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Influence of Delayed Metamorphosis on the Growth and Metabolism of Young *Crepidula fornicata* (Gastropoda) Juveniles

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Abstract. Larvae of most marine invertebrates delay metamorphosis if they fail to contact an appropriate environmental stimulus. We conducted seven experiments with the slipper shell snail, *Crepidula fornicata,* to determine if delaying metamorphosis decreases juvenile fitness. Larvae were reared in the laboratory at 25°C on the unicellular alga *Isochrysis* sp. (clone T-ISO, 18×10^4 cells ml^{-1}), and were induced to metamorphose after long, medium, or short periods of delayed metamorphosis. Long delay larvae were reared until they metamorphosed spontaneously in acid-cleaned glassware. Medium and short delay larvae were induced to metamorphose with adult-conditioned seawater or 20 m*M* elevations of KCl. Juveniles were subsequently reared for about one to two weeks at 25°C in the laboratory on a diet of T-ISO. Delaying metamorphosis generally did not lower juvenile weight-specific feeding rates, increase juvenile weight-specific respiration rates, or lower juvenile shell or tissue growth rates, any of which effects would suggest that delaying metamorphosis reduces juvenile fitness. Moreover, there was no indication that delaying metamorphosis reduced juvenile tolerance of temperature-salinity stress. Delaying metamorphosis of *C. fornicata* does not appear detrimental, at least for the first few weeks of juvenile life.

Introduction

Many sedentary marine invertebrates have a free-swimming, dispersive larval stage (Thorson, 1950). Invertebrate larvae must generally develop for a time in the plankton before they become capable of metamorphosis. Once larvae are "competent," metamorphosis is commonly triggered by contact with chemical and physical cues characteristic of suitable adult habitat (Crisp, 1974; Gray, 1974; Scheltema, 1974; Hadfield, 1977; Jaccarini *et al.,* 1983; Olson, 1983; Sebens, 1983; Høeg, 1984).

If such cues are not encountered by competent larvae, metamorphosis may be delayed (*e.g.,* Knight-Jones, 1953; Wilson, 1958; Scheltema, 1961; Bayne, 1965; Hadfield, 1978; Pechenik, 1980). The ability to distinguish among substrates and to delay metamorphosis promotes preferential recruitment of larvae into habitats favorable for juvenile and adult survival and for reproduction (Thorson, 1950, 1966; Crisp, 1974). As a byproduct of this discriminatory capability, delaying metamorphosis enhances dispersal potential, which should influence a species' geographic range, the degree of genetic isolation among populations, rates of speciation, and species longevity (Shuto, 1974; Strathmann, 1974, 1978, 1980; Scheltema, 1977; Hansen, 1978; Beaumont and Budd, 1982; Jablonski and Lutz, 1983). This presumes that juvenile fitness (specifically, the probability of successfully giving rise to the next generation of reproductive adults) is not adversely affected by delayed metamorphosis (Pechenik, 1985). However, larvae of some invertebrate species exhibit morphological degeneration while they delay metamorphosis (Wilson, 1935; Bayne, 1965; Sastry, 1965; Hinegardner, 1969; Highsmith and Emlet, 1986) and may actually lose the ability to metamorphose in response to suitable substrates after a lengthy delay period (Wilson, 1935; Grave, 1936; Knight-Jones, 1953; Hinegardner, 1969; Barker, 1977; Sebens, 1983). Other delaying larvae, including those of the gastropod *Crepidula fornicata,* that fail to show such dramatic morphological and physiological degeneration during delayed metamorphosis (Pechenik, 1980, 1984) may nevertheless experience subtle declines in fitness that could be car-

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ried into juvenile life. Any such decrease in fitness would lessen the importance of delay capability in maintaining genetic homogeneity and influencing rates of extinction and speciation.

Characterizing such evolutionary "design constraints" is a major goal of life history research (Stearns, 1980; Reznick, 1982). The possibility that fitness is reduced following an extended delay of metamorphosis is readily testable for *C. fornicata* in the laboratory. Reduced fitness could result in higher juvenile mortality, decreased rates of energy acquisition, increased rates of energy utilization, delayed maturation, reduced fecundity, or reduced tolerance to physical and biological stress (Stearns, 1976; Bayne, 1975; Newell and Branch, 1980; Berven, 1982). This report focuses on the influence of delayed metamorphosis on survival, energy accumulation, and tolerance of physical stress by juveniles of *Crepidula fornicata.*

Crepidula fornicata is a rapidly growing (Calabrese and Rhodes, 1974), sedentary, suspension-feeding gastropod. These characteristics facilitate measuring growth, respiration, and feeding rates. Moreover, the larvae of this species can be laboratory-reared with very low mortality (Pechenik, 1980; Pechenik and Lima, 1984), competent larvae can be readily induced to metamorphose (Pechenik and Heyman, 1987), and even young juveniles are large enough to allow accurate determinations of individual dry tissue weights. Finally, *C. fornicata* is one of the few temperate molluscs whose larvae have been demonstrated to delay metamorphosis in the field (Pechenik, 1986).

Materials and Methods

Obtaining and rearing animals

Adult *Crepidula fornicata* were collected from Narragansett, Rhode Island (1985), Barnstable, Massachusetts (1986), and Nahant, Massachusetts (1987) and fed the unicellular flagellate *Dunaliella tertiolecta* daily until veliger larvae were released from the brooded egg cases. We reared larvae at 25°C in Percival incubators (12L:12D photoperiod), with about one larva per ml of 0.45 μm filtered seawater, and fed them the alga *Isochrysis* sp. (clone T-ISO; Bigelow Laboratory Culture Collection) at 18–20 $\times$ 10^4 cells ml^{-1}. Water was changed and food was replenished every other day. Seawater was aerated by vigorous agitation just before adding larvae and phytoplankton, but not between water changes.

About 10 days after hatching, larvae were measured at 50$\times$ using a dissecting microscope fitted with an ocular micrometer. Larvae with shell lengths between 800 and 1200 μm were segregated by pipette and randomly divided into two groups; Pechenik and Heyman (1987) demonstrated that most *C. fornicata* larvae can metamorphose by the time they are 800–1000 μm long. Larvae in one group were triggered to metamorphose over the next 6–8 h using either adult-conditioned seawater or seawater whose KCl concentration was elevated by 20 m*M* (Pechenik and Heyman, 1987); Eyster and Pechenik (1988) showed that shell growth, weight-specific respiration, and weight-specific feeding rates of young juveniles are comparable following both modes of induction. These larvae are referred to as "short delay" individuals, since they probably had delayed metamorphosis for less than two days.

Larvae in the second group were maintained until each individual metamorphosed "spontaneously." By spontaneous metamorphosis, we refer to metamorphosis that eventually occurs despite our best efforts to prevent it; larvae of *C. fornicata* inevitably undergo such spontaneous metamorphosis in laboratory culture despite daily cleaning of glassware (Pechenik, 1984; Pechenik and Lima, 1984). These juveniles were designated "long delay" individuals, resulting from the maximum possible extent of delayed metamorphosis.

In two experiments, we reared three groups of juveniles: short delay individuals (as above), long delay individuals (as above), and medium delay individuals (triggered to metamorphose using KCl 4–6 days after metamorphosis was triggered in short delay individuals).

All juveniles were reared at 25°C for 6–13 days in individual glass dishes containing 45 ml of T-ISO feeding suspension at an initial concentration of 18 $\times$ 10^4 cells ml^{-1}. Initial shell lengths were measured at 50$\times$, usually within 12 h of metamorphosis. Feeding suspension in each bowl was changed daily, but algal cell density nevertheless declined markedly between water changes. Therefore, we conducted an experiment to estimate the phytoplankton cell density below which feeding rates decline for juveniles of *C. fornicata.* Preliminary studies (Eyster and Pechenik, 1988) suggested that this critical cell density was about 8 $\times$ 10^4 cells ml^{-1} for 1.7 mm long juveniles feeding on T-ISO.

Determining critical food concentration

To determine the critical phytoplankton cell concentration for recently metamorphosed juveniles, 24 juveniles (mean shell length $\pm$ one standard deviation = 2.84 $\pm$ 0.33 mm) were selected for the experiment. Anterior-posterior shell lengths were measured at 25$\times$ using an ocular micrometer. All juveniles were allowed to pre-feed for 30 min at 18 $\times$ 10^4 T-ISO cells ml^{-1} in individual 5-ml glass beakers. The pre-feeding suspension was then replaced with 4 ml of suspension at the same algal concentration, 18 $\times$ 10^4 cells ml^{-1}. After 1 h at 25°C, the final cell concentrations were determined in all 24 beakers. Cell concentrations were also determined in five

controls at the start of the experiment and five controls at the end of the experiment. All incubations were conducted in dim light. Cell concentrations in control beakers did not change appreciably during this experiment. All cell concentrations were determined using a Model ZM electronic particle counter (Coulter Electronics), and occasionally double-checked using hemacytometers. Feeding rates were determined from the rate of disappearance of cells from suspension (Pechenik, 1980; Marin *et al.*, 1986; Eyster and Pechenik, 1988).

After feeding rates were determined for all individuals fed at 18×10^4 cells ml^{-1}, feeding suspensions were replaced in all beakers. Twelve animals were repeatedly fed at 18×10^4 cells ml^{-1}. The other 12 animals were fed at progressively lower initial food levels for each one-hour feeding bout. Animals in the two groups did not differ in mean shell length ($P < 0.05$; one-way analysis of variance). The following algal concentrations were tested: 18×10^4, 11×10^4, 9×10^4, 7×10^4, and 4×10^4 cells ml^{-1}. Feeding rates were then again determined for all 24 individuals at an initial concentration of 18×10^4 cells ml^{-1}.

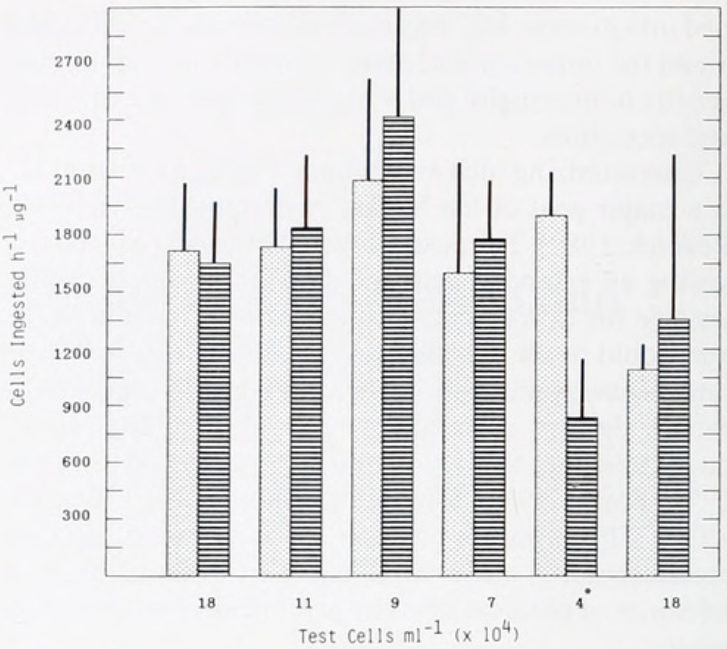

Figure 1. The influence of algal cell concentration (*Isochrysis* sp., Tahitian isolate) on weight-specific ingestion rate of juvenile *Crepidula fornicata*. One group of 12 juveniles (dotted bars) was fed at an initial cell concentration of 18×10^4 cells ml^{-1} in each 1-h feeding bout. The other group of 12 juveniles (striped bars) was fed at progressively lower food concentrations every 1 h as indicated. To complete the experiment (extreme right), both groups of snails were offered 18×10^4 cells ml^{-1}. *Represents a significant difference between means ($P < 0.05$, one-way analysis of variance). Error bars indicate one standard deviation above the mean.

Effects of delayed metamorphosis on growth and metabolism

We performed six experiments testing the influence of delayed metamorphosis on juvenile development. Larvae were induced to metamorphose after different amounts of delayed metamorphosis, as detailed above. Rates of individual juvenile shell growth were then monitored by periodic microscopic examination. Three to 13 days after triggering metamorphosis, we carefully transferred the juveniles to individual 5 ml glass bottles to measure individual respiration and feeding rates at 25°C, as described elsewhere (Eyster and Pechenik, 1988). After a 1–2 h gut evacuation period in 0.45 µm filtered seawater, respiration rates were determined from the decline in oxygen concentration over 4–15 h periods, the length of each incubation period depending on juvenile size. Oxygen concentrations were determined by injecting 1-ml samples into a Strathkelvin Instruments Model MC100 glass microrespiration cell equipped with a Model 1302 electrode; the electrode was coupled to a Strathkelvin Model 781 oxygen meter. Individual feeding rates were determined from declines in algal cell concentration over 1–5 h periods, the experimental duration again varying inversely with juvenile size.

After measuring respiration and feeding rates, we measured the final shell length of each snail and then determined the individual's ash free dry weight (AFDW). This enabled us to calculate weight-specific respiration and feeding rates. To obtain dry weights, each juvenile was removed from its test container, measured at 25×, rinsed free of salts, transferred to preweighed aluminum foil pans, and desiccated over indicating $CaSO_4$ for approximately one week. Individual dry weights were determined to the nearest 1 µg using a Cahn Model 21 electrobalance, with desiccant placed in the weighing chamber to prevent specimen rehydration. Samples were then ashed at 500°C for 6 h and reweighed to estimate tissue content by weight loss (Paine, 1964; Pechenik, 1980).

Linear regression analysis (method of least squares) was used to determine the relationship between final juvenile shell length (mm) and tissue weight (µg); this enabled us to estimate weight at metamorphosis from shell length data, and to express metabolic data on a weight-specific basis when direct tissue weight measurements were not available, due to loss or mishandling. In one experiment (No. 10), juvenile shell length measurements were made twice: once on day 7 and later on day 13, at which time actual individual weight determinations were made. Thus, we could calculate shell growth rate for the first 7 days of juvenile development, the first 13 days of juvenile development, and between days 7–13. The regression equation was used to estimate individual biomass on day 7 so that tissue growth rates could be calculated separately for the first and second weeks of juvenile growth.

Effects of delayed metamorphosis on stress tolerance

In addition to examining the influence of delayed metamorphosis on juvenile growth and metabolism, we

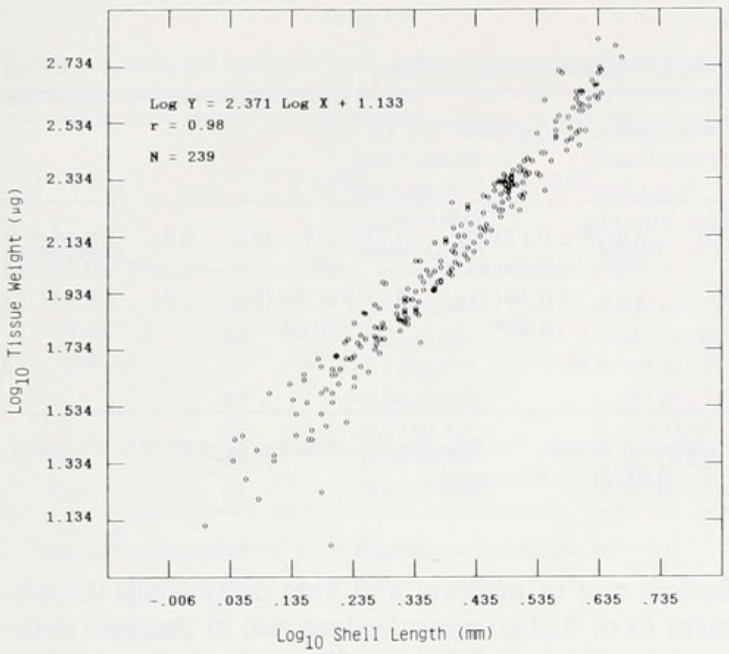

Figure 2. The relationship between tissue weight and longest shell length for juvenile *Crepidula fornicata*. Tissue weights were estimated as ash-free dry weight after combustion at 500°C. Each point represents data from a single individual.

also conducted experiments to determine whether delayed individuals were less tolerant of physical stress than were individuals triggered to metamorphose soon after they became competent. Larvae reared at 25°C were divided into two groups: short delay individuals were triggered to metamorphose soon after they developed shell brims (Pechenik, 1984; Pechenik and Lima, 1984); long delay individuals were either triggered to metamorphose about one week later or were reared through spontaneous metamorphosis. All juveniles were reared at 25°C for 5–7 days at about 18×10^4 cells ml^{-1} on a mixed diet of T-ISO and *Dunaliella tertiolecta* (clone DUN). They were then distributed among combinations of five osmotic concentrations (460, 610, 770, 830, and 925 mOsm kg^{-1}—approximately 15, 20, 25, 27, and 30 ppt salinity, with full strength seawater being the highest concentration) and five temperatures covering the range 25°–35°C. Osmotic concentrations were determined using a freezing-point depression osmometer (Advanced Instruments, Inc). Test solutions were prepared by diluting full strength Nahant-collected seawater with glass-distilled water. Five to eight juveniles were tested at each stress level, without replication, and examined after 24 and 36 h of exposure. Tested juveniles were 1–2 mm in shell length. Four separate stress experiments were conducted, each using juvenile snails from separate hatches.

Results

Critical food concentration

Juvenile weight-specific feeding rates were unaffected by phytoplankton cell concentrations between about 7 and 18×10^4 cells ml^{-1}. However, decreasing cell concentration to 4×10^4 cells ml^{-1} caused a 56% reduction in average feeding rate (Fig. 1). Clearly, the critical food level for these juveniles lies somewhere between 4 and 7 $\times 10^4$ cells ml^{-1}, confirming previous indications (Eyster and Pechenik, 1988). Spot checks indicated that algal cell concentrations rarely declined below about 8–10 $\times 10^4$ cells ml^{-1} during our juvenile growth studies. This suggests that juveniles were not food limited during our study and that growth rates among these juveniles can be reasonably compared.

Survival

Juveniles survived well following metamorphosis. Of the 320 juveniles studied in these experiments, only about 12% died; one-third of these died only because they crawled out of the water and desiccated. The incidence of this "suicidal" behavior was unrelated to the extent of delayed metamorphosis.

Table I

Influence of delayed larval metamorphosis on subsequent juvenile weight-specific respiration rates in Crepidula fornicata

	Mean respiration rates ($\mu l\ O_2$ consumed h^{-1} μg tissue^{-1}) $\times 10^{-3}$				
Treatment	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 9
Short delay	3.6 ± 0.6 (17)	2.7 ± 0.5 (27)	2.8 ± 0.5 (20)	3.6 ± 0.5 (18)	3.5 ± 0.85 (26)
Medium delay	NT	NT	NT	4.1 ± 0.9 (13)	NT
Long delay	2.8 ± 0.5 (7)	3.6 ± 0.8 (7)	2.1 ± 0.7 (8)	3.5 ± 0.5 (11)	3.9 ± 1.2 (10)
ANOVA F-value	F = 8.72*	F = 13.30*	F = 9.23*	F = 2.87	F = 1.61
(degrees of freedom)	(1, 22)	(1, 32)	(1, 26)	(2, 39)	(1, 34)

Short delay and medium delay animals were induced to metamorphose using adult-conditioned seawater or elevated levels of KCl. Long delay individuals metamorphosed spontaneously. Rates are mean ± one standard deviation (sample size). * = Significant difference exists among treatments, ANOVA, $P < 0.05$. NT = Not tested.

Table II

Influence of delayed metamorphosis on subsequent weight-specific feeding rates of Crepidula fornicata *juveniles*

	Mean feeding rates (cells ingested h^{-1} μg tissue^{-1}) $\times 10^3$					
Treatment	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 9	Expt. 10
Short delay	1.46 ± 0.55 (17)	1.06 ± 0.18 (27)	1.54 ± 0.68 (20)	0.86** ± 0.17 (19)	0.73 ± 0.35 (25)	0.86 ± 0.26 (13)
Medium delay	NT	NT	NT	1.26 ± 0.23 (14)	NT	1.36** ± 0.23 (18)
Long delay	1.36 ± 0.58 (8)	0.93 ± 0.48 (7)	1.75 ± 0.67 (8)	1.11 ± 0.24 (11)	0.75 ± 0.26 (10)	0.94 ± 0.23 (16)
ANOVA F-value (degrees of freedom)	F = 0.16 (1, 23)	F = 1.22 (1, 32)	F = 0.56 (1, 26)	F = 14.80* (2, 41)	F = 0.04 (1, 33)	F = 20.19* (2, 44)

Rates are mean ± one standard deviation (sample size). See Table I for treatment details. * = Significantly different by ANOVA, $P < 0.05$. ** = Treatment that is significantly different by Duncan's multiple range test, $P < 0.05$. NT = Not tested.

Weight-length relationship

The relationship between juvenile tissue weight and shell length in *C. fornicata* was well defined by the following equation: $\log_{10}$ μg tissue = 2.371 ($\log_{10}$ mm shell length) + 1.133 (N = 239; $r^2 = 0.96$) (Fig. 2). Therefore, the units of growth rate could be converted from amount of shell added per day to μg tissue added per day using this equation.

Effect of delayed metamorphosis on respiration and feeding

Weight-specific respiration rates were significantly different ($P < 0.05$) for long and short delay individuals in three of the five experiments conducted. They were significantly higher in only one experiment (experiment 4) (Table I). Weight-specific feeding rates were not significantly lower ($P > 0.10$) for long delay individuals in any of the six experiments (Table II); significant differences in mean feeding rates were recorded in two experiments, but these differences reflect either higher mean feeding rate in medium and long delay snails (experiment 6) or higher mean feeding rate in medium delay snails (experiment 10).

Effect of delayed metamorphosis on juvenile growth

Determining the influence of delayed metamorphosis on juvenile growth rate is potentially complicated by an unavoidable difference in the sizes at which individuals metamorphose in the different treatments. Long delay individuals were typically larger at metamorphosis (Table III), since *C. fornicata* larvae continue to grow as metamorphosis is delayed (Pechenik, 1980, 1984; Pechenik and Lima, 1984). This size difference was not a problem when comparing postmetamorphic shell growth, since rates of juvenile shell growth were not correlated ($P > 0.10$) with shell size at metamorphosis. Since no more than 10% of the variation in individual juvenile shell growth could be accounted for by differences in shell length at metamorphosis ($r^2 < 0.10$, Table IV), shell growth rates for snails in the different treatment groups

Table III

Shell sizes (mean μm ± one standard deviation) at metamorphosis for Crepidula fornicata *larvae delaying metamorphosis for different lengths of time*

Treatment	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 10
Short delay	1343 ± 187 (N = 17)	1118 ± 207 (N = 28)	1234 ± 141 (N = 20)	1100 ± 149 (N = 20)	891 ± 128 (N = 20)	942 ± 165 (N = 13)
Medium delay	NT	NT	NT	1075 ± 108 (N = 14)	NT	982 ± 123 (N = 18)
Long delay	1541 ± 186 (N = 8)	1311 ± 163 (N = 7)	1550 ± 166 (N = 8)	1189 ± 204 (N = 11)	1256 ± 109 (N = 12)	1202** ± 217 (N = 16)
ANOVA F-value (degrees of freedom)	F = 6.1* (1, 23)	F = 5.2* (1, 32)	F = 25.9* (1, 26)	F = 1.8 (2, 42)	F = 67.4* (1, 30)	F = 10.3* (2, 44)

Metamorphosis was triggered using adult-conditioned seawater or 20 m*M* elevations of KCl for "short delay" and "medium delay" animals; "long delay" individuals were allowed to metamorphose spontaneously. * and ** = Significant differences ($P < 0.05$), ANOVA and Duncan's multiple range test, respectively. NT = Not tested.

Table IV

Extent of correlation between Crepidula fornicata *juvenile growth rate and initial postmetamorphic shell length*

Experiment	N	Shell growth rate (μm d^{-1}) vs initial shell length (μm)	Tissue growth rate (μg d^{-1}) vs initial shell length (μm)
3	45	−0.04 (0.002)	0.39* (0.15)
4	34	−0.23 (0.05)	0.44* (0.20)
5	29	0.16 (0.03)	0.53* (0.28)
6	45	−0.04 (0.002)	0.39* (0.15)
7	32	0.31 (0.10)	0.69* (0.48)
10a	47	0.18 (0.03)	0.53* (0.28)
10b	47	−0.03 (0.001)	0.53* (0.28)

Data are presented as r(r^2). * = Significant correlation ($P < 0.05$).

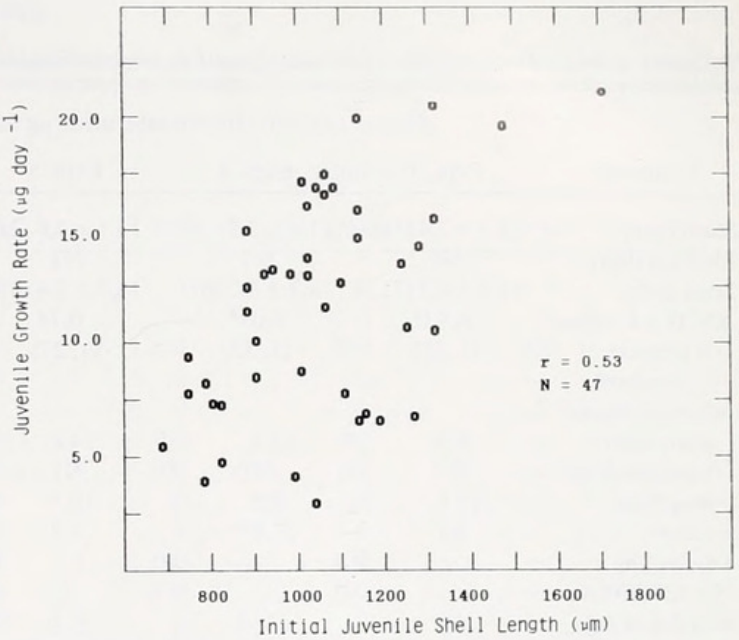

Figure 3. Representative relationship between size at metamorphosis and daily tissue growth in juvenile *Crepidula fornicata.* These data are from experiment 10a, in which juveniles grew for 7 days following metamorphosis.

could be compared directly, despite the size differences at metamorphosis. Rates of shell growth were significantly reduced ($P < 0.05$) for delayed individuals in only two of the seven determinations (experiments 4 and 10b); they were actually higher ($P < 0.05$) in one case (experiment 7) (Table V).

In contrast to results for shell growth, tissue growth rates correlated significantly ($P < 0.05$) with shell size at metamorphosis in all experiments (Table IV). That is, individuals that were larger at metamorphosis tended to add biomass more rapidly following metamorphosis (Fig. 3). Thus, direct comparison of tissue growth rate among treatment groups could be misleading: any tendency of long delay individuals to add biomass more slowly than short delay individuals could be masked by the larger average size of the long delay animals at metamorphosis. Therefore, mean tissue growth rates within an experiment were adjusted by analysis of covariance, with shell length at metamorphosis serving as the covariate.

Without adjustment, long delay individuals added biomass more slowly than medium or short delay individuals in only one of the six experiments (experiment 4) (Table VI). Adjusting for differences in initial shell lengths among treatment groups, long delay individuals grew significantly more slowly than other individuals in two experiments (experiments 4 and 10) (Table VI).

The data for experiment 10 are susceptible to separate analysis since juvenile size was measured thrice, on days 1, 7, and 13 after metamorphosis. Thus, juvenile growth rates can be examined separately for the first and second weeks of development. Although at metamorphosis shell sizes (and biomass) differed among treatment groups in experiment 10 (Table III), these differences had eva-

Table V

Influence of delayed larval metamorphosis on shell growth rate of juvenile Crepidula fornicata

	Growth rates: mean μm day^{-1} ± one standard deviation (sample size)						
Treatment	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 10a	Expt. 10b
Short delay	126 ± 31 (18)	208 ± 24 (28)	183 ± 43 (21)	201 ± 39 (20)	220 ± 43 (20)	167 ± 30 (13)	200 ± 31 (13)
Medium delay	NT	NT	NT	195 ± 37 (14)	NT	188 ± 24** (18)	194 ± 32 (18)
Long delay	150 ± 46 (8)	141 ± 51 (6)	173 ± 13 (8)	166 ± 37 (11)	256 ± 28 (12)	150 ± 53 (16)	146 ± 30** (16)
ANOVA F-value (degrees of freedom)	F = 2.31 (1, 23)	F = 25.2* (1, 32)	F = 1.64 (1, 27)	F = 2.39 (2, 41)	F = 6.56* (2, 29)	F = 7.18* (2, 44)	F = 9.86* (2, 44)
Number of days	5	3–4	5–6	4–5	9	7	13

Larval treatments are given in Table I. Juvenile growth rates were compared over 3–13 days as indicated. * = Significantly different by ANOVA, $P < 0.05$. ** = Significantly different by Duncan's multiple range test, $P < 0.05$. NT = Not tested.

Table VI

Influence of delayed metamorphosis on tissue growth in juvenile Crepidula fornicata

	Growth rate: mean μg tissue day^{-1} ± one standard deviation (sample size)						
Treatment	Expt. 3	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 10a	Expt. 10b
Short delay	8.0 ± 2.4 (18)	11.8 ± 3.2 (28)	13.1 ± 4.6 (21)	11.6 ± 3.8 (20)	24.5 ± 8.4 (20)	10.1 ± 3.9 (13)	20.3 ± 7.4 (13)
Medium delay	NT	NT	NT	11.1 ± 3.1 (14)	NT	13.3 ± 3.5 (18)	20.7 ± 6.3 (18)
Long delay	11.7 ± 4.7 (7)	8.3 ± 3.2 (6)	14.6 ± 2.4 (8)	9.7 ± 3.2 (11)	39.1 ± 7.0 (12)	11.0 ± 6.1 (16)	17.2 ± 9.4 (16)
ANOVA F-value	6.93*	6.02*	0.74	1.08	25.7*	1.92	0.96
(degrees of freedom)	(1, 23)	(1, 32)	(1, 27)	(2, 42)	(1, 30)	(2, 44)	(2, 44)
Adjusted means							
short delay	8.0	12.2	14.6	11.8	27.0	12.1	23.4
medium delay	NT	NT	NT	11.5	NT	14.5	22.6
long delay	11.1	6.3	10.7	8.9	35.0	8.0	12.5
F-value:	3.4	27.9**	3.2	3.1	4.28**	12.4**	11.5**
Adjusted by ANCOVA							
Number of days	5	3–4	5–6	4–5	9	7	13

Larval treatments are given in Table I. Tissue weights at metamorphosis were estimated from shell length data using the relationship illustrated in Fig. 2. * = Significant differences ($P < 0.05$) by one-way analysis of variance (ANOVA) or by analysis of covariance (**) (ANCOVA). ANCOVA was performed with initial postmetamorphic shell length as the covariate, to adjust for differences in mean size at metamorphosis among treatment groups within an experiment. NT = Not tested.

nesced by day 7, as did any differences in mean tissue weight (Table VII). Therefore, mean rate of tissue growth can be compared for the subsequent 6 days of juvenile growth without data adjustment. Delaying metamorphosis had no effect on rate of shell or tissue growth for the second week following metamorphosis (Table VII).

In all experiments juveniles grew rapidly following metamorphosis. Shell growth rates averaged about 150–200 μm day^{-1}, the equivalent of accumulating biomass at about 10–20 μg dry weight day^{-1} (Tables V, VI).

Effect of delayed metamorphosis on stress tolerance

In the temperature-salinity stress study, mortality was always 100% at 35°C (all salinities). Mortality was always 0% at 25°C and 30°C (all salinities). None of our experiments indicated that delaying metamorphosis reduced tolerance of *C. fornicata* to temperature-salinity stress. Indeed, the data for 24 h (Table VIII) and 36 h (Table IX) survival suggest that long delay individuals are more tolerant of stress than short delay individuals.

Discussion

Delaying the metamorphosis of the gastropod *Crepidula fornicata* typically had no detectable detrimental influence on juvenile survival, feeding rate, respiration rate, shell growth rate, tissue growth rate, or tolerance to temperature-salinity stress for at least the first few weeks following metamorphosis (Tables IX, X). Similarly, de-

Table VII

The lack of influence of delayed metamorphosis on juvenile growth rates of Crepidula fornicata *from days 7–13 after metamorphosis in experiment 10*

	N	Actual 7d shell length (mm)	Estimated 7d tissue wt. (μg)	Actual 13d tissue wt. (μg)	Tissue growth rate (μg d^{-1})
Short delay	13	2.11 ± 0.35	83.3 ± 31.4	275.7 ± 100.1	32.0 ± 12.6
Medium delay	18	2.36 ± 0.26	106.0 ± 26.7	282.2 ± 83.3	29.4 ± 10.3
Long delay	16	2.24 ± 0.50	99.1 ± 50.6	245.9 ± 130.0	24.5 ± 16.1
F-value (2, 44)		1.60	1.40	0.54	1.32
P-value		0.21	0.26	0.58	0.28

Data are given as mean ± one standard deviation.

Table VIII

Percent mortality of juvenile Crepidula fornicata *exposed to various salinity-temperature combinations for 24 h*

Experiment	Length of delay	Temp °C	Percent morality: Salinities 15‰	20‰	25‰	27‰	30‰
A, B, C, D	short, long	25°	0	0	0	0	0
A, B, C, D	short, long	30°	0	0	0	0	0
A, B, C, D	short, long	35°	100	100	100	100	100
A	short	32°	100	0	0	—	—
	long		100	0	0	—	0
A	short	33.5°	100	100	60	—	—
	long		100	100	60	—	60
B	short	32°	**80**	0	0	—	—
	long		**0**	0	0	—	0
B	short	33.5°	100	100	100	—	—
	long		100	100	100	—	100
C	short	32°	0*	0	0	—	0
	long		0*	0	0	—	0
C	short	33.5°	100	100	100	—	0*
	long		100	100	100	—	0*
D	short	32°–32.8°	100	0*	**0***	—	**25**
	long	32°–33.2°	100	0*	**0**	—	**0**
D	short	33.5°	100	100	100	100	100
	long		100	100	100	100	100

— = Not tested. * = None dead but all moribund. Experiments where mortality varied between short delay and long delay treatments are shown in boldface.

laying metamorphosis did not significantly alter juvenile growth rates for the sand dollar *Dendraster excentricus* (Highsmith and Emlet, 1986, p. 350 and their fig. 4). Highsmith and Emlet (1986) also worked with the sand dollar *Echinarachnius parma* and found that delaying metamorphosis rarely depressed juvenile growth rates

Table IX

Percent mortality of juvenile Crepidula fornicata *exposed to various salinity-temperature combinations for 36 h*

Experiment	Length of delay	Temp °C	Percent mortality: Salinities 15‰	20‰	25‰	27‰	30‰
A	short	32°	100	**100**	**50**	—	—
	long		100	**0**	**0**	—	0
A	short	33.5°	100	100	100	—	—
	long		100	100	100	—	60
B	short	32°	**100**	0	0	—	—
	long		**25**	0	0	—	0
C	short	32°	**100**	0	0	—	0
	long		**0***	0	0	—	0
C	short	33.5°	100	100	100	—	100
	long		100	100	100	—	100
D	short	32°–32.8°	100	**25**	**40**	—	**25**
	long	32°–33.2°	100	**40**	**0**	—	**0**

— = Not tested. * = None dead but all moribund. As in 24 h exposures, mortality was 0% at 25°C and 30°C (all salinities, even for 48 h). Those experiments in which mortality was already 100% in all treatments by 24 h are not relisted in this table (see Table VIII). Experiments where mortality varied between short delay and long delay treatments are shown in boldface.

Table X

Summary table: effects of delayed metamorphosis on juvenile growth and metabolism in Crepidula fornicata

	Number of experiments showing increase in delayed individuals	Number of experiments showing decrease in delayed individuals	Number of experiments showing no effect
Shell growth rate	1	2	4
Tissue growth rate	2	1	4
Adjusted tissue growth rate	1	3	3
Weight-specific feeding rate	1	0	5
Weight-specific respiration rate	1	2	2

In this table, determinations 10a and 10b are considered separate experiments. Summary is based on comparisons of short delay versus long delay individuals only.

(their Table 6, p. 356). Moreover, they found no correlation between juvenile growth rate and the number of days that metamorphosis was delayed. Their data for *D. excentricus* suggest a possible relationship between extent of delayed metamorphosis and rate (but not necessarily extent) of juvenile mortality. However, their results may reflect inadequate culture conditions more than a direct effect of delayed metamorphosis on survival: the water in larval cultures was changed only once or twice per week, the water in juvenile cultures was changed as seldom as once every two weeks, larvae and juveniles were fed unknown amounts of food as infrequently as once per week, and unfed juveniles showed dramatically less mortality than fed individuals from the same experiments. If the substantial mortality Highsmith and Emlet (1986) report for juvenile *D. excentricus* does reflect inhospitable culture conditions, then, as the authors suggest, delaying metamorphosis appears to have made those juveniles more susceptible to that stress. Of those individuals that died, long delay animals tended to die before short delay animals. In contrast, our experiments suggest that juvenile *C. fornicata* may actually be more tolerant of physical stress if metamorphosis has been delayed.

In contrast to these results for *C. fornicata,* we recently found that delaying metamorphosis of the bryozoan *Bugula stolonifera* by only 8–10 h significantly reduced the rate of early colony development (Woollacott *et al.,* in press). Unlike the gastropod and echinoid larvae discussed above, the bryozoan larvae are non-feeding, and the impact of delaying metamorphosis likely reflects depletion of stored nutrients. Since *C. fornicata* larvae continue to feed while metamorphosis is delayed (Pechenik, 1980; Pechenik and Lima, 1984), delaying metamorphosis is unlikely to impose nutritional stress in this species.

Critical food concentrations for the early juveniles (about 2–3 mm shell length) feeding on the alga *Isochrysis* sp. (clone T-ISO) were between 4–7 $\times$ 10^4 cells ml^{-1}, confirming preliminary studies with slightly smaller juveniles of this species (Eyster and Pechenik, 1988). Because we usually succeeded in maintaining food levels above about 8 $\times$ 10^4 cells ml^{-1} between water changes in our experiments, we can assume that all juveniles were growing at their maximal rates and that juvenile growth rates were independent of food level. Pechenik (1978) reported a higher critical food concentration—somewhere between 10–12 $\times$ 10^4 cells ml^{-1}—for larvae of this species.

The growth rates we report here (150–200 μm day^{-1}) for juveniles of *C. fornicata* are somewhat higher than those reported earlier for juveniles reared in this laboratory at the same temperature and on the same diet (Pechenik and Lima, 1984). The maximum growth recorded previously at 25°C was about 130 μm day^{-1}. Our data demonstrating rapid growth in this species are consistent with earlier reports of rapid development; sexual maturity in *C. fornicata* is reached, in the laboratory at 25°C, in about only 105 days after metamorphosis (Calabrese and Rhodes, 1974).

Comparable metabolic data for juvenile *C. fornicata* are available only for much larger individuals (20–40 mm long; Newell and Kofoed, 1977). At 25°C, weight-specific respiration rates of these larger snails were typically one-half to one-quarter the values reported here for individuals only a few mm long.

Pechenik (1985) suggested that a gradual decline in fitness could explain why natural selection might not act to prolong larval life indefinitely in *C. fornicata.* It is therefore puzzling that spontaneous metamorphosis occurs in the absence of a demonstrable cost to delaying metamorphosis. Although we found no consistent negative influence of delayed metamorphosis on early juvenile development, we cannot rule out the possibility that negative effects on survival, growth, or metabolism appear in later development. Other potential costs of delaying metamorphosis include effects on fecundity, age at first reproduction, and resistance to predation. These have not yet been investigated for any marine invertebrate.

The results of this study have implications for molluscan mariculture. Molluscan larvae develop at widely different rates even within a single culture (*e.g.,* Hadfield, 1977; Newkirk *et al.,* 1977; review by Pechenik, 1987). Therefore, when most of the larvae in a culture are competent, many of the larvae in that culture will have already been competent to metamorphose for some time.

Based on this work for *C. fornicata,* it appears that stimulating metamorphosis after a lengthy delay period will not reduce juvenile survival or growth, at least for species with feeding larvae. Even so, similar experiments on commercially important molluscan species should be conducted before generalizations are made.

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