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The influence of food concentration and temperature on growth and morphological differentiation of blue mussel *Mytilus edulis* L. larvae

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Abstract: The relationship between growth rate, rate of morphological development, and length of larval life, was examined for larvae of the blue mussel *Mytilus edulis* L. The larvae were reared at phytoplankton (*Isochrysis galbana*, clone T-ISO) concentrations between 0.5×10^4 and 30×10^4 cells \cdot ml $^{-1}$ at either 12 or 16 °C. Growth rates generally increased with increasing food concentration and were highest at the higher temperature; maximum shell growth rate was $\approx 8 \mu\text{m} \cdot \text{day}^{-1}$. The number of days required for larvae to develop recognizable eyespots sometimes varied proportionately with changes in growth rate, but often did not; temperature was especially effective at uncoupling rate of growth from rate of morphological development. Maintained in clean glass dishes at 16 °C, many eyespotted larvae survived until the end of the study, as long as 8 wk after they first developed eyespots (≈ 80 days after fertilization). Nearly 30% of those larvae that survived under these conditions eventually metamorphosed in the absence of filamentous substratum, suggesting that metamorphosis can be postponed at least 45 days at 16 °C. Food concentration had no effect on (1) the time elapsed between eyespot development and noninduced attachment; (2) the average shell length of individuals that did attach; or (3) the mean size of larvae remaining at the end of the study.

Key words: Development; Larva; Metamorphosis; Mussel; *Mytilus edulis*

INTRODUCTION

Partly because of a strong recent focus on the ecological implications of energetics on molluscan development (Pechenik, 1980; Jespersen & Olsen, 1982; Bayne, 1983; Sprung, 1984a,b; Mann & Gallager, 1985; Whyte et al., 1987; MacDonald, 1988) and partly because mollusc larvae lack discrete developmental stages, biologists have typically examined the effects of temperature, food concentration, and other environmental factors on the larval development of marine molluscs only with respect to survival and growth, with growth being measured by changes in shell size or ash-free dry weight (reviewed by Pechenik, 1987). The effects of environmental factors on rates of larval differentiation remain largely unexplored for marine molluscs, although there is good reason to suspect that rates of growth and differentiation may be affected to

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different degrees by any given environmental perturbation. Anatomical and physiological differentiation clearly can proceed in the absence of growth. In the European oyster *Ostrea edulis*, for example, larvae deprived of particulate food continue to develop morphologically for some time after they cease growing (Holland & Spencer, 1973). Similarly, in at least some opisthobranch larvae, substantial anatomical changes, and the development of competence for metamorphosis, take place many days after shell growth ceases (Switzer-Dunlap & Hadfield, 1977; Kempf, 1981; Paige, 1988). As further evidence for considerable independence between the processes of growth and differentiation during larval development, larvae of the gastropod *Crepidula fornicata* developed gill filaments at a smaller mean shell length when reared at a lower temperature (Pechenik & Lima, 1984). If the time required to develop gill filaments had increased in the same proportion as rate of shell growth had decreased, gills should have developed later, but at the same shell size; rates of shell growth were apparently decreased more by the low temperature than were rates of gill differentiation, so that larvae developed gills at a smaller average size. Similarly, larvae of *C. fornicata* reared at higher temperatures grew faster and metamorphosed at a smaller average size (Pechenik, 1984), again implying a differential effect of temperature on rates of growth and differentiation. The extent to which rates of larval growth and differentiation are coupled has not been specifically studied in molluscs.

Larvae of the marine bivalve *Mytilus edulis* L. have been reared routinely for > 40 yr. One discrete developmental characteristic that may be used to monitor the morphological differentiation of mussel larvae is the larval eyespot (Bayne, 1971), which develops shortly before the larvae first become competent to metamorphose (Bayne, 1964b, 1976). In this paper, we consider the influences of food concentration and temperature on rates of larval growth and time taken to develop the larval eyespot. We also document the relationship between food concentration and length of time that larvae can persist following eyespot development.

Past studies concerning effects of food concentration on larval growth rate of *M. edulis* have produced conflicting results: Sprung (1984) reports maximum growth rate at 1×10^4 cells \cdot ml $^{-1}$, Jespersen & Olsen (1982) report maximum growth at $3\text{--}5 \times 10^4$ cells \cdot ml $^{-1}$, and Bayne (1965) found that growth rates increased with every increase in food concentration to the highest concentration he tested, 10×10^4 cells \cdot ml $^{-1}$. All three workers used the alga *Isochrysis galbana* as a food source (mixed with *Monochrysis lutheri* in the work of Jespersen & Olsen). We used the same alga in our study, rearing larvae at food concentrations ranging from 0.5×10^4 to 30×10^4 cells \cdot ml $^{-1}$.

MATERIALS AND METHODS

Adult *M. edulis* were collected in March and May 1986 from Whitsand Bay, southeast Cornwall, U.K. The mussels were induced to spawn in the laboratory by rapidly elevating seawater temperature from ≈ 16 to ≈ 28 °C for ≈ 10 min and then placing the

animals individually in dishes containing seawater at 16 °C. As individuals spawned, we pipetted gametes to freshly filtered seawater.

≈ 1000 sperm · ml⁻¹ from two or more males and 15–30 eggs · ml⁻¹ from two or more females were gently mixed and then left undisturbed. After 4–5 h, the supernatant was poured off and the embryos were gently resuspended in freshly aerated seawater filtered to 0.45 μm. The following day, swimming larvae were decanted from the top of each beaker and reared at a density of 10–15 larvae · ml⁻¹ in 2-l glass beakers half-filled with 0.45-μm Millipore-filtered seawater. We aerated the seawater before adding larvae, but not between water changes. Every other day, larvae were filtered out and moved to clean glass 2-l beakers with freshly filtered and aerated seawater. These cultures are referred to below as batch cultures.

Larvae were fed the unicellular flagellate *Isochrysis galbana* Parke (supplied by the Culture Centre for Algae and Protozoa, UK) at concentrations of ≈ 0.5, 1, 3, 9, 15, or 30 × 10⁴ cells · ml⁻¹. Larvae from Fertilizations I and II were fed algae cultured in artificial seawater (Tropic Marin, UK); algae were cultured in natural seawater for Fertilization III experiments. Algal concentrations were routinely determined using a model D electronic particle counter (Coulter Electronics) and were periodically verified using a hemacytometer. Larvae were removed to clean glassware and freshly mixed algal suspension every other day for up to 8 wk after fertilization.

Concurrent measurements of growth and differentiation were obtained twice using larvae from two separate spawnings; spawning dates were 15 April (Fertilization II) and 14 May (Fertilization III). Growth rates at several food concentrations were also obtained for an earlier spawning (Fertilization I, 27 March), but larvae became moribund and the experiment was terminated before differentiation rates could be adequately determined. All experiments were conducted at either 12 or 16 °C with a photoperiod of ≈ 12 L:12 D. Larvae cultured at 12 °C were fertilized at 16 °C and maintained for 2 days at this temperature before transfer to 12 °C.

In one further experiment larvae were reared at 16 °C at two different concentrations (15 and 50 · ml⁻¹) to compare the effect of larval concentration on rates of growth and morphological differentiation. Some larvae from Fertilization II were cultured at each density at 16 °C and 3 × 10⁴ algal cells · ml⁻¹ for ≈ 6 wk postfertilization and were examined every few days until 20% of larvae in each culture had eyespots.

Growth rates of larvae reared under the various conditions were determined by removing ≈ 30 (range 10–50) larvae at random and measuring all larvae removed. Shell lengths and widths of larvae taken from each culture were measured about every four days (range 2–9 days) at 100 ×, using a compound microscope equipped with a drawing attachment; images were digitized using a MOP-AM03 system and a Kontron digitizing tablet. To determine whether shell length alone was an adequate measure of size for larvae reared under various conditions, we measured both the lengths and widths of > 1600 larvae from Fertilization III reared at six different food concentrations and two temperatures and compared the length/width ratios for individuals reared under the different food and temperature regimes.

To monitor rate of morphological differentiation in the large 2-l beakers, some larval cultures were examined at each water change immediately after concentrating the larvae in the filter and before larvae resumed swimming. 50 randomly selected larvae from each culture were quickly examined at $50\times$, using a dissecting microscope, and the number with a detectable eyespot was recorded. This information was used to determine the time required (D) for larvae in each batch culture to develop eyespots. Differentiation rate was then approximated as $(D)^{-1}$ as in previous studies on amphibian development (Smith-Gill & Berven, 1979).

An additional experiment was conducted to examine (1) the influence of food concentration on the size at which larval eyespots formed, and (2) the influence of food concentration on the duration of larval life following eyespot formation. We also obtained additional data from Fertilization II animals on the rate at which larvae developed eyespots in the various treatments. Larvae were batch cultured at 16°C and $1, 3, 9, \text{ or } 15 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$ for 3 wk. Our preliminary studies and those by Lucas et al. (1986) had shown that under these conditions eyespots first become detectable at $\approx 3\text{--}3.5$ wk postfertilization. 72–90 larvae from each food concentration were then distributed among six small glass dishes (150 ml vol.) each containing 45 ml of algal suspension for each of the four food concentrations tested: 1, 3, 9, and $15 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$. The larvae in these small dishes were thus maintained at $\approx 1 \text{ ind} \cdot 3 \text{ ml}^{-1}$. Food and water were changed every other day, and dishes were cleaned thoroughly at each water change. Each day for 81 days, all larvae in all six of the dishes maintained at each food concentration were examined at $50\times$ for the presence of eyespots. Larvae from three of the dishes maintained at each food concentration were killed and measured as soon as they developed visible eyespots. Newly eyed larvae in the other three dishes from each food concentration were removed to a separate container (45 ml algal suspension, with $1\text{--}15 \text{ larvae} \cdot \text{container}^{-1}$ – usually 2–8 – depending on how many larvae developed eyespots at a given food concentration on a given day) for further rearing at the same food concentration. We then monitored larval mortality daily, changing food and water every other day. The dishes were thoroughly cleaned at each water change to discourage attachment and metamorphosis, which is normally triggered by filamentous algae (Bayne, 1964a; Eyster & Pechenik, 1987). During this time, some of these eyed larvae secreted byssal threads and initiated metamorphosis (Bayne, 1965; Eyster & Pechenik, 1987); these individuals were removed from culture and some of them were measured. The experiment was terminated ≈ 53 days after larvae developed eyespots and the shell lengths of all unmetamorphosed larvae were then determined. The effects of food concentration and temperature on the growth and differentiation of larvae were analysed by linear regression and ANOVA, following the inverse transformation of shell length and width to normalize the variance.

RESULTS

For individuals of $< 300 \mu\text{m}$ in shell length, the ratio between larval shell length and shell width was not significantly affected by food concentration or temperature (larvae aged 2–54 days, $N = 1616$ larvae measured from Fertilization III, $P > 0.05$), so that change in shell length will serve as an adequate index of growth under all rearing conditions tested. Similarly, temperature has no apparent effect on the ratio of shell length to shell width in larvae of the hardshell clam *Mercenaria mercenaria* (Loosanoff, 1959). For larvae of *M. edulis*, the relationship between shell length (Y , μm) and shell width (X , μm) was: $Y = 34.95 + 0.998X$ ($r = 0.987$, $F = 61,558$, $df = 1,614$) (Fig. 1).

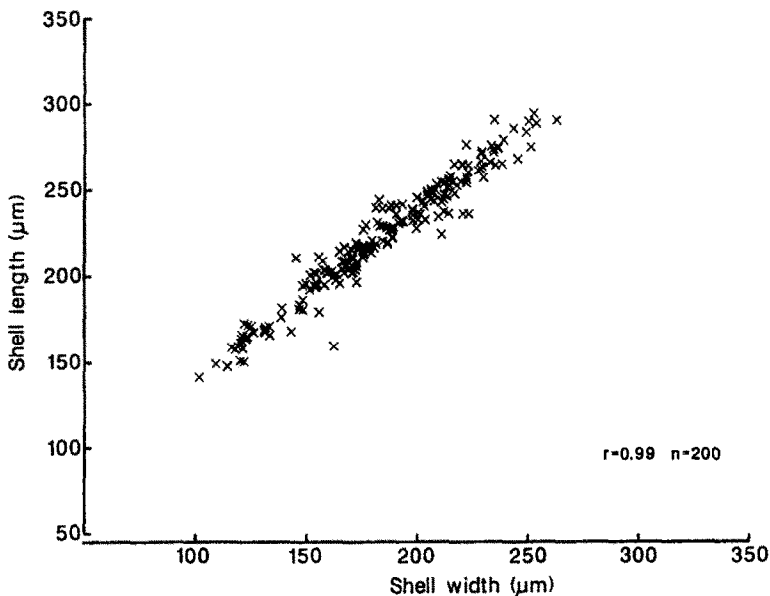


Fig. 1. Representative relationship between shell length and width for *M. edulis* larvae. These 200 individuals were cultured at 15×10^4 cells \cdot ml $^{-1}$ at 16°C ; $r = 0.99$. Comparable data were obtained at other food concentrations and at 12°C .

For larvae reared in the laboratory at 16°C on six distinct concentrations of algae, mean growth rates (measured as changes in shell length) ranged from 1 to $8.1 \mu\text{m} \cdot \text{day}^{-1}$ (Table I). The effect of food concentration on larval growth rate varied among the three fertilizations; regression and covariance analysis was used to test for significant differences in growth rate (slope = $\mu\text{m shell length} \cdot \text{day}^{-1}$). Specifically, larvae from Fertilizations I and II grew at equivalent rates at 9×10^4 and 15×10^4 cells \cdot ml $^{-1}$ ($P > 0.05$, Table I, Fig. 2); however, in Fertilization III, growth rates were significantly higher at 15×10^4 than at 9×10^4 cells \cdot ml $^{-1}$ ($P < 0.05$, Table I, Fig. 3).

In addition to comparing overall growth rates, we compared larval shell sizes on

TABLE I

Mean growth rates of *M. edulis* larvae provided different concentrations of *I. galbana*. Growth rates were determined from slopes after regressing shell length on days in culture. Number of larvae measured ranged from 10 to 50 ($\bar{x} = 30$). Larvae were reared at 16 °C unless otherwise indicated.

Algal concentration (cells · ml ⁻¹)	Growth rate (μm · day ⁻¹ ± 95% CI)		
	Fertilization I	Fertilization II	Fertilization III
0.5 × 10 ⁴	—	—	1.0 ± 0.10
1 × 10 ⁴	—	2.7 ± 1.05	1.3 ± 0.11
3 × 10 ⁴	2.9 ± 0.3	3.1 ± 0.39	—
9 × 10 ⁴ (12 °C)	—	—	5.0 ± 0.49
9 × 10 ⁴	5.2 ± 0.4	5.5 ± 0.58	4.1 ± 0.35
15 × 10 ⁴	5.5 ± 0.4	4.7 ± 1.07	6.6 ± 0.72
30 × 10 ⁴	—	—	8.1 ± 0.79

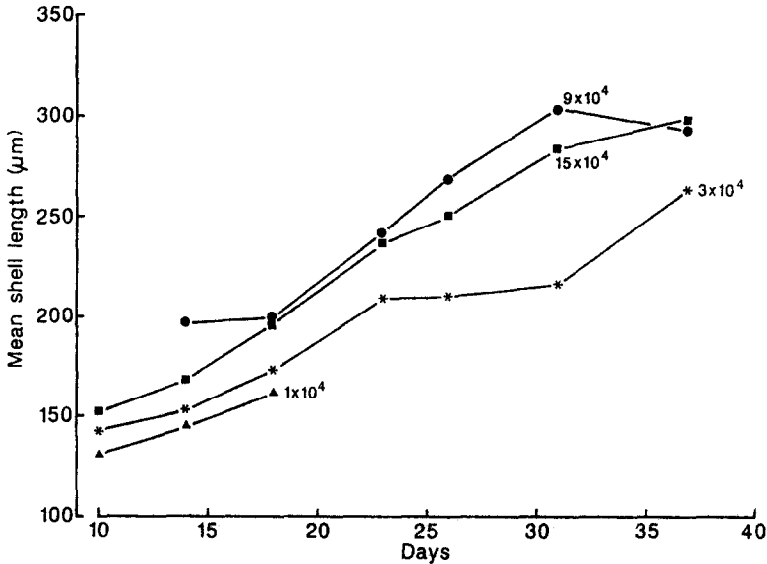


Fig. 2. Growth of *M. edulis* larvae at different food concentrations (*I. galbana*) from Fertilization II. All larvae were cultured at 16 °C. Each point represents the average of ≈ 30 measurements made at 100 × magnification. SD values were generally between 12 and 25.

specific days. For example, we compared shell lengths of Fertilization III larvae about every 4 days between Days 8 and 54 after fertilization (Fig. 3); significant effects of food ration on shell size were apparent by Day 8 (one-way ANOVA) ($P < 0.001$). At this time, larvae fed 0.5×10^4 cells · ml⁻¹ were significantly smaller than those reared at other food concentrations, and larvae fed 15×10^4 and 30×10^4 cells · ml⁻¹ were significantly larger. After Day 24, larvae fed 1×10^4 cells · ml⁻¹ were also consistently

larger than those fed 0.5×10^4 cells \cdot ml $^{-1}$. Although overall growth rates (Days 8–20) of larvae fed 15×10^4 and 30×10^4 cells \cdot ml $^{-1}$ were not significantly different from each other, the final average shell length (Day 24) of larvae fed at 30×10^4 cells \cdot ml $^{-1}$ was significantly larger ($P < 0.05$, ANOVA).

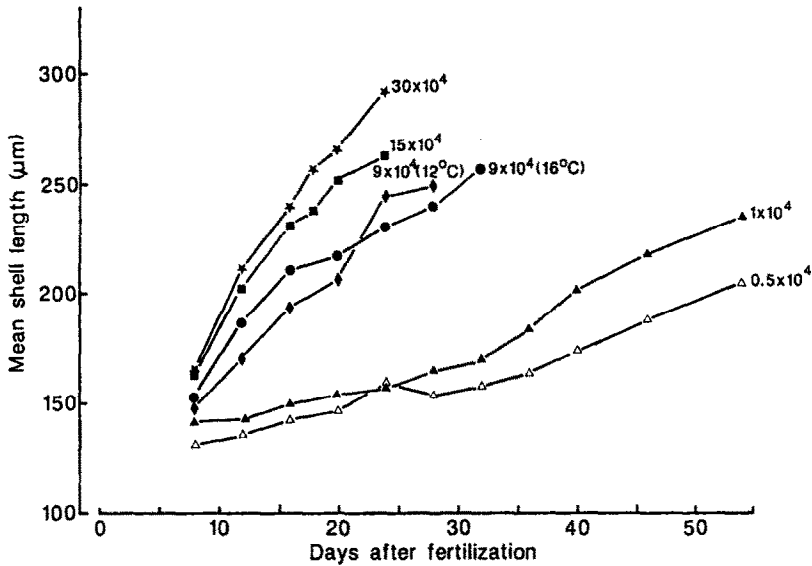


Fig. 3. Growth of *M. edulis* larvae from Fertilization III at different food concentrations (*I. galbana*). At 9×10^4 cells \cdot ml $^{-1}$, larvae were reared at 12 and 16 °C. All other cultures were maintained at 16 °C. Each data point represents the average of ≈ 30 measurements made at $100\times$ magnification. SD values rarely exceeded 25.

Larvae usually developed a single left and single right eyespot near the base of developing gill filaments as described by Bayne (1965, 1971). Preliminary study showed that formation of the left and right eyespots usually occurred synchronously so that we

TABLE II

Influence of container size and larval density on the rate at which *M. edulis* larvae developed eyespots at different food concentrations (Fertilization II). Cultures were established at 12–15 larvae \cdot ml $^{-1}$ in large cultures (1000 ml) and < 1 larva \cdot ml $^{-1}$ in small cultures (45 ml).

Food level (cells \cdot ml $^{-1}$)	Number of days for 50% of larvae to develop eyespots	
	Large cultures	Small cultures
1×10^4	—	35
3×10^4	32.5	29
9×10^4	25	24
15×10^4	24	24

only had to examine one side of each larva to determine if larval eyespots were present. Occasionally we observed eyespot formation on only one side, which could indicate either asynchronous eyespot formation or failure of the eyespot to develop on one side. On rare occasions we also observed larvae with two well-developed eyespots on one side and zero to two eyespots on the other side. Larvae apparently developed eyespots at comparable rates in the large (1-l) and small (45-ml) cultures, at least when reared at the higher food concentrations (Table II).

The effect of food concentration on rates of growth and eyespot development varied with food concentration, temperature, and experiment. Fertilization II larvae cultured at 9×10^4 and 15×10^4 cells \cdot ml $^{-1}$ grew at comparable rates (Table I, Fig. 2), and formed eyespots at comparable ages (Table III, Fig. 4, $P > 0.10$) and at comparable

TABLE III

Size and age at initial eyespot formation for *M. edulis* larvae cultured in small glass dishes at 16 °C (except where noted otherwise). Values are $\bar{x} \pm$ SD; NT, not tested.

Food level (cells \cdot ml $^{-1}$)	Fertilization II			Fertilization III		
	Length (μ m)	Age (days)	<i>N</i>	Length (μ m)	Age (days)	<i>N</i>
1×10^4	255.9 ± 14.1	36.0 ± 5.3	22	NT	NT	—
3×10^4	270.7 ± 17.4	29.1 ± 3.6	32	253.7 ± 13.4	33.8 ± 3.1	20
9×10^4 (12 °C)	NT	NT	—	284.6 ± 18.8	29.9 ± 3.4	29
9×10^4	262.5 ± 12.6	24.7 ± 1.7	32	253.3 ± 13.8	24.3 ± 2.4	30
15×10^4	266.6 ± 15.2	24.1 ± 1.2	39	NT	NT	—

shell lengths (Table III). Larvae grew and differentiated more slowly at 3×10^4 and at 1×10^4 cells \cdot ml $^{-1}$ (Fig. 2, Fig. 4). Despite the substantially lower mean growth rate at 3×10^4 cells \cdot ml $^{-1}$, larvae reared in small dishes at this food concentration formed eyespots at the same average shell size as did larvae reared at the higher food concentrations, even though they took longer to do so (Table III). In this second fertilization, although growth rates for larvae cultured at 1×10^4 and 3×10^4 cells \cdot ml $^{-1}$ were statistically equivalent (for at least the first 18 days of development) (Table I, $P > 0.10$), larvae cultured at the lowest food concentration took much longer to develop eyespots, and did so at a significantly smaller mean shell length (Table III, $P < 0.05$); in these larvae, rate of eyespot differentiation was apparently not suppressed by the low food concentration to the same degree as rate of shell growth (see Discussion).

Similarly disproportionate effects of food concentration on rates of growth and morphological differentiation were seen at some food concentrations for Fertilization III larvae. Although larvae at the two highest food concentrations grew at statistically comparable rates until Day 20 (Fig. 3), the larvae developed eyespots substantially faster when reared at the higher food concentration (30×10^4 cells \cdot ml $^{-1}$) (Fig. 5, $P < 0.05$). Although we have no direct measurements of size at eyespot formation at

these two highest food concentrations (30×10^4 and 15×10^4 cells \cdot ml $^{-1}$), estimates derived from Figs. 3 and 5 indicate that 50% of larvae developed eyespots at shell lengths of ≈ 270 and $260 \mu\text{m}$, respectively. For larvae reared in small dishes at 16°C , the individuals reared at 3×10^4 and 9×10^4 cells \cdot ml $^{-1}$ developed eyespots at $\approx 254 \mu\text{m}$ (one-way ANOVA, $P > 0.05$) despite dramatic differences in time required to develop eyespots at these two food concentrations (Table III, 34 vs. 24 days).

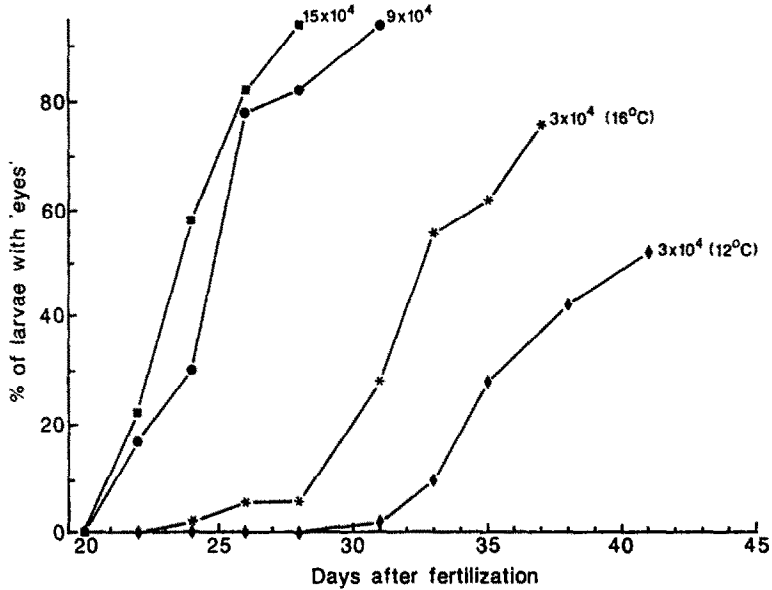


Fig. 4. Influence of food and temperature on rate of eyespot formation for *M. edulis* larvae reared in 1-l cultures (Fertilization II). Individuals from same the cultures were used to determine larval growth rates (Fig. 2). In one treatment, eggs were fertilized at 16°C and then transferred to 12°C 2 days later to monitor rate of eyespot formation at 12°C ; this culture was subsampled for shell measurements twice, 38 and 41 days after fertilization. All other larvae were cultured at 16°C . Each point represents observations made at $50 \times$ magnification on exactly 50 larvae.

Temperature had dramatically different effects on rates of growth and rates of eyespot development (Fertilization III). Larvae reared at 9×10^4 cells \cdot ml $^{-1}$ had equivalent average growth rates at 12 and 16°C (Table I, Fig. 3). But the larvae reared at 12°C did not develop eyespots until ≈ 5 days after those reared at 16°C (Table II) and were on average $32 \mu\text{m}$ larger when they finally did so (Table III) ($P < 0.001$, ANOVA followed by Scheffé's test of least significant differences; $F = 34.0$, $df = 2,76$). The reduced temperature apparently decreased rate of eyespot development without affecting rate of shell growth, as discussed below.

Eyespot-bearing larvae from Fertilization II were maintained at each food concentration to monitor their fates – remained swimming as larvae; attached and became

juveniles; or died – in the absence of filamentous substratum. A substantial number (40–70% at the different food concentrations) of these larvae were still alive and actively swimming 7–8 wk after they formed eyespots, up to 81 days after the eggs were fertilized

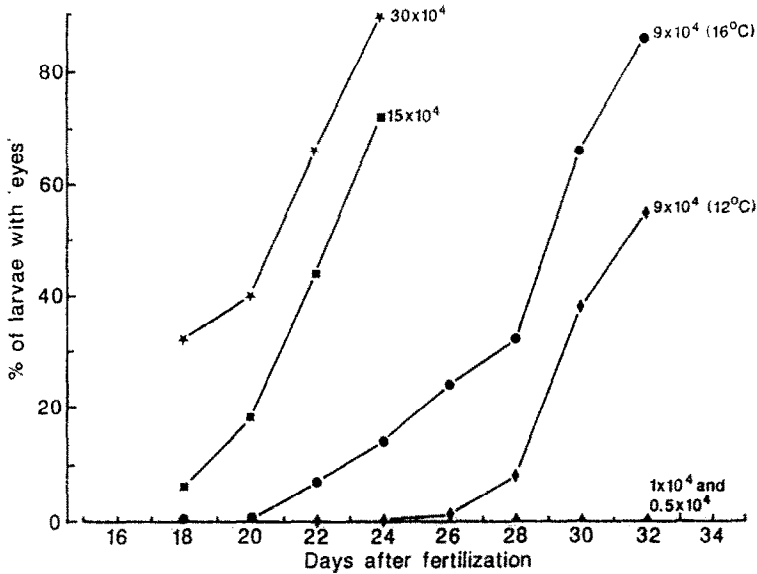


Fig. 5. Influence of food concentration and temperature on rate at which *M. edulis* larvae developed eyespots in Fertilization III. Larvae from these same batch cultures were used to obtain growth rate data shown in Fig. 3. Larvae maintained at 9×10^4 cells \cdot ml⁻¹ were reared at two temperatures: 12 and 16 °C. All other larvae were reared at 16 °C. Each point represents observations made at $50 \times$ magnification on exactly 50 larvae.

(Table IV). Of the 110 larvae individually monitored, $\approx 25\%$ died; but nearly 30% secreted byssal threads onto the glass surface of their containers and initiated metamorphosis. Food concentration had no effect on the average time (days after eyespot formation) required for larvae to attach ($\bar{x} = 30.7$ days, $F = 1.5$, $df = 3,28$, $P = 0.25$), the average size of individuals that did attach ($\bar{x} = 321$ μ m, $F = 1.68$, $df = 3,17$, $P = 0.21$), or on the size of larvae remaining at the end of the study ($\bar{x} = 347$ μ m, $F = 1.01$, $df = 3,47$, $P = 0.40$) (Table V). However, of those larvae that never attached and eventually died, those fed at the highest food concentration (15×10^4 cells \cdot ml⁻¹) survived significantly longer (≈ 60 days) than those maintained at all lower food concentrations (≈ 27 days) ($F = 12.2$, $df = 2,23$, $P = 2.4 \times 10^{-4}$; followed by Scheffe's test for significant differences). Larvae that either attached or died did so, on average, at comparable rates (all food concentrations combined; $F = 1.05$, $df = 1,56$, $P = 0.31$). However, the average shell length of the 51 individuals that survived as larvae until the end of the study was significantly larger than that of the 21 individuals that attached to the surface of the glass during this time (one-way ANOVA, $F = 9.2$, $df = 1,70$,

TABLE IV

Fate of larvae kept after eyespot formation in small glass dishes, cleaned daily (Fertilization II). Several dishes of larvae were held at each food level, usually with 2-8 ind · dish⁻¹. Final column shows time elapsed from eyespot formation to end of experiment for those individuals that survived as larvae to end of experiment. All larvae were maintained at 16 °C.

Food level (cells · ml ⁻¹)	Initial number of larvae	Number died (%)	Number attached (%)	Number of larvae remaining (%)	Days reared after eyespot formation ($\bar{x} \pm SD$)
1 × 10 ⁴	20	9 (43%)	3 (10%)	8 (40%)	42.9 ± 4.4
3 × 10 ⁴	20	1 (5%)	5 (25%)	14 (70%)	51.9 ± 2.2
9 × 10 ⁴	35	11 (31%)	10 (29%)	14 (40%)	56.6 ± 2.1
15 × 10 ⁴	35	6 (17%)	14 (40%)	15 (43%)	56.9 ± 1.1
	\bar{x}	24%	26%	48%	

TABLE V

Comparison of shell lengths and ages between individuals (from Fertilization II) that attached by byssal threads in clean glass dishes and individuals that remained larvae until experiment was terminated. Sample sizes (N) for days from fertilization are same as given for days from eyespot formation.

Food level (cells · ml ⁻¹)	Size (μm) at attachment [$\bar{x} \pm \text{SD}$ (N)]	Final size of larvae (μm) [$\bar{x} \pm \text{SD}$ (N)]	Days from eyespot formation [$\bar{x} \pm \text{SD}$ (N)]		Days from fertilization ($\bar{x} \pm \text{SD}$)	
			To attachment	To death	To attachment	To death
1×10^4	341 (2)	343.9 ± 47.9 (8)	35.3 ± 5.1 (3)	25.6 ± 14.5 (9)	70.7 ± 7.8	61.1 ± 15.5
3×10^4	301.3 ± 44.7 (3)	356.2 ± 21.5 (4)	27 ± 13 (5)	49 (1)	56.4 ± 12.3	75
9×10^4	304 ± 10 (5)	335.2 ± 33.4 (14)	25.2 ± 10 (10)	29.3 ± 16.4 (11)	50.2 ± 9.7	53.9 ± 16.5
15×10^4	330.6 ± 27.9 (11)	350.5 ± 33.9 (15)	35.0 ± 14.7 (14)	60.2 ± 8 (6)	59.8 ± 13.9	84.2 ± 7
	321 ± 31.1 (21)	346.8 ± 33.5 (51)	30.7 ± 12.9 (32)	35.6 ± 19.4 (27)	57.3 ± 13	63.8 ± 18.2

$P = 3.5 \times 10^{-3}$), suggesting that attachment to glass was probably not initiated by the mussel larvae reaching a particular shell size.

DISCUSSION

Based on the time required to reach an average shell length of $250 \mu\text{m}$, the mussel larvae in our experiments grew as quickly as $8 \mu\text{m} \cdot \text{day}^{-1}$. The highest growth rates were recorded at 16°C , $15\text{--}30 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$ (Fertilization III, Table I). Comparable mean growth rates ($6.6\text{--}8.7 \mu\text{m} \cdot \text{day}^{-1}$) have been recorded by others (Beaumont & Budd, 1982; Jespersen & Olsen, 1982). Sprung (1984a) reported growth rates up to almost $12 \mu\text{m} \cdot \text{day}^{-1}$ (at 18°C) for larvae reared at only $1 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$. Bayne (1965) recorded mean growth rates nearly as high (his Fig. 5, p. 13), but only at the highest food concentration tested, $10 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$. Jespersen & Olsen (1982) reported maximal larval growth at $\approx 1 \times 10^4 \text{ algal cells} \cdot \text{ml}^{-1}$. Increasing food concentration $> \approx 1 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$ also had little further effect on larval growth rates in Sprung's work (1984a), whereas we found growth rates continuing to increase as food concentrations exceeded $9 \times 10^4 \text{ cells} \cdot \text{ml}^{-1}$. Similarly, Bayne (1965) reported increas-

TABLE VI

Manner in which age and size at which larval eyespots form can be influenced by shifts in relative rates of growth and differentiation.

Change in growth rate	Change in rate of eyespot development	Effect on age at eyespot formation	Effect on size at eyespot formation
Increased growth rate	Disproportionately smaller increase in differentiation rate	Eyespots form sooner	Larger at eyespot formation
Increased growth rate	Increased differentiation rate in same proportion	Eyespots form sooner	No effect
Increased growth rate	No change	No effect	Larger at eyespot formation
Relatively little (or no) increase in growth rate	Increased rate of differentiation	Eyespots form sooner	Smaller at eyespot formation
Growth rate increases or remains the same	Decreased rate of differentiation	Eyespots form later	Larger at eyespot formation
Decreased growth rate	No decrease in rate of differentiation	No effect	Smaller size at eyespot formation
Decreased growth rate	Disproportionately smaller decrease in rate of eyespot differentiation	Eyespots form later	Smaller size at eyespot formation
Decreased growth rate	Decreased differentiation in same proportion	Eyespots form later	No effect

TABLE VII
 Influence of food and temperature on relative rates of larval growth and differentiation. *Signifies data for *C. virginica*; **signifies data for *M. mercenaria*; all other data are for *M. edulis*.

Parameter	Conditions tested	% increase in mean growth rate ($\mu\text{m} \cdot \text{day}^{-1}$)	% increase in mean differentiation rate	Aspect of differentiation measured	Reference
Temperature	10 vs. 18 °C	179% (to 200 μm)	54%	(Days to 50% pediveliger) ⁻¹	Bayne (1965)
Temperature	12 vs. 16 °C	-15% (to 250 μm)	21%	(Days to 50% eyed) ⁻¹	This paper (Fertilization III)
Temperature*	20 vs. 27.5 °C	300% (% size increases over 8d)	153%	(Days to begin metamorphosis) ⁻¹	Davis & Calabrese (1964)
Temperature**	18 vs. 27 °C	55%	71%	(Days to begin metamorphosis) ⁻¹	Loosanoff (1959)
Food level	2.5 × 10 ⁴ vs. 10 × 10 ⁴ cells · ml ⁻¹	36% (to 200 μm)	95%	(Days to 50% pediveliger) ⁻¹	Bayne (1965)
Food level	3 × 10 ⁴ vs. 15 × 10 ⁴ cells · ml ⁻¹	93% (to 250 μm)	21%	(Days to 50% eyed) ⁻¹	This paper (Fertilization II)
Food level	9 × 10 ⁴ vs. 30 × 10 ⁴ cells · ml ⁻¹	88% (to 250 μm)	41%	(Days to 50% eyed) ⁻¹	This paper (Fertilization III)
Larval density	15 vs. 50 · ml ⁻¹ (Day 24)	26% (length by Day 24)	43%	(Days to 20% eyed) ⁻¹	This paper (Fertilization II)

ed larval growth with increased food concentration up to the highest concentration he tested, 10×10^4 cells \cdot ml $^{-1}$. Differences in larval culture density probably explain at least some of the discrepancy in results obtained by different biologists. Jespersen & Olsen (1982) and Sprung (1984a,b) cultured larvae at densities of < 1 larva \cdot ml $^{-1}$, while larval concentrations were at least 10 times higher in our work and in that of Bayne (1965).

Our finding of a linear relationship between larval shell length and width in this species at all temperatures and food concentrations confirms and extends previous findings for mussel larvae presumably reared at a single temperature and food concentration (Loosanoff et al., 1966). However, our larvae were ≈ 20 μ m wider than those described by Loosanoff et al. (1966) for a given shell length. This discrepancy is difficult to explain, since we found no influence of food concentration or temperature on the relationship between larval shell length and width.

Bayne (1964) noted that mussel larvae cultured at 15 °C developed eyespots in ≈ 24 days, at shell lengths of 240–245 μ m. Loosanoff et al. (1966) reports that mussel larvae reared under unspecified conditions form eyespots at ≈ 215 –230 μ m shell length. Our study indicates considerable variation in both the size at which larval eyespots develop in *M. edulis* and the time required for larvae to develop them (Table III). The average size at which larvae developed eyespots in our studies ranged between ≈ 250 and 285 μ m.

Changes in larval growth rates often failed to predict the effects of food concentration or temperature changes on time required for larvae to form eyespots. In some cases, larvae cultured under different conditions grew at comparable rates but developed eyespots at different ages and at different shell lengths. In other cases, rates of shell growth and eyespot development were altered in the same direction but to markedly different degrees. In still other cases, rates of shell growth and eyespot development were apparently affected to identical degrees. The manner in which size and age at eyespot formation can indicate the relative effects of environmental change on rates of growth and morphological differentiation is outlined in Table VI. If, for example, eyespots form sooner and at a smaller shell size, rates of eyespot development must have been accelerated to a greater extent than rates of shell growth (Table VI, line 4).

Other data for *M. edulis* and for two other bivalve species (*M. mercenaria* and *C. virginica*) similarly indicate a poor correspondence between effects of environmental factors on rates of growth and other aspects of development (Table VII). Moreover, based on these few data, it appears that food concentration and temperature generally affect the relationships quite differently. In Bayne's (1965) studies, for example, increased temperature accelerated growth rate more than overall rate of development to the pediveliger stage. In contrast, increasing food concentration had considerably more impact on differentiation than on growth in his experiments; larvae became pediveligers much sooner than would have been predicted from the effect of increased food concentration on growth rate. Similarly, recent experiments with marine crustaceans indicate that certain environmental factors alter larval growth and differen-

tiation to different degrees (Scheltema & Williams, 1982; Laughlin et al., 1983; Sanders & Jenkins, 1984; Harms, 1986; West & Costlow, 1987). For example, Laughlin et al. (1983) and Sanders & Jenkins (1984) report that exposing mud crab *Rhithropanopeus harrisi* zoeae to low concentrations of certain pollutants (tributyltin oxide and Cu^{2+}) affects individual weight of megalopa larvae (i.e., growth) without significantly affecting the time required to reach the megalopa stage. Similarly, salinity has a pronounced effect on the rate at which nonfeeding larvae of the horseshoe crab *Limulus polyphemus* lose weight, without affecting the duration of larval development (Laughlin, 1983).

The magnitude, and even the direction of the effects of environmental factors on growth and morphological differentiation may vary among species (compare data for oyster and clam, Table VII), and within a species seem to depend on what aspect of differentiation is being monitored. For example, Bayne (1965) found that food concentration altered differentiation rate [(time to pediveliger stage)⁻¹] for mussel larvae more than growth rate, in contrast to results reported here for time to eyespot formation (Table VII, rows 5–7). Several aspects of differentiation must be monitored simultaneously in future experiments if this discrepancy is to be resolved.

Previous data (Bayne, 1964b, 1965) indicated that mussel larvae cultured at 15–16 °C and deprived of filamentous substrate typically survived only \approx 15–30 days after they developed eyespots. Most of the larvae in Bayne's (1965) study died during this period of "delayed metamorphosis" and only a few larvae ever attached to the glass of the culture vessels. In contrast, we found that larvae typically survived well for > 50 days after they formed eyespots, especially at the higher food concentrations (Table IV). If mussel larvae become competent to metamorphose \approx 5 days after forming eyespots (Bayne, 1964), our data imply that they are capable of delaying metamorphosis for > 45 days at this temperature, far longer than previously estimated (Table 11 of Bayne, 1965). Moreover, substantial numbers of delaying larvae eventually attached to the glass of our culture containers (Table IV); this shows a higher incidence of "spontaneous" metamorphosis on glass than previously reported for mussel larvae (Bayne, 1965; Eyster & Pechenik, 1987).

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