1994), larvae of at least some other species may be able to obtain sufficient food under the same conditions, through, for example, more efficient food collection, lower rates of energy expenditure, higher assimilation efficiencies, different nutritional requirements, or exploitation of different nutrient sources (Pechenik 1987; Morgan 1995).

The mechanism through which transfer of larvae to suspensions of reduced phytoplankton concentrations provoked metamorphosis in our experiments is uncertain, partly because we do not yet know what causes spontaneous metamorphosis in this or any other species. Moreover, food-limited metamorphosis and normal "spontaneous" metamorphosis do not necessarily operate through the same mechanisms in these larvae. In any event, the algal medium itself did not inhibit metamorphosis in our experiments, so the prolonged competent period exhibited by larvae of *Crepidula fornicata* in the presence of abundant food is not an artifact of our methodology. Spontaneous metamorphosis presumably reflects either a greatly increased sensitivity to some external inducing chemical present in seawater in exquisitely low concentration (Coon et al. 1990), the programmed eventual release of some endogenous stimulatory internal factor in the absence of external cues (Pechenik 1980, 1984, 1990), or the degradation of some internal factor inhibiting metamorphosis (Chia 1978). It is easy to imagine a stimulatory neurosecretory substance being sequestered within the larva during development, its release normally triggered by contact with the appropriate external cue(s). For *C. fornicata* (and *C. plana*), such external cues are associated with microbial films and soluble substances produced by adults (Lima and Pechenik 1985; McGee and Targett 1989). In such a case, the substance might be released prematurely in starved individuals, perhaps as a by-product of self-digestion. Another possibility is that starving or food-limited competent larvae are unable to produce enough of some endogenous inhibitory substance to prevent spontaneous metamorphosis. The fact that larvae metamorphosed soon after being transferred to low food

concentrations ( $1$  to  $5 \times 10^4$  cells  $\text{ml}^{-1}$ ) at which growth continues, albeit slowly, weakens both hypotheses, although not ruling either one out. We have no other testable hypotheses to offer in their place as yet. In any event, it may be the sudden transfer to food-limiting conditions that provokes metamorphosis rather than the low food concentrations per se; larvae of *C. fornicata* reared continuously at  $5 \times 10^4$  cells  $\text{ml}^{-1}$  do not seem to metamorphose prematurely in the absence of external cues (Pechenik 1985 and unpublished data).

We do not know how often larvae of *Crepidula fornicata* experience declines in food concentration that are comparable in magnitude and duration to those tested in our study. Larvae that are carried for days or weeks in ship ballast water (Carlton and Geller 1993) probably experience marked declines in ambient food concentration and might be especially likely to demonstrate some of the consequences documented here. In the field, phytoplankton is patchily distributed vertically, horizontally, and temporally (e.g., Seliger et al. 1981; Mackas et al. 1985; Villafane et al. 1995), and the phytoplankton concentrations that we used in our study are within the range of particle concentrations of the appropriate sizes (approximately  $1$  to  $20 \mu\text{m}$ ) reported from coastal waters (Walne 1965; Seliger et al. 1981; Fenaux et al. 1994; Baldwin and Newell 1995). The fact that gastropod veligers alter their vertical position in the water column, either diurnally (Richter 1973) or as they age (Fretter and Shale 1973), increases the likelihood that they will experience substantial fluctuations in food concentration as they develop, although the length of time they spend at any particular concentration will be hard to access directly.

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**Note added in proof** DeClerck (1995) discusses several energetic and biomechanical constraints that should prevent juvenile gastropods from being effective suspension feeders. Juveniles of *Crepidula fornicata* (and *C. plana*) apparently bypass such constraints and suspension-feed effectively within hours of losing the velum. Further studies specifically comparing the relative contributions of suspension-feeding and radular rasping to juvenile growth seem warranted.

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