

RELATIONSHIP BETWEEN GROWTH, DIFFERENTIATION, AND
LENGTH OF LARVAL LIFE FOR INDIVIDUALLY REARED LARVAE
OF THE MARINE GASTROPOD, *CREPIDULA FORNICATA*

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ABSTRACT

Larvae of the gastropod *Crepidula fornicata* were reared individually through spontaneous metamorphosis in clean glass containers at constant temperatures ranging from 15°C to 29°C; each larva was examined daily. Growth rates were determined from periodic measurements of individual shell length. Differentiation rates were estimated as (days to development of gill rudiments)⁻¹ and as (days until shift from larval to adult shell geometry)⁻¹. Growth ceased abruptly in a majority of the larvae in each treatment, over the size range 900-1100 μm shell length. Larvae continued to ingest phytoplankton during this period, and growth resumed at a normal rate following spontaneous metamorphosis. An inverse correlation was observed between rates of larval growth and length of larval life through spontaneous metamorphosis; faster-growing larvae generally had shorter larval lives than did slower growing larvae. Individual growth rate ($\mu\text{m}/\text{day}$) prior to competence was significantly correlated with rate of individual differentiation. However, rates of differentiation and growth as measured in this study were comparable predictors of when spontaneous metamorphosis would occur. The results are consistent with the hypothesis of a pre-programmed end to larval life in the planktotrophic larvae of *C. fornicata*, although the factors responsible for initiation of gill development and the shift in shell morphology are apparently not directly related to progress towards the point at which the larva spontaneously metamorphoses to the benthos.

INTRODUCTION

Metamorphosis of many marine invertebrate larvae involves the loss of a specialized swimming organ or structure, and the consequent exchange of a free-living, planktonic life for a relatively sedentary, benthic one. Larvae of most marine benthic invertebrates are able to postpone this time of morphological and ecological transition in the absence of environmental cues characteristic of the appropriate adult habitat (Crisp, 1974; Scheltema, 1974). Species differ significantly in the length of time that metamorphosis can be delayed, and thus differ in maximum dispersal capability (Pechenik, 1980; Jackson and Strathmann, 1981). The length of time that metamorphosis can be delayed often appears to be related to rate of larval development (Pechenik, 1980, 1984; Jackson and Strathmann, 1981), even for species with feeding (planktotrophic) larvae. In particular, a significant inverse correlation between rate of growth and maximum duration of planktonic life has been documented for larvae of the prosobranch gastropod *Crepidula fornicata* (Pechenik, 1984). This relationship is observed for larvae within a single culture, and among cultures held at different temperatures.

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The relationship is also apparent for larvae of *C. fornicata* reared at different food concentrations at a single temperature (unpub. data). An end to larval life appears to be somehow developmentally pre-determined for these species; the duration of planktonic existence may thus be limited by the rate at which individuals progress towards this pre-determined end point (Chia, 1978; Pechenik, 1980, 1984). Similar findings have been reported for larval amphibians (Smith-Gill and Berven, 1979) and insects (Nijhout and Williams, 1974), although we do not yet know which, if any, of the physical and hormonal mechanisms responsible for terminating larval life in these organisms apply to the metamorphosis of marine invertebrates (Hadfield, 1978; Highnam, 1981; Pechenik, 1984).

Although the correlation between growth rate and maximum length of larval life was significant for larvae of *C. fornicata*, a great deal of scatter in the data was observed; less than 50% of the variation in the timing of spontaneous metamorphosis was explained (statistically) by variation in estimated individual growth rates (Pechenik, 1984). I suggested that the scatter may reflect a limited correspondence between rates of growth and rates of differentiation, as demonstrated for amphibian development by Smith-Gill and Berven (1979). I could not discuss the relationship between rates of growth and differentiation in *Crepidula* since the larvae were reared in batch cultures and the development of individuals could not be followed. There was also some uncertainty as to whether growth of larval *C. fornicata* was constant throughout the development or whether it declined sometime prior to spontaneous metamorphosis (Pechenik, 1984). Again, the question could not be resolved because larvae were reared in batch cultures, and individual growth could not be followed.

In this paper we report on the development of individually-reared larvae of *C. fornicata*. In particular, we examine the relationships between growth rates, rates of morphological differentiation, and length of planktonic life.

MATERIALS AND METHODS

Adult *Crepidula fornicata* were collected from Woods Hole and Nahant, Massachusetts, and maintained in the laboratory at 20–22°C on a mixed diet of *Dunaliella tertiolecta* and *Isochrysis galbana* or a Tahitian strain of *I. galbana* (T-Iso). All larvae used in this study were released on a single day (Day 0), but not necessarily from a single adult. Metamorphosis, signaling the irrevocable transition from a planktonic to a benthic habitat, was defined by loss of the ciliated velum. Individual larvae were maintained through spontaneous metamorphosis on a uni-algal diet of *I. galbana* or T-Iso at a cell density of approximately 18×10^4 cells per ml in sea water collected from Nahant, Massachusetts, and filtered to 0.45 μm . Larvae were reared individually in glass dishes containing 40–50 ml of algal suspension. Each day, water and food were changed and dishes were thoroughly cleaned with cleanser and acid. Larvae were held under constant light in incubators stable to 0.1°C (Percival Manufacturing).

Rates of growth and morphological differentiation were examined as a function of rearing temperature and diet in this study. Four experiments were conducted over a 14°C range of temperatures (15–29°C). In each experiment, 10–15 larvae were reared at each temperature. The shell length of each larva was measured at 1–2 day intervals at 63 \times using a dissecting microscope equipped with an ocular micrometer. Growth rates were determined by two different methods (Pechenik, 1984). Average individual growth rates were estimated from the amount of shell growth which occurred between release from the parent and metamorphosis. This was divided by the total length of larval life, providing an estimated average growth rate in $\mu\text{m}/\text{day}$. Average growth rates were also calculated by regressing shell length over time for the first

7–10 days of larval life for each larva. Data were analyzed by One-way Analysis of Variance followed by Fisher's test of Least Significant Difference for comparisons among pairs of means (Ott, 1977). Slopes of regression lines were analyzed according to Kleinbaum and Kupper (1978). Statistical analyses were completed with the aid of the Statistical Package for the Social Sciences (SPSS) (Nie *et al.*, 1975).

The development of two conspicuous morphological features were monitored in these experiments. The shell geometry of *C. fornicata* shifts from a spiral conformation to a more flattened, linear pattern of shell growth during larval life. In particular, a shelf ("brim") develops at the rear of the shell (Pechenik, 1980; Thiriot-Quievreux and Scheltema, 1982). The shell size and date at which this shell brim formed was recorded for each individual larva. The shell length and date at which gill rudiments became apparent were also recorded. These characteristics (brim development and formation of gill rudiments) appear to be the only major morphological alterations which can be monitored non-destructively in larvae of *C. fornicata*.

Most larvae in these experiments ceased growth for one to several days before the occurrence of spontaneous metamorphosis. Feeding rates were determined after growth stopped for three individuals at 20°C and three at 25°C, by determining the rate of disappearance of algal cells from a known volume of algal suspension; appropriate controls were included (Pechenik and Fisher, 1979; Pechenik, 1980). Individual larvae which had stopped growing for two days were placed in a 1.1 ml suspension of *I. galbana* at an initial cell density of 16.1×10^4 cells per ml for 6 h in dim light. Cells per ml were determined using a hemacytometer. Feeding rates were expressed as number of cells eaten per h per larva, for comparison with previous determinations (Pechenik, 1980). Data obtained from the few individuals that metamorphosed during the determination of feeding rate were excluded from analysis. The average shell length (\pm S.D.) of the larvae (at T_0) used in this experiment was $1032.4 \pm 25.2 \mu\text{m}$ ($n = 6$).

Biomass determinations were made on 10 individuals following their metamorphosis to establish the relationship between shell length and tissue weight. This relationship is known to be linear for larvae of this species (Pechenik, 1980, 1984). Individuals which had been reared at 24°C were maintained on a diet of *I. galbana* for three to seven days following spontaneous metamorphosis. The juveniles were then preserved in buffered formalin for later determinations of shell length and tissue weight. Dry tissue weights were determined by decalcifying the shells in 2% HCl, then rinsing the tissue free of salts with deionized water and transferring the tissue into pre-weighed aluminum pans (Pechenik, 1980). After 48 h of desiccation over indicating CaSO_4 (Drierite), dry tissue weight was determined using a Cahn Model 21 electrobalance in the presence of additional desiccant.

RESULTS

Survivorship and rates of development

Larval survivorship was high. Only one or two individuals died in most treatments. However, at 15°C, mortality was 70% on a diet of T-Iso, compared with 0% on a diet of the non-tropical strain. These results suggest that 15°C is very close to the lowest temperature tolerated by larvae of *Crepidula fornicata*; alternatively, there may be subtle nutritional differences between the two strains of *I. galbana* at different temperatures.

There was generally good agreement between the two methods of estimating rates of shell growth (Table I). Where major discrepancies exist (*e.g.*, 25°C, experiments II and IV), average growth rates as determined from sizes at metamorphosis were

TABLE I
Rates of growth and differentiation before and after spontaneous metamorphosis as a function of food and temperature

Diet	Expt. no.	Rearing temp °C	Growth rate (µm/day) from size at metamorphosis	Growth rate (µm/day) from linear regression	Days to gill	Days to brim	Days to metamorphosis	Growth rate µm/day after metamorphosis
<i>I. galbana</i>	I	18	40.1 ± 9.4 (n = 10)	37.6 (n = 8)	8.5 ± 1.9 (n = 13)	12.1 ± 1.9 (n = 13)	26.5 ± 5.0 (n = 10)	—
<i>I. galbana</i>	I	24	55.5 ± 9.1 (n = 10)	65.7 (n = 8)	5.5 ± 1.4 (n = 15)	6.6 ± 1.6 (n = 14)	17.6 ± 2.8 (n = 10)	—
<i>I. galbana</i>	II	15	27.9 ± 3.3 (n = 8)	33.3 (n = 5)	9.9 ± 1.3 (n = 11)	16.2 ± 1.3 (n = 12)	28.5 ± 2.4 (n = 10)	—
<i>I. galbana</i>	II	20	46.0 ± 13.5 (n = 10)	50.9 (n = 6)	6.4 ± 1.0 (n = 13)	8.2 ± 0.7 (n = 13)	20.5 ± 6.5 (n = 12)	52.5 ± 4.6 (n = 2)
<i>I. galbana</i>	II	25	63.5 ± 9.1 (n = 10)	83.2 (n = 5)	4.4 ± 0.9 (n = 13)	6.2 ± 0.4 (n = 13)	12.7 ± 4.8 (n = 11)	116.7 ± 31.8 (n = 9)
<i>I. galbana</i>	II	29	67.2 ± 28.2 (n = 12)	97.5 (n = 6)	3.5 ± 0.5 (n = 13)	5.5 ± 0.7 (n = 13)	12.3 ± 4.1 (n = 12)	89.1 ± 29.2 (n = 7)
T-Iso	III	15	—	15.5 (n = 5)	12.1 ± 1.4 (n = 2)	—	23.0 (n = 1)	—
T-Iso	III	20	40.6 ± 10.4 (n = 6)	52.3 (n = 6)	7.1 ± 1.3 (n = 9)	8.75 ± 0.5 (n = 8)	23.0 ± 5.7 (n = 6)	93.6 (n = 1)
T-Iso	III	25	57.5 ± 18.3 (n = 8)	63.4 (n = 6)	6.6 ± 1.1 (n = 10)	7.9 ± 0.9 (n = 10)	17.9 ± 5.1 (n = 8)	133.1 (n = 1)
T-Iso	IV	20	24.4 ± 4.8 (n = 3)	38.5 (n = 6)	9.2 ± 0.8 (n = 5)	12.0 ± 1.2 (n = 5)	34.7 ± 5.1 (n = 3)	—
T-Iso	IV	25	40.4 ± 11.1 (n = 8)	71.4 (n = 6)	5.0 ± 1.6 (n = 11)	7.3 ± 1.9 (n = 11)	19.9 ± 4.0 (n = 9)	128.8 ± 45.9 (n = 4)
T-Iso	IV	29	28.9 ± 14.2 (n = 8)	74.2 (n = 6)	4.3 ± 0.8 (n = 12)	8.3 ± 3.8 (n = 12)	21.1 ± 3.9 (n = 8)	—

* Indicates significant differences between means ($P < 0.05$, Sheffe's test).

always less than those determined directly from repeated measurements of shell length made on individual larvae (Table I). Note that the discrepancy observed at 25°C in experiment III was relatively small.

Individual growth was reasonably linear over time until larvae reached shell lengths of approximately 900–1100 μm ; about 75–100% of the larvae in each treatment then abruptly stopped growing for a number of days prior to spontaneous metamorphosis (Table II). Temperature had no obvious effect on the duration of this hiatus in shell growth. Feeding continued during the period of suspended growth. The average feeding rate (\pm one S.D.) of six larvae from experiment II (Table I) which had stopped growing for two days was $1.03 \times 10^4 \pm 0.50 \times 10^4$ cells of *I. galbana* eaten per larva per h. This is comparable to feeding rates determined previously for *C. fornicata* larvae of similar size ($\sim 1032 \mu\text{m}$), and is less than the average feeding rate for competent larvae of ~ 800 – $900 \mu\text{m}$ (Pechenik, 1980).

Following spontaneous metamorphosis, shell growth occurred for individuals in all treatments, except at 29°C in experiment IV. In this treatment, all post-metamorphic individuals died. A temperature of 29°C is near the lethal limit for larvae of *C. fornicata* (Lucas and Costlow, 1979). In all other treatments, post-metamorphic shell growth was a linear function of time, and growth resumed at rates equal to or exceeding rates of shell growth recorded for the larvae (Table I). The relationship between shell length (Y) and tissue biomass (X) for post-metamorphic individuals reared at 24°C was found to be linear: $Y = 0.043X - 36.28$, $r^2 = 0.76$. The implication is that for at least one week following spontaneous metamorphosis, constant growth resumes in terms of both shell length and biomass.

Increased temperature generally accelerated the rate of shell growth. The slopes of the lines relating larval shell length to days in culture were significantly different ($\alpha = 0.05$) from each other at both temperatures in experiment I and for the lowest three temperatures of experiment II; larvae were fed *I. galbana* in both experiments (Table I). In experiment III, rates of shell growth for larvae fed T-Iso were significantly different at 15 and 20°C, but were not significantly different at 20 and 25°C. In experiment IV, in which the diet was also T-Iso, shell growth rates were significantly different from each other at 20 and 25°C, but not at 25 and 29°C (Table I).

All conditions which altered rates of shell growth also altered time to formation of gill rudiments and shell brims, and maximum length of larval life ($P < 0.05$) (Table I). Conditions which did not alter growth rates did not alter length of planktonic life or time to brim and gill formation. For example, in experiment II (Table I), growth rates were essentially the same at 25 and 29°C. Length of larval life, time to appearance of shell brims, time to appearance of gill rudiments were also comparable for larvae reared at these two temperatures. In the same experiment, however, growth rates recorded at 15 and 20°C were significantly different from those recorded at the higher temperatures, and were significantly different from each other as well. These differences in growth rates were correlated with differences in time to spontaneous metamorphosis, and in time to formation of gill rudiments and shell brims (Fig. 1; Table I).

Effect of temperature on the relative rates of growth and differentiation

Over the range 18–25°C, temperature did not affect ($\alpha = 0.05$) the sizes at which gill rudiments or shell brims first became visible, or at which spontaneous metamorphosis occurred (Table III). However, mean shell length at first appearance of gill filaments was significantly reduced at 15°C in experiment II, and both shell length at first appearance of gill filaments and size at metamorphosis were significantly reduced at 29°C in experiment IV. Rates of growth and differentiation (at least with

TABLE II

Cessation of growth prior to spontaneous metamorphosis

Diet	Expt. no.	Temperature (°C)						
		15°	18°	20°	24°	25°	29°	
<i>I. galbana</i>	I	—	1.9 ± 1.9 (100%) n = 10	—	4.6 ± 3.0 (70%) n = 10	—	—	
<i>I. galbana</i>	II	4.4 ± 2.0 (80%) n = 8	—	4.0 ± 3.4 (83%) n = 10	—	1.4 ± 1.4 (73%) n = 10	2.6 ± 2.5 (75%) n = 12	
T.-Iso	III	3.0 (100%) n = 1	—	3.5 ± 2.4 (100%) n = 6	—	3.8 ± 2.8 (87%) n = 8	—	
T.-Iso	IV	—	—	6.0 ± 1.7 (100%) n = 3	—	7.1 ± 2.9 (100%) n = 9	8.9 ± 3.9 (100%) n = 8	

Data are average number of days (±S.D.) that growth stopped.

Percentage of larvae which ceased growth for ≥1 day is given in parentheses for each treatment.

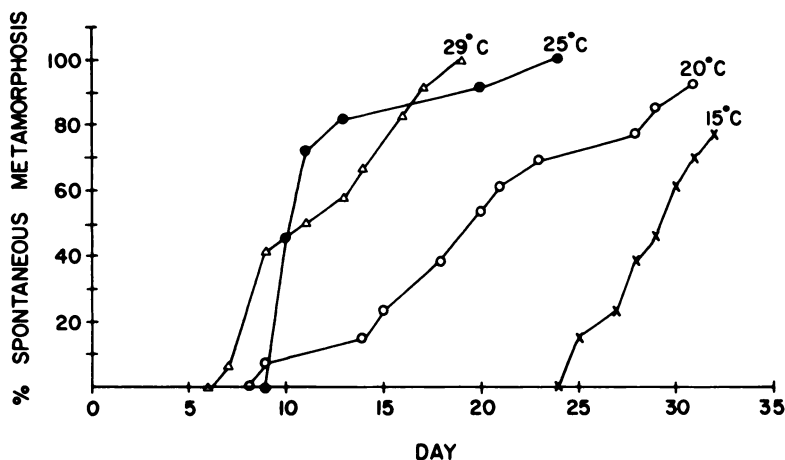


FIGURE 1. The influence of temperature on the number of days elapsed between larval release and spontaneous metamorphosis of *C. fornicata*.

respect to gill differentiation) thus appear to be differentially sensitive to major changes in temperature.

Relationship between growth rate, differentiation rate, and length of planktonic life

Average rates of morphological differentiation were expressed as (days to first appearance of gill filaments)⁻¹ and as (days to first appearance of a shell brim)⁻¹.

TABLE III

Influence of temperature on size at which morphological features develop and size of spontaneous metamorphosis

Diet	Expt.	Rearing temp. °C	Mean size at gill formation	Mean size at brim formation	Mean size at spontaneous metamorphosis
<i>I. galbana</i>	I	18	767.7 ± 48.0 (n = 13)	900.6 ± 70.3 (n = 13)	1475.4 ± 118.6 (n = 10)
<i>I. galbana</i>	I	24	784.6 ± 58.9 (n = 15)	881.6 ± 89.1 (n = 14)	1404.5 ± 57.1 (n = 10)
<i>I. galbana</i>	II	15	637.5 ± 30.5 (n = 11)	843.7 ± 71.2 (n = 12)	1167.9 ± 94.5 (n = 8)
		20	704.2 ± 23.3 (n = 13)	863.6 ± 62.7 (n = 13)	1237.0 ± 166.8 (n = 10)
		25	700.3 ± 33.4 (n = 13)	905.5 ± 68.2 (n = 13)	1095.2 ± 92.8 (n = 10)
		29	717.0 ± 40.5 (n = 13)	907.0 ± 73.5 (n = 13)	1111.2 ± 109.7 (n = 12)
T-Iso	III	15	747.1 ± 32.9 (n = 2)	—	977.4 (n = 1)
		20	779.5 ± 57.6 (n = 9)	897.0 ± 37.4 (n = 8)	1302.7 ± 100.2 (n = 6)
		25	817.3 ± 76.8 (n = 10)	961.4 ± 59.9 (n = 10)	1364.2 ± 71.5 (n = 8)
T-Iso	IV	20	812.4 ± 36.9 (n = 5)	916.8 ± 53.7 (n = 5)	1241.2 ± 46.3 (n = 3)
		25	793.4 ± 58.7 (n = 11)	877.8 ± 76.7 (n = 11)	1203.8 ± 249.1 (n = 8)
		29	712.2 ± 62.8 (n = 12)	853.9 ± 79.8 (n = 12)	986.1 ± 172.0 (n = 8)

Data are means (µm) ± one S.D.

* Indicates significant differences between means (P < 0.05).

Pooling the 120 data points for all temperatures indicates that both measures of differentiation rate are positively correlated with growth rate (Figs. 2a, b). Approximately 75% of the variation in growth rate was explained (statistically) by variation in differentiation rate in both cases.

Pooling the data for the 102 larvae which survived to spontaneous metamorphosis reveals an inverse correlation between the day on which spontaneous metamorphosis occurred (Y) and the rate of growth (X) as determined by periodic measurements of shell length (Fig. 3). The relationship is approximately linear ($Y = 34.83 - 0.23X$, $r = -0.72$) and the slope of the relationship is significantly different from zero ($P < 0.05$). Approximately 50% of the variation in the timing of metamorphosis was explained by variation in shell growth rate. Treating day of spontaneous metamorphosis as a function \ln growth rate does not improve the strength of the relationship: $Y = 71.58 - 12.55X$, $r^2 = 0.47$.

The day of spontaneous metamorphosis is inversely related to the rate of morphological differentiation (Figs. 4a, b). If the relationship is treated as linear, ap-

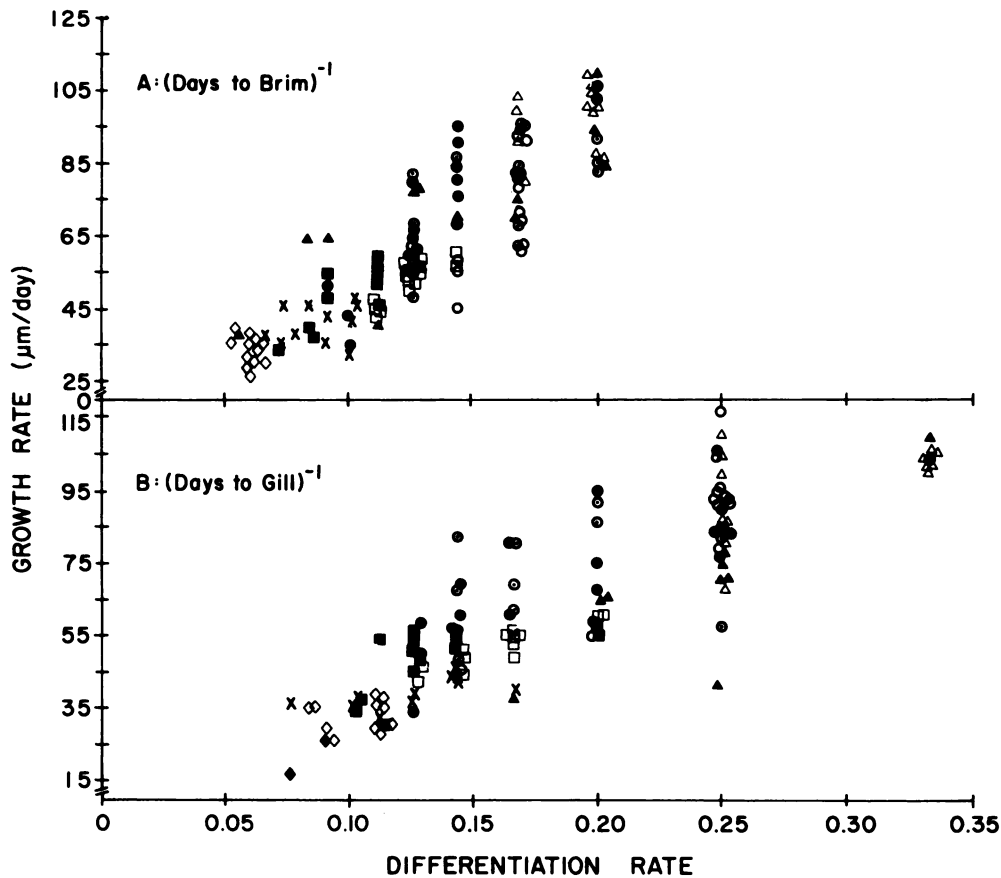


FIGURE 2. Relationship between rates of growth ($\mu\text{m}/\text{day}$) and rates of morphological differentiation (days^{-1}) for larvae of *C. fornicata*. Differentiation rates were measured as (days required to develop a brimmed shell)⁻¹ (A) and as (days required to develop conspicuous gill rudiments)⁻¹ (B). Legend: Iso diet— \diamond = 15°C, \times = 18°C, \square = 20°C, \circ = 24°C, \bigcirc = 25°C, Δ = 29°C. T-Iso diet— \blacklozenge = 15°C, \blacksquare = 20°C, \bullet = 25°C, \blacktriangle = 29°C.

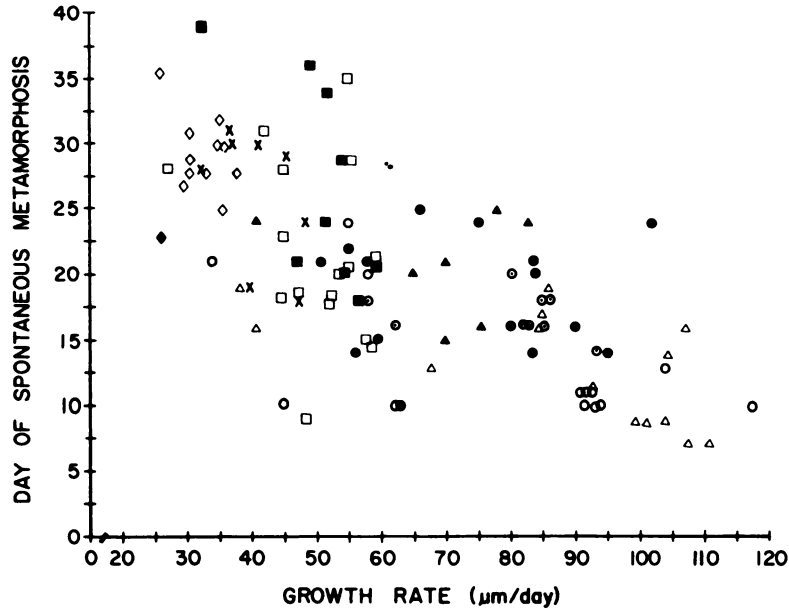


FIGURE 3. Relationship between growth rate and duration of planktonic life for larvae of *C. fornicata*. Symbols are defined in Figure 2.

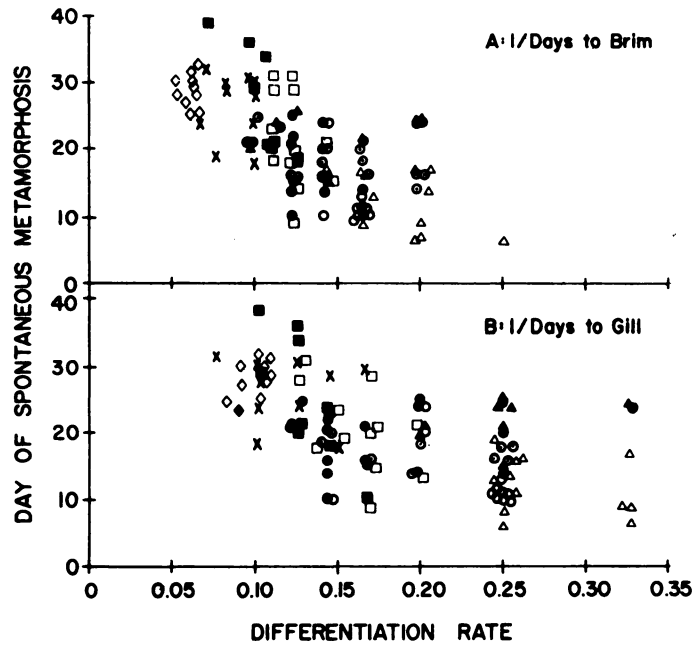


FIGURE 4. Relationship between rates (day^{-1}) of morphological differentiation and duration of larval life in *C. fornicata*. Symbols are as defined in Figure 2.

proximately 46% and 37% of the variation in when metamorphosis occurs is explained by variation in the timing of brim formation and gill formation, respectively. Considering the relationship to be logarithmic, approximately 48% and 42% of the variation in the timing of metamorphosis is explained by variation in differentiation rate.

DISCUSSION

Larvae of most marine invertebrates studied to date have the capacity to prolong the planktonic stage beyond the point at which metamorphosis first becomes possible (Crisp, 1974; Scheltema, 1974). Although most studies on the induction and delay of metamorphosis have been conducted in the laboratory, there is evidence that the larvae of several mollusc species also delay metamorphosis in the field (Bayne, 1965; Scheltema, 1971; Pechenik, 1978). The ecological significance of this ability has often been pointed out (*e.g.*, Scheltema, 1961; Bayne, 1965; Crisp, 1974; Doyle, 1975; Obrebski, 1979; Jackson and Strathmann, 1981). However, few studies have been concerned specifically with defining ability to delay metamorphosis for any particular species, or determining the factors which affect delay capability.

Bayne (1965), working with larvae of the blue mussel, *Mytilus edulis*, was the first to study the influence of environmental factors on delay potential, and was the first to consider a relationship between rate of growth and duration of the delay period. He found that both salinity and temperature had a pronounced effect on the length of time that metamorphosis could be delayed by mussel larvae. However, no correlation between rate of growth to the pediveliger stage and length of time that metamorphosis of mussel larvae could be delayed was detected (Bayne, 1965). This is in sharp contrast to results reported here and elsewhere (Pechenik, 1984; Lima and Pechenik, in review) for larvae of *C. fornicata* and *C. plana*, in which slower growth is significantly correlated with a greater capacity for delayed metamorphosis. Absence of such a relationship in the larvae of *M. edulis* is most likely explained by the fact that these larvae exhibit degeneration of the velum and ciliary feeding tracts as metamorphosis is delayed; larvae eventually become unable to feed at all (Bayne, 1965). Delaying larvae of *M. edulis* are thus forced to obtain all nutrients from internal energy stores, and the length of time that metamorphosis can be delayed is limited by the size of these energy stores and the rate at which these reserves are depleted. Environmental factors such as increased temperature and altered salinity presumably increase the rate at which energy stores of delaying mussel larvae are depleted and foreshorten the delay period accordingly. At 10–11°C, for example, a temperature range at which metabolic rates are likely to be low, mussel larvae could postpone metamorphosis for 43–46 days. The delay period was reduced to only two days at 21–22°C, conditions apt to produce much higher metabolic rates (Vernberg, 1972).

In contrast to larvae of *M. edulis*, larvae of *C. fornicata* (in batch culture) continue to feed, and show no sign of morphological degeneration or energy imbalance after becoming competent to metamorphose in batch culture (Pechenik, 1980). The present paper indicates that even when growth of individuals ceases prior to spontaneous metamorphosis, feeding rates remain high. However, more detailed measurements of feeding rates, respiration rates, and assimilation efficiencies will have to be made for larvae which have stopped growing, to determine whether the cessation of growth in larvae of *C. fornicata* has a nutritive/energetic basis. The abruptness with which growth ceased suggests that dietary insufficiency was not the cause. Growth resumed following spontaneous metamorphosis of all individuals under most temperature conditions, again arguing against a pathological basis for cessation of growth by *C. fornicata* larvae. That growth ceases prior to spontaneous metamorphosis was

anticipated from results of experiments with batch cultures of larvae (Pechenik, 1984), but the abruptness of the transition was not.

Some opisthobranch gastropod larvae also cease growth during development, generally prior to the onset of competence (Kriegstein, 1977; Switzer-Dunlap and Hadfield, 1977; Kempf, 1981). This state of no-growth can then be maintained for many weeks, or even months, and is presumed to play a role in prolonging larval life in gastropods (Scheltema, 1966; Kempf, 1981). It should be pointed out, however, that growth of *C. fornicata* did not always stop for all individuals within a treatment (Table II), and that even when growth did cease, spontaneous metamorphosis generally occurred within a few days. The significance of the cessation of larval shell growth in *C. fornicata* thus remains unclear. Present indications for larvae reared in batch culture are that the congener *C. plana* does not cease growth prior to spontaneous metamorphosis. That is, for larvae of *C. plana*, growth rates estimated from size at metamorphosis were comparable to growth rates determined by direct measurements made earlier in development (Lima and Pechenik, in review).

The present study confirms the inverse correlation between growth rate and duration of planktonic life reported previously for larvae of *C. fornicata* reared in batch culture, and supports the hypothesis that length of larval life in this species is limited by the rate of development towards a pre-determined end point (Pechenik, 1984). The same must also be true of the larvae of *Mytilus edulis*; the immediate cause of the end to larval life in that species may well be nutritional limitation (Bayne, 1965), but the loss of feeding ability itself would seem to be the result of a developmentally programmed degeneration of larval structure.

The ability to predict when spontaneous metamorphosis will occur in *C. fornicata* has not been improved by the results of this study. Although growth rates are seen to be well-correlated with rate of progress towards gill development and shell brimming, neither measure of differentiation was better correlated with length of larval life than was growth rate. This is in contrast to results reported for amphibian larvae by Smith-Gill and Berven (1979). As with *Crepidula* larvae, growth rate was found to be a statistically significant, but mediocre predictor of the onset of amphibian metamorphosis. Prediction capabilities for larval amphibians improved dramatically when rate of morphological development was monitored instead of growth rate. The tight correlation between rate of morphological development and date of metamorphosis obtained in their study is explained by the fact that the morphological changes measured are controlled by the same hormonal mechanism responsible for metamorphosis (Smith-Gill and Berven, 1979). Most likely, the factors responsible for brim formation or gill proliferation in delaying larvae of *C. fornicata* have little to do with progress towards the point at which the velum is lost. Additional interpretation must await clarification of the internal mechanisms involved in terminating larval life in marine invertebrates.

Kempf's (1981) study also focused on the duration of delayed metamorphosis for a gastropod species with feeding larvae. In his experiments with larvae of the opisthobranch *Aplysia juliana*, a few individuals survived as long as 316 days after release from the egg mass, and showed no sign of morphological degeneration or loss of ability to metamorphose in response to inducer. However, most of the larvae died during the experiment; mortality was approximately 50% during the first eight weeks of laboratory culture. As the author suggests, mortality may have resulted from inadequate culture conditions, perhaps a dietary insufficiency. Relative incidence of mortality and spontaneous metamorphosis in molluscan larvae may also be affected by temperature regime (Pechenik, 1984a). However, the possibility that the mortality of delaying opisthobranch larvae is somehow developmentally programmed, *i.e.*, that

there is a larval endpoint, cannot be ruled out. Since mollusc larvae develop at a variety of different rates within a single laboratory culture (*e.g.*, Bayne, 1965; Hickman and Gruffydd, 1971; Lucas and Costlow, 1979; Pechenik, 1984), the logarithmic mortality curve reported by Kempf (1981) may in part reflect such differences in rates of development towards the hypothesized larval end point. Possibly the few larvae which survived the 316 days are those which developed the most slowly throughout the study; because larvae were reared in batch culture, individual rates of development could not be followed. Even had the larvae been reared individually, rates of differentiation are likely to be better predictors of delay periods than rates of growth (Pechenik, 1984), and these are not easily measured. Moreover, as in the present study, actual delay periods cannot be known with certainty because the time at which an individual larva first becomes competent to metamorphose can be determined only by inducing metamorphosis (Pechenik, 1984). Our ability to further explore the proximal factors controlling delay of metamorphosis by gastropod larvae is thus hindered by the difficulty of ascertaining individual competence and monitoring rates of individual differentiation non-destructively. The ideal species for future study will be one demonstrating a pronounced morphological or behavioral correlate of competence, and a series of discrete morphological or behavioral changes which are under the influence of the same internal factors responsible for the termination of larval life.

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