Evaluating whether velar lobe size indicates food limitation among larvae of the marine gastropod *Crepidula fornicata*

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**Abstract**

Disproportionately large feeding structures have been used to infer food limitation in some marine invertebrate larvae, but few studies have investigated whether other factors alter larval morphology in similar ways. In this study, larvae of *Crepidula fornicata* were reared either at five different food concentrations of *Isochrysis galbana* (clone T-ISO) at a single temperature (22°C) (Experiments I and II); or on three different phytoplankton species (*Isochrysis galbana*, *Dunaliella tertiolecta*, and *Pavlova lutheri*) at both high and low concentrations at a single temperature (22°C) (Experiment III); or at high and low concentrations of *Isochrysis galbana* at four different temperatures between 16 and 25°C (Experiment IV). Shell lengths and velar lobe dimensions were determined for individual larvae at intervals to monitor relative rates of velar and shell growth. In addition (Experiment V), fast growing and slow growing larvae in Experiment I were examined separately to determine whether velar lobes developed at similar rates (relative to shell growth) for fast and slow growing larvae within individual cultures. In general, velar lobes grew significantly larger, relative to shell length, when larvae were reared at low food concentrations ($P < 0.0001$); for larvae of similar shell length, the velar lobes of those fed $1 \times 10^4$ cells ml$^{-1}$ were on average 17.7% larger than those of larvae fed $18 \times 10^4$ cells ml$^{-1}$ of T-ISO. In contrast, larvae fed different phytoplankton species at equivalently high food concentrations did not differ in relative velum size ($P = 0.2666$), even though shell growth rates differed significantly for larvae raised on the different diets, indicating substantial variation in food quality. We also found that relative rates of velum and shell growth differed among fast and slow growing individuals within treatments. Temperature had no significant effect on relative rates of velar and shell growth within the 16–25°C range tested ($P = 0.121$), but may have altered the relationship between food concentration and relative velar growth. These results indicate that dramatically reduced food concentration induces disproportionate growth in the velar lobes of *C. fornicata*, but that interpretation of data from field-collected individuals of this species will be made difficult by the potentially confounding effects of temperature, food quality, and differences in individual growth.

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potential. Assessments of food limitation using morphological measurements for field-collected larvae will need to be supplemented with other indicators before convincing conclusions about the extent of food limitation in *C. fornicata* can be drawn. © 2000 Elsevier Science B.V. All rights reserved.

**Keywords:** Crepidula; Food limitation; Larvae; Larval growth; Veliger

### 1. Introduction

Larvae are food limited when natural food concentrations are below those needed to support maximal rates of growth and differentiation (Olson and Olson, 1989; Fenaux et al., 1994). Rates of larval development can also be suboptimal if the diet is nutritionally inadequate (reviewed by Pechenik, 1987), which is a different, and less-often considered, form of food limitation. In the laboratory, low food concentrations and nutritionally inadequate diets have been shown to decrease growth rate and prolong development for many invertebrate larvae, including those of crustaceans (West and Costlow, 1987; Wehrtmann, 1991; Anger, 1995), polychaetes (Hansen, 1993), sea stars (Allison, 1994; Basch, 1996), gastropods (Pillsbury, 1985; Pechenik et al., 1996a), and oysters (His and Seaman, 1992). By delaying development to metamorphic competence and prolonging exposure to pelagic predators and offshore currents, food limitation can indirectly decrease recruitment into benthic populations (Thorson, 1950; Vance, 1973; Pechenik and Fisher, 1979; Pechenik, 1987; Forrester, 1990; Widdows, 1991).

Food limitation can also affect recruitment more directly. For the oyster *Crassostrea gigas*, food limitation during the first 6–8 days of development led to substantial larval mortality even after larvae resumed feeding (His and Seaman, 1992). Food limitation can also diminish the ability of at least some species to delay metamorphosis in the laboratory (Pechenik et al., 1996a), possibly impairing their ability to select favorable substrates in the field. In addition, larvae of *C. fornicata* that were food limited during late precompetent stages metamorphosed at smaller sizes than well fed larvae (Pechenik et al., 1996b) and the juveniles grew more slowly than control individuals that had been well fed as larvae (Pechenik et al., 1996a). Similar results have been reported for echinoderm larvae (Miller and Emlet, 1999). Smaller sizes at metamorphosis and slower juvenile growth rates may influence recruitment by reducing the ability of small juveniles to escape size-specific predation (Ray et al., 1994; reviewed by Pechenik, 1999). These laboratory studies demonstrate the potential impact of larval food limitation on larval abundance, recruitment, and postmetamorphic survival of marine benthic invertebrates.

Despite the deleterious effects of food limitation demonstrated for marine invertebrate larvae in the laboratory, it has been difficult to determine whether planktotrophic larvae are food limited in the field. Larvae are often presumed to experience food limitation because phytoplankton concentrations vary both spatially (Seliger et al., 1981; Mackas et al., 1985) and temporally (Villafane et al., 1995). However, larvae may be able to utilize other resources besides phytoplankton, such as heterotrophic ciliates (Baldwin
and Newell, 1991), ultraplankton (Ayukai, 1994), bacteria (Rivkin et al., 1986) or dissolved organic material (Manahan, 1990), so that low phytoplankton concentration cannot in itself demonstrate that larvae are food limited. In addition, phytoplankton cells may be too large or too small for larvae to ingest and can vary considerably in nutritional quality (reviewed by Pechenik, 1987). Thus, larvae can be food limited even when phytoplankton concentrations are high. Food limitation cannot be inferred exclusively from measurements of phytoplankton concentration.

A few workers have attempted to assess degrees of food limitation by comparing growth rates of larvae reared on natural seawater in the laboratory with those reared in seawater artificially enriched with additional phytoplankton (e.g., Paulay et al., 1985; Fotel et al., 1999). Certain morphological, biochemical, or histological characteristics can potentially distinguish food-limited larvae from those that have been well fed (Olson and Olson, 1989). This approach has been used to assess food limitation in the field for the larvae of several fish (Bulow, 1987; Garcia et al., 1998; Kawakami et al., 1999), crustacean (i.e. Anger, 1995; Juinio and Cobb, 1994; Wagner et al., 1998), and echinoderm (Fenaux et al., 1994) species. In particular, Fenaux et al. (1994) determined that larvae of the sea urchin *Paracentrotus lividus* were food limited in the Mediterranean, relying in part on evidence that echinoplutei develop longer larval arms at low food concentrations (Boidron-Mjtairon, 1988; Hart and Scheibling, 1988). Similarly, larvae of the oyster *Crassostrea gigas* develop disproportionately large velar lobes in response to relatively low food concentrations (Strathmann et al., 1993). Although morphological criteria are being used to deduce the extent of food limitation in field-collected larvae, there have been no attempts to determine whether other factors can influence the same aspects of larval morphology to the same degree. In order for these morphological criteria to be reliably applied to field-collected larvae, the criteria must be sensitive to nutritional limitation, but insensitive to other abiotic and biotic factors that vary in the natural environment. If other factors can alter larval morphology in the same ways, then these morphological alterations may be misleading indicators of whether larvae are food limited in the field.

The goal of the present study was to determine whether changes in the relative rates of velum and shell growth are sufficient to indicate food limitation in veligers of the slippershell snail, *Crepidula fornicata*. Specifically, we examined the influence of phytoplankton concentration, phytoplankton species, temperature, and inherent differences in individual growth rate on rates of velar lobe growth relative to rates of shell growth. Larvae of *C. fornicata* are well-suited to such studies in that they grow rapidly and survive well in laboratory culture (Pechenik, 1984; Pechenik et al., 1996a,b), exhibit linear shell growth over time, and exhibit a linear relationship between shell length and biomass (Pechenik, 1980, 1984). Moreover, the larvae grow and survive well over a wide range of temperatures, approximately 16 to 29°C (Lucas and Costlow, 1979; Pechenik and Lima, 1984), and exhibit a wide range of growth rates at any single temperature, even when all larvae are produced by one female (Pechenik, 1984; Pechenik and Lima, 1984; Pechenik et al., 1996c). Thus, although larvae of this species are very similar in size at hatching, within several days a wide range of shell sizes is found in each culture. Also, the larvae of *C. fornicata* grow at markedly different rates on different phytoplankton species; in preliminary studies, the larvae grew 40–60%
faster when fed *Isochrysis galbana* (clone T-ISO) than when fed any of the three concentrations tested of *Dunaliella tertiolecta* (clone DUN), and grew at intermediate rates on a diet of another naked flagellate, *Pavlova lutheri* (clone MONO) (Klinzing, 1997). Thus, shell growth rates can be manipulated by altering phytoplankton concentration, phytoplankton species, and temperature, without increasing larval mortality. Moreover, a wide range of individual growth rates will be observed within each treatment. Finally, the larvae of this species are common and readily identified components of the summer plankton in New England (Pechenik, 1986).

### 2. Materials and methods

#### 2.1. Collection and maintenance of adults and larvae

Stacks of adult *Crepidula fornicata* were collected either from Wickford, Rhode Island or Nahant, Massachusetts, or were ordered from the Marine Biological Laboratory (Woods Hole, MA, USA). Adults were maintained in 2 l glass jars of filtered seawater and fed ad libitum a combination of the naked flagellates *Dunaliella tertiolecta* (clone DUN) and *Isochrysis galbana* (clone T-ISO) until they released larvae. The larvae used in each experiment were all released from one female, but they may have had more than one father. For all experiments, larvae were collected using 120 µm Nitex mesh and then transferred to a 2 l glass jar with approximately 1 l of seawater. Larvae were then fed the naked flagellate *Isochrysis galbana* (clone T-ISO) at a concentration of $1.8 \times 10^{2}$ cells ml$^{-1}$. Larvae of *C. fornicata* survive and grow well at this concentration of T-ISO (Pechenik, 1980, 1984; Pechenik et al., 1996a,b,c). Larvae were maintained at room temperature (22–24°C) at ambient photoperiod and transferred to fresh feeding suspension ($1.8 \times 10^{2}$ cells ml$^{-1}$ of T-ISO) every other day until each experiment began. In all experiments, seawater was passed through a 0.45 µm filter before use, and larvae were incubated at 22°C and a photoperiod of 12L:12D.

#### 2.2. The effect of food concentration on relative velum growth (Experiments I and II)

Two experiments were conducted to determine how the concentration of T-ISO affected relative rates of shell and velum growth. Adults were obtained from the Marine Biological Laboratory (Woods Hole) on March 25, 1996 (Experiment I) and Wickford, RI, on July 7, 1996 (Experiment II), and held in the laboratory until they released larvae. Larvae from each hatch were reared in a 2 l glass jar and fed T-ISO at $1.8 \times 10^{4}$ cells ml$^{-1}$ for 1 or 3 days, respectively, before being pipetted into 3.5 inch diameter glass dishes; eight larvae were pipetted into each of 30 glass dishes with 45 ml of T-ISO feeding suspension at $1.8 \times 10^{4}$ cells ml$^{-1}$. The following day, the dishes were randomly distributed among four treatments, with six replicates per treatment. In Experiment I, the treatments were 1, 5, 10, and $1.8 \times 10^{4}$ cells ml$^{-1}$. For Experiment II, the $1 \times 10^{4}$ cells ml$^{-1}$ treatment was replaced with $2 \times 10^{4}$ cells ml$^{-1}$ to discourage spontaneous metamorphosis of larvae (Pechenik et al., 1996a).

Larvae were pipetted to clean glass dishes and freshly prepared feeding suspension
daily. Since larvae were growing at different rates at the four phytoplankton concentrations, measurements of shell length and velum dimensions were staggered so that similar ranges of shell lengths were sampled from each treatment by the end of each experiment. The velum circumference and shell length of larvae in one dish were determined daily for larvae growing at the highest phytoplankton concentration \((18 \times 10^4 \text{ cells ml}^{-1})\), every other day for larvae growing at \(10 \times 10^5 \text{ cells ml}^{-1}\), and approximately every 2 days and every 3 days for larvae growing at \(5 \times 10^4\) and \(1 \times 10^5 \text{ cells ml}^{-1}\), respectively. Shell lengths were determined nondestructively at 50× using a dissecting microscope equipped with an ocular micrometer (Pechenik, 1984; Pechenik and Lima, 1984). Velum dimensions were then determined by videotaping larvae from each treatment while they were swimming in 0.45 µm filtered seawater. Larvae growing at \(1 \times 10^4 \text{ cells ml}^{-1}\) were measured and videotaped only five times, because they started to metamorphose before they grew to the largest shell lengths sampled from the other treatments. No larvae spontaneously metamorphosed in any other treatment, allowing measurements to be made over a wider range of shell sizes for larvae reared at higher phytoplankton concentrations.

NIH Image software was used to measure the length and width of one velar lobe for each videotaped larva. In the few instances when the two velar lobes of a given larva were of different sizes, the length and width of both velar lobes were measured and the average size was used for subsequent analysis. Velum circumference was estimated using the formula for an ellipse \((C = 2[\{(L/2)^2 + (W/2)^2\}/2]^{1/2})\) (Strathmann et al., 1993). To test for effects of food concentration on relative rates of velar growth, velum dimensions among the different treatments were analyzed using Model 1 Analysis of Covariance (ANCOVA), using shell length as the covariate. The assumptions of ANCOVA were tested using residual plot analysis. Subsequent pairwise comparisons using the GT2 method (Sokal and Rohlf, 1995) were used to test for significant differences in adjusted least square mean velar dimensions among larvae reared at the different phytoplankton concentrations. Images of at least 32 larvae were analyzed for each treatment.

2.3. The effect of food quality on relative velar growth rates (Experiment III)

Preliminary data (Klinzing, 1997) showed that larvae of \(C. fornicata\) grew substantially more quickly on a diet of \(Isochrysis galbana\) (clone T-ISO) than on unialgal diets of either \(Dunaliella tertiolecta\) (DUN) or \(Pavlova lutheri\) (clone MONO) over a wide range of phytoplankton concentrations. Thus, food quality varied substantially among the three phytoplankton species. To investigate whether food quality can influence the rate of velar growth relative to the rate of shell growth, larvae were reared at optimal and suboptimal concentrations (as determined by preliminary growth experiments) of T-ISO, DUN, MONO, and a 1:1 mixture of MONO and T-ISO at one temperature (22°C). Larval shell lengths and velar dimensions were determined at intervals as already described. Because increasing phytoplankton concentrations of MONO above \(18 \times 10^4 \text{ cells ml}^{-1}\) did not increase rates of larval shell growth (Klinzing, 1997), \(18 \times 10^4 \text{ cells ml}^{-1}\) was used as the optimal concentration for both T-ISO and MONO.

In order to standardize the amount of food provided to the larvae, given the difference in cell sizes among the three phytoplankton species (Fretter and Montgomery, 1968;
Pechenik and Fisher, 1979), we determined the number of cells of each species required to form a pellet of given volume in a centrifuge tube. To provide roughly the same volume of phytoplankton cells of each species, DUN was given at a concentration of $7 \times 10^4$ cells ml$^{-1}$, while the two other species were offered at $18 \times 10^4$ cells ml$^{-1}$. This is roughly in agreement with estimates made by Walne (1963). A mixed algal food treatment was included in one experiment because molluscan veligers typically grow faster on a mixed diet (Davis and Guillard, 1958; Pilkington and Fretter, 1970; Helm, 1977).

For this experiment, adults collected from Wickford, Rhode Island, on June 26, 1996 released larvae on July 9, 1996. Larvae were reared on T-ISO at $18 \times 10^4$ cells ml$^{-1}$ for 2 days before being distributed among 52 3.5 inch diameter glass dishes, each containing $45$ ml of either T-ISO (at 18 or $5 \times 10^4$ cells ml$^{-1}$), MONO (at 18 or $5 \times 10^4$ cells ml$^{-1}$), DUN (at 7 or $2 \times 10^3$ cells ml$^{-1}$), or a mixture of T-ISO and MONO (at $18 \times 10^4$ cells ml$^{-1}$). Each treatment consisted of seven replicate dishes with eight larvae each. Larvae were videotaped at intervals over 15 days, the intervals depending on the larval growth rates observed among treatments; a similar range of shell lengths was sampled within each treatment group over the course of the experiment. On any particular sampling day, all the larvae in one dish of a particular treatment were individually videotaped while swimming upwards in a depression slide in 0.45 $\mu$m filtered seawater. In a previous study, larval growth rates for this species did not differ significantly among replicate glass dishes (Pechenik et al., 1996a).

Effects of food quality on rate of velar development were analyzed using ANCOVA with shell length as the covariate to detect significant differences among treatments. In this experiment and subsequent ones, we confined analysis to velum circumference since the effects of food concentration were qualitatively similar whether velum length, width or circumference was used. Not all videotaped records of larvae were suitable for subsequent analysis, but at least 38 larvae were included in the analysis for each treatment.

2.4. The effect of temperature on relative rate of velum growth (Experiment IV)

To investigate whether temperature affects the relative rate of velar and shell growth, thereby confounding the interpretation of data from field-collected larvae, larvae were reared at four temperatures (16, 19, 22, and 25°C) on a unialgal diet of T-ISO at the optimum food concentration ($18 \times 10^4$ cells ml$^{-1}$) or at one suboptimum food concentration ($5 \times 10^4$ cells ml$^{-1}$). These temperatures represent the range of temperatures at which these larvae are found in Narragansett Bay over the course of the reproductive season (unpubl. observations) and have been shown to induce significantly different growth rates among batches of *C. fornicata* larvae in the laboratory (Pechenik, 1984; Pechenik and Lima, 1984).

Adult *C. fornicata* were collected from Wickford, Rhode Island, on June 8, and fed *Dunaliella tertiolecta* at 19°C until they released larvae on June 12, 1996. Larvae were reared for 1 day at one larva ml$^{-1}$ on a diet of T-ISO at $18 \times 10^4$ cells ml$^{-1}$ T-ISO in a 2 l glass jar, and then distributed among 56 glass dishes each containing 45 ml of T-ISO at
either 5 or 18×10³ cells ml⁻¹. Fourteen dishes of larvae (seven dishes containing each of the two food concentrations) were placed into each of four incubators set to 16, 19, 22, or 25°C. Larvae were allowed to swim for 48 h at the various temperatures before they were first videotaped and measured. They were then videotaped and measured every 1–3 days over 19 days, the taping interval for particular treatments depending on larval growth rate, in order to sample a comparable range of shell lengths for each treatment.

2.5. The effect of inherently differing growth rates on relative rates of velar and shell growth (Experiment V)

To determine whether fast and slow growing larvae reared under uniform conditions in the same containers varied in their relative rates of shell and velar growth, some of the data from Experiment I were reanalyzed separately for fast and slow growing individuals; all larvae used in this reanalysis had been reared on a unialgal diet of T-ISO at 18×10³ cells ml⁻¹ at 22°C. Growth rate was determined by subtracting the mean shell length of a sample of six larvae measured at the beginning of the experiment from the final shell length of each larva, and then dividing by the number of days the larva had been growing. As mentioned in the Introduction, there is a linear relationship between shell length and tissue weight in this species (Pechenik, 1980, 1984), so that rate of shell growth accurately represents rate of tissue growth. Median growth rates of larvae in each treatment were used in the data analysis because the median growth rate is affected less than the mean growth rate by a few very fast or very slow growing individuals; thus the median gives a less biased measure of growth. The relationship between shell growth rate and velar growth rate was assessed using two-way ANCOVA, with growth rate (fast or slow) and food concentration as factors, and shell length as the covariate.

3. Results

3.1. Larval survival and growth

Fewer than 1% of larvae died during the course of this study in any treatment. A few larvae were lost or damaged during transfers and thus were not measurable, but generally at least 95% of larvae at the start of each experiment remained to be measured at the end of each study. Because shell growth rates were generally constant in all treatments (see below) as reported previously (Pechenik, 1980, 1984; Pechenik and Lima, 1984), a mean rate of shell growth was computed for all larvae in each treatment. Mean larval growth rates decreased at successively lower concentrations of T-ISO (P < 0.0001) and successively lower temperatures (P < 0.0001) (Table 1). Mean growth rates were significantly higher with T-ISO as the food source, at both the high food concentration (Expt. IIIa, F = 8.64, d.f. = 2,103, P = 0.0003) and the low food concentration (Expt. IIIb, F = 22.2, d.f. = 3,150, P = 0.0003) than for any of the other two
Table 1
Mean shell growth rates (±95% CI) of Crepidula fornicata larvae under the conditions of each experiment. Different letters in right-hand column indicate means that differ significantly from the highest mean growth rate (designated ‘a’) in each experiment or sub-experiment (P < 0.05, Tukey–Kramer multiple comparisons test)

<table>
<thead>
<tr>
<th>Expt No.</th>
<th>Variable tested</th>
<th>Temp. (°C)</th>
<th>Food species</th>
<th>Food conc. (cells ml⁻¹)</th>
<th>Mean growth rate ±95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Food concentration</td>
<td>22</td>
<td>T-ISO</td>
<td>1×10⁶</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5×10⁶</td>
<td>85.3±5.68 (35)</td>
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<td></td>
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<td></td>
<td></td>
<td>10×10⁶</td>
<td>104.4±7.00 (26)</td>
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<td></td>
<td></td>
<td></td>
<td>18×10⁶</td>
<td>113.5±8.68 (44)</td>
</tr>
<tr>
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<td>Food concentration</td>
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<td>T-ISO</td>
<td>2×10⁶</td>
<td>28.1±4.45 (49)</td>
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<td>Food quality at optimal concentration</td>
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<td>Temperature, optimal food concentration</td>
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<td>78.4±4.74 (30)</td>
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<td>5×10⁶</td>
<td>65.0±5.05 (35)</td>
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</table>

unialgal diets tested (Table 1). Mean growth rate for larvae reared on the mixed diet of MONO and T-ISO was intermediate between the growth rates recorded when the two phytoplankton species were given singly (Table 1). Growth rates observed during the course of the study were within the range of values reported previously for this and related species (Pechenik, 1980, 1984; Pechenik and Lima, 1984; Zimmerman and Pechenik, 1991; Pechenik et al., 1996c).

Mean shell growth rates varied among the different batches of larvae used in this study and individual growth rates varied considerably about the mean, as has been commonly observed in larval cultures of this and many other invertebrate species (reviewed by Pechenik, 1987; Pechenik et al., 1996c).

3.2. The effect of food concentration on relative rates of shell and velum growth (Experiments I and II)

Velum dimensions increased as a linear function of increasing shell size at all
Fig. 1. Experiment I. The effect of lowered food concentration on relative rates of velum and shell growth in larvae of *C. fornicata*. Larvae were all reared on the naked flagellate *Isochrysis galbana* (clone T-ISO) at 22°C. The same $18 \times 10^3$ cells ml$^{-1}$ data are presented in all three graphs.

Fig. 2. Experiment II. The effect of lowered food concentration on relative rates of velum and shell growth in larvae of *C. fornicata*. Larvae were all reared on the naked flagellate *Isochrysis galbana* (clone T-ISO) at 22°C. Food concentrations mimic those used in Experiment I, except for the lowest concentration tested.
concentrations of T-ISO at 22°C (Figs. 1 and 2). Most importantly, larvae reared at lower food concentrations had larger velar lobes than larvae of comparable shell size that were reared at higher food concentrations (Figs. 1 and 2). Although there was considerable overlap among treatments in Experiments I and II, the effect of food concentration on velar circumference was statistically significant, with shell length as the covariate (ANCOVA for Experiment I: $F = 18.812$, d.f. = 3,147, $P < 0.0001$; ANCOVA for Experiment II: $F = 16.299$, d.f. = 3,192, $P < 0.001$); the interaction between shell length and food concentration was not significant (ANCOVA for Experiment I: $F = 1.116$, d.f. = 3,144, $P = 0.345$; ANCOVA for Experiment II: $F = 0.691$, d.f. = 3,189, $P = 0.559$). The relative difference in rates of velum and shell growth was greatest for larvae reared at the highest and lowest food concentrations tested in each experiment (Figs. 1 and 2).

When larvae that were raised at each phytoplankton concentration were compared using the GT2 multiple comparison method, mean velum circumferences (adjusted least squared means for a mean shell length of 841.5 μm) were always significantly different between larvae raised at the highest and lowest food concentrations tested. However, differences in adjusted mean velar circumferences were not significant for larvae reared at adjacent food concentrations within an experiment, except for larvae raised at 5 vs. $10 \times 10^3$ cells ml$^{-1}$ in Experiment I (Fig. 3) and for larvae raised at 10 vs. $18 \times 10^3$ cells ml$^{-1}$ in Experiment II (Fig. 4). Because the results for velum width, length or...

![Graph](image)

**Fig. 3.** Experiment I. The effect of food concentration (T-ISO) on mean velum circumference (least squares means adjusted for shell length) for larvae of *C. fornicata* reared at 22°C. Error bars represent 95% confidence intervals computed using GT2 method of multiple comparison among treatments, after finding a significant effect of food concentration (ANCOVA, $P < 0.0001$). The number of larvae measured at each treatment is given above each bar.
circumference were not qualitatively different (Fig. 4), comparisons of shell and velar growth in all subsequent experiments were based solely on determinations of velar circumference.

To examine in greater detail the differences in velar lobe circumference for different-sized larvae reared at different food concentrations, larvae were partitioned into arbitrary shell length size classes, as given in Table 2. The effect of food concentration on growth of the velum compared with growth of the shell was strikingly clear for larvae ranging in size between 600 and 899 μm. For example, the mean velar circumference for larvae between 700 and 799 μm was about 37% larger when larvae were reared at $2 \times 10^4$ cells ml$^{-1}$ than when they were reared at $18 \times 10^4$ cells ml$^{-1}$ (Table 2).

The effect of food concentration on differential velar growth was less pronounced for larvae larger than about 900 μm, because velum size tended to plateau as larval shell size increased, especially for food-limited larvae (Fig. 5). At the lowest food concentration tested ($2 \times 10^3$ cells ml$^{-1}$) in Experiment II, for example, velum growth ceased after about 1 week in culture (Fig. 5d: the slope of the line describing velum growth rate during the final four sampling days was not significantly different from zero by linear regression analysis: $F = 2.372$, d.f. = 1.27, $P = 0.1352$), after larvae reached approximately 800 μm in shell length; shell growth, however, continued (slope of linear regression line differs from zero: $F = 11.05$, d.f. = 1.27, $P = 0.0026$).
Table 2
Mean ± standard error of velum circumference within nine arbitrarily chosen shell length classes of *C. fornicata* larvae reared at 22°C at five different concentrations of *Isochrysis galbana* (T-ISO). Mean velar circumferences were calculated only for size classes that had at least three larvae. (--) denotes no larvae sampled in a particular size class; (<3) denotes less than three larvae sampled from this size class.

<table>
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<th>Size class (µm)</th>
<th>Food concentration (cells ml⁻¹)</th>
<th>18×10⁴</th>
<th>10×10⁴</th>
<th>5×10⁴</th>
<th>2×10⁴</th>
<th>1×10⁵</th>
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<td>500–599</td>
<td>1228.15 ± 13.364</td>
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<td>(n = 11)</td>
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<td>1235.42</td>
<td>&lt;3</td>
<td>1257.66</td>
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<td>1296.32</td>
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<td>(n = 7)</td>
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<td>1538.28</td>
<td>1616.51</td>
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3.3. The effect of food quality on relative rates of velar and shell growth (Experiment III)

Because the experimental design was not fully factorial, data for larvae growing at the optimal food concentration on each diet (T-ISO, DUN, MONO, and an equal mixture of
Fig. 5. Experiment II. Velar and shell growth rates of *Crepidula fornicata* larvae reared at four concentrations of T-ISO at 22°C. The open circles represent velum circumference, while the filled circles represent shell length.
T-ISO and MONO) were analyzed separately from those for larvae growing at the respective suboptimal food concentrations. Shell length served as the covariate in these analyses. Although larvae grew at significantly different rates when the different diets were provided at optimal concentrations (Table 1, Experiment IIIa, \( P < 0.0003 \)), diet at these high concentrations did not significantly affect the relationship between shell growth and velum growth (ANCOVA: \( F = 1.325 \), d.f. = 3,223, \( P = 0.2666 \)) (Fig. 6); this was true whether or not the interaction between food type and shell length was included in the ANCOVA model (the interaction term was almost significant: \( P = 0.0582 \)). However, diet had a significant effect on relative rates of velar and shell growth for larvae reared at suboptimal food concentrations (Fig. 7) (ANCOVA: \( F = 9.567 \), d.f. = 2,152, \( P < 0.05 \)). In particular, the mean shell-size adjusted velar circumference (GT2: adjusted least squares mean) for larvae reared at \( 2 \times 10^4 \) cells ml\(^{-1} \) DUN was significantly smaller than that for larvae reared at \( 5 \times 10^4 \) cells ml\(^{-1} \) MONO (\( P < 0.05 \)), though not significantly smaller than that for larvae reared at \( 5 \times 10^5 \) cells ml\(^{-1} \) T-ISO (\( P < 0.05 \)) (Fig. 8). In contrast, shell growth rates on DUN and MONO at these low concentrations were statistically equivalent (\( P > 0.10 \)), while growth rate of larvae reared on DUN was significantly below (\( P < 0.05 \)) that for larvae reared on T-ISO (Table 1, Experiment IIIb). Mean growth rate for larvae at the lowest concentration of DUN (Table 1) did not differ significantly from that at the highest concentration.

Fig. 6. Experiment IIIa. The influence of food quality on the relative rates of velum and shell growth for larvae of *C. fornicata* reared at 22°C. Three naked flagellate species (*Isochrysis galbana* (T-ISO), *Dunaliella tertiolecta* (DUN), *Pavlova lutheri* (MONO)) or an equal mixture of T-ISO and MONO (T/M) were provided at high concentrations that produced optimal shell growth rates.
Fig. 7. Experiment IIIb. Relationship between velum circumference and shell length (both in μm) of *C. fornicata* larvae reared at 22°C, as in Fig. 6. This figure differs from Fig. 6 in that, in this part of the experiment, phytoplankton were provided at low, suboptimal concentrations, and mixed diets were not tested.

Fig. 8. Experiment IIIb. The influence of food quality on relative rates of mean velar growth for larvae of *C. fornicata* reared at 22°C at suboptimal phytoplankton concentrations. Data are least squares means adjusted for shell length differences. Error bars represent 95% confidence intervals computed using G T2 method of multiple comparison among treatments after finding a significant effect of food species on relative rate of velar growth (ANCOVA, *P* = 0.023). The number of larvae measured in each treatment is given above each bar.
suggesting that growth-limiting concentrations of this phytoplankton species are below those tested in this study.

3.4. The effect of temperature on the relationship between food concentration and relative rates of velar growth (Experiment IV)

Temperature had no significant effect on relative velar growth rates (two-way ANCOVA on log$_{10}$ transformed data: $F = 1.948$; d.f. = 3,390, $P = 0.1213$), and no significant difference was detected among size-adjusted mean velar circumferences (GT2 test: adjusted least squares means, $P > 0.10$) for larvae growing at different temperatures at either the high or the low food concentration (Fig. 9). Thus, temperature over the range 16–25°C did not alter the relationship between velar and shell growth rates among larvae at any given food concentration, even though temperature had a pronounced effect on mean larval growth rate (Table 1, Experiments IVa and IVb, $P < 0.0001$).

In contrast, food concentration had a strong effect on relative rates of mean velar and shell growth in this experiment over the temperature range tested (Fig. 9) (ANCOVA: $F = 68.308$, d.f. = 1,390, $P < 0.0001$).

Except for larvae reared at 25°C, adjusted mean velum circumferences were significantly larger for larvae reared at the low food concentration than for larvae reared at the high food concentration (Fig. 9). The anomaly at 25°C apparently reflects the cessation of velum growth for larvae reared at the low food concentration after day 8, when larvae were approximately 950 μm in shell length (Fig. 10, 25°C; velum growth rate using only the last four samples was not significantly different from zero: $F = 1.025$, d.f. = 1,21, $P = 0.3229$). This cessation of velar growth may have biased the

Fig. 9. Experiment IV. The influence of temperature on relative rate of velar and shell growth for larvae of C. fornicata reared at optimal (18×10$^5$ cells ml$^{-1}$) or suboptimal (5×10$^5$ cells ml$^{-1}$) concentrations of T-ISO. Each point represents the least squares mean adjusted for shell length differences. Error bars represent 95% confidence intervals computed using GT2 method of multiple comparison among treatments.
Fig. 10. Experiment IV. The influence of temperature on rates of shell (SL) and velar growth (VC) for *C. fornicata* larvae reared at either high (H: $18 \times 10^3$ cells ml$^{-1}$) or low (L: $5 \times 10^3$ cells ml$^{-1}$) phytoplankton concentrations (T-ISO). Solid triangles and open circles represent larvae grown at the high and low phytoplankton concentrations, respectively. The lines were generated using a locally weighted regression (Lowess) curve.
resulting adjusted mean velum circumference for larvae reared at 25°C at the lowest food concentration. Cessation of velar growth was not observed for larvae reared at other temperatures (Fig. 10). When the data were reanalyzed using only samples taken during the first 8 days — the linear portion of the growth rate curve — larvae grown at the low food concentration had a significantly larger mean velar circumference with respect to shell length than did larvae grown at the high food concentration. Thus, food concentration had a major impact on the relative rates of velar and shell growth at all temperatures tested.

3.5. The effect of inherently differing shell growth rates on relative rates of velar and shell growth (Experiment V)

Mean size-adjusted velar circumference differed significantly between fast and slow growing larvae within a treatment (two-way ANCOVA: \( F = 5.323, \, \text{d.f.} = 1,120, \, P = 0.023 \)). The interaction between larval growth rate and food concentration was significant (ANCOVA: \( F = 4.246, \, \text{d.f.} = 3,120, \, P = 0.007 \)), which means that the relationship between shell growth rate and velar growth rate varied with food concentration. This was because adjusted mean velar circumferences (GT2 test: adjusted least squares means) of slow growing larvae were generally larger than those of fast growing larvae, except for larvae growing at \( 1 \times 10^4 \) cells ml\(^{-1} \) (Fig. 11). The effect of shell growth rate on the relationship between shell size and velar circumference was significant only at the highest food concentration tested, \( 18 \times 10^4 \) cells ml\(^{-1} \) (Fig. 11); slow growing larvae had disproportionately large velar lobes (\( P < 0.05 \)), even though both fast and slow growing larvae were reared in the same dishes under identical conditions of food and temperature.

4. Discussion

In general, larvae of \( C. fornicata \) reared on T-ISO at the lowest concentrations tested grew disproportionately large velar lobes. Moreover, relative rates of velar and shell growth were not appreciably altered by temperature over the range experienced by larvae in the field (16–25°C), even though larval shell growth rate was itself affected significantly; temperature differences explained only 1% of the variation in velum circumference in this study. Similarly, McEdward (1984) found no significant effect of rearing temperature on ciliated band length, arm length, or total arm length for larvae of the sand dollar \( Dendraster excentricus \) reared under nonlimiting food conditions. McEdward (1984) speculated that, under food-limiting conditions, the increase in metabolic rate at higher temperature might induce a compensatory increase in ciliary band length to accommodate increased metabolic demand. This was not evident in larvae of \( C. fornicata \): at growth-limiting phytoplankton concentrations, larvae reared at the highest temperature (25°C) did not grow larger velums than they did at the lower temperatures (Fig. 9). The general failure of increased temperature to alter relative rates of velar and shell growth in \( C. fornicata \) is surprising in that rates of growth and differentiation are often affected to different extents by altered temperature in this and
Fig. 11. Experiment V. The influence of inherent differences in larval growth rate on relative rate of velar growth for larvae of *C. fornicata* reared at 22°C at four different phytoplankton concentrations. Fast growing larvae were delineated from slow growing larvae based on the median shell growth rate (μm/day) at each phytoplankton concentration. Each bar represents the least squares mean velar circumference as adjusted for shell length differences. Error bars represent 95% confidence intervals computed using GT2 method of multiple comparison among treatments after finding a significant effect of food concentration (ANCOVA: *P* < 0.0001) and inherent growth rate (ANCOVA: *P* = 0.023). The number of larvae measured in each treatment is given above each bar. (*) denotes a significant difference between means.


The differential allocation of nutrients toward velar growth in response to low food concentration is similar to that exhibited by larvae of the oyster *Crassostrea gigas* (Strathmann et al., 1993). However, larval survival was not recorded in the oyster study, so that increased average velum dimensions under food-limiting conditions might have reflected differential mortality of individuals with smaller velar lobes. In our experiment, however, fewer than 1% of the larvae died in each treatment; thus, the larvae of *C. fornicata* were clearly responding to low food availability by preferentially allocating resources to velum growth rather than to shell growth. Low food concentration also caused the differential allocation of resources to larval feeding organs (i.e. arm lengths, ciliated band length) in planktotrophic larvae of sea urchins (Fenaux et al., 1994; Hart and Scheibling, 1988; Strathmann et al., 1992) and sand dollars (Boidron-Métairon, 1988; Hart and Strathmann, 1994). The response is probably adaptive, in that larger food-collecting surfaces will increase potential rates of water processing and particle collecting (Strathmann and Liese, 1979; Strathmann et al., 1993; Hart and Strathmann, 1994). Clearly, however, the increased size of larval feeding organs does not fully
compensate for the lower phytoplankton concentration, since growth rates of larvae reared at low food concentrations remained below those observed for larvae reared at higher food concentrations.

For *C. fornicata* larvae larger than about 900 μm in shell length and experiencing low food concentration at both 22 and 25°C, velar growth rate plateaued while shell growth continued (Table 2, Figs. 5 and 10). The trajectory of velum growth stimulated by low food concentrations in young larvae may be difficult to maintain in larger larvae experiencing limited resources and increased maintenance energy costs. On the other hand, for larvae of *C. fornicata* smaller than about 900 μm in shell length, velum size relative to shell length can potentially indicate the extent to which field-collected larvae have been growing at growth-limiting phytoplankton concentrations over a wide range of temperatures. There are, however, a number of factors potentially complicating the interpretation of measurements made on field-collected larvae, none of which have been considered previously.

One difficulty is that significant differences between average velar and shell growth rates were generally detectable only if the differences in food concentration were large, even though mean shell growth rates varied significantly over a smaller range of food concentrations. It appears that food concentrations must be at least 50% below optimal concentrations before that difference in food availability will be accurately reflected in average relative rates of velar growth. Even so, individual larvae varied substantially in the degree of velar response to reduced food concentration, so that a substantial number of larvae would need to be measured in order to reliably infer whether they have experienced growth-limiting food concentrations or not. Another potential complicating factor is that we could document no effect of food quality on the relative growth of the velar lobes and shell in *C. fornicata* when the three different phytoplankton species were given at optimal food concentrations, even though mean shell growth rates were reduced up to 41% on the two poorer diets (DUN, MONO: Table 1). In one sense this is an attractive result: reduced larval growth induced by poor diet does not mimic the effect of low food concentration in increasing the relative rate of velar growth. On the other hand, for *C. fornicata* larvae growing at relatively high phytoplankton concentrations, food-limiting conditions caused by variation in food quality will not be revealed from measurements of shell length and velar dimensions made on field-collected larvae.

An additional complication is that slower growing larvae of *C. fornicata* grew larger velar lobes than did faster growing larvae obtained from the same hatch and reared simultaneously under identical conditions, at the same temperature (22°C) and the same optimal food concentration (18×10^4 cells ml⁻¹ T-ISO). Thus, larvae that are genetically predisposed to grow more slowly — because of greater size-adjusted rates of food collection, lower rates of energy expenditure, or higher assimilation efficiency (Garton, 1984; Holley and Foltz, 1987; Hedgecock et al., 1995; Hawkins and Day, 1996) — may exhibit the morphological characteristics of food-limited individuals even when they have been growing under ideal conditions in the field. In such a case, measurements of shell and velar dimensions made on field-collected larvae could create an impression of food limitation when none was actually experienced. The relative magnitude of the confounding effect on mean rates of velar and shell growth will vary with the proportion of fast and slow growing individuals in the sample of larvae, which would likely vary from sample to sample and from week to week.
How reduced food concentration promotes disproportionately greater velar growth in molluscan larvae is not known. Larvae may be sensitive to external factors such as differential rates of contact with phytoplankton cells or differences in the concentration of stimulatory or inhibitory organic molecules secreted by phytoplankton cells (Wilson, 1981; Strathmann et al., 1992; Shilling, 1995), or to internal factors such as differences in gut processing times at different food concentrations; at low food concentrations, the larval gut is never filled and feeding is continuous, but at high food concentrations the gut fills quickly and feeding is periodic (Fretter and Montgomery, 1968). In addition, phytoplankton can contain thyroid hormone compounds that might selectively modify growth rates of different body parts after ingestion (Chino et al., 1994). As yet, it is not possible to distinguish among these different mechanisms.

5. Conclusion

At substantially lower than optimal phytoplankton concentrations, the larvae of Crepidula fornicata produced significantly larger velar lobes at any given shell length. Similar effects have been reported for at least some larval echinoids (Boidron-Métaireon, 1988; Hart and Scheibling, 1988; Fenaux et al., 1994) and one bivalve species (Strathmann et al., 1993). Growth-decreasing reductions in temperature and food quality did not have comparable effects on relative rates of velar and shell growth, suggesting that food limitation might be deduced for C. fornicata from measurements of velar dimensions and shell length on field-collected individuals. However, temperature did alter the relationship between food concentration and velar morphology, so that, at higher temperatures, low food concentration may not enlarge the velar lobes to the same extent that they are enlarged at lower temperatures at the same low food concentration. Thus, field-collected larvae may be seriously food limited even when their morphology is comparable to that of well-fed larvae reared in the laboratory.

Moreover, the effect of phytoplankton concentration varied markedly among individuals at every concentration tested, and at most phytoplankton concentrations, particularly the highest one, faster growing individuals tended to develop disproportionately larger velar lobes. In addition, measurements of relative velum size on field-collected individuals would be unable to detect growth limitation caused by poor phytoplankton quality, and any detected shifts in larval morphology may incorrectly suggest food limitation when caused instead by differences in inherent growth potential of different batches of larvae.

Fenaux et al. (1994) were able to conclude convincingly that echinoid larvae were food limited in the Mediterranean at certain times of year, because five different indicators all pointed in the same direction. Similarly, larval morphology alone will apparently not suffice as a means of assessing whether larvae of C. fornicata are food limited in the field. Measurements of velar lobe dimensions and shell size must be supplemented with histological or biochemical indicators, such as determinations of RNA/DNA ratios (e.g., Juinio and Cobb, 1994; Garcia et al., 1998; Wagner et al., 1998; Esteves et al., 2000), before conclusions can be drawn regarding the extent of food limitation for larvae of this species in the field. For future studies with larvae of other species, it will be important to determine whether morphological alterations induced by
limited food availability are also induced to the same extent by other factors: ‘B causes A’ does not necessarily mean ‘If A, then B’.

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References


