



Effects of larval starvation and delayed metamorphosis on juvenile survival and growth of the tube-dwelling polychaete *Hydroides elegans* (Haswell)

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Abstract

Competent larvae of the serpulid polychaete, *Hydroides elegans* (Haswell), were induced to metamorphose by either 10^{-4} M 3-isobutyl 1-methylxanthine (IBMX), adult homogenate, or 30 mM excess K^+ . Treatment with excess K^+ had adverse effects ($P < 0.05$) on juvenile growth while IBMX and adult homogenate had no detectable effects ($P > 0.1$). Metamorphosis was triggered using IBMX in subsequent studies. Competent larvae were forced to delay metamorphosis for up to 12 days by preventing the formation of biofilm in glass beakers. Juvenile growth was assessed by increases in tube length and dry tissue weight. The larvae remained fully responsive to IBMX while delaying metamorphosis up to 11 days but lost the ability to respond to adult homogenates within only 3 days, suggesting that the two chemicals act at different points in the metamorphic pathway and that only part of the pathway degrades as metamorphosis is delayed. Metamorphic responses were not affected by starvation during the competent phase. Delaying metamorphosis significantly reduced juvenile survival whether the larvae were fed or starved. However, there was no apparent effect of starvation on juvenile growth as juveniles developed from the larvae that were starved while delaying metamorphosis grew as fast as those developed from the larvae that were fed during the delay period. Our results suggest that *Hydroides elegans* cannot delay metamorphosis without measurable adverse effects on juvenile survival and growth. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Delay of metamorphosis; *Hydroides elegans*; Larvae; Polychaetes; Juvenile survival; Juvenile growth; Starvation

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1. Introduction

For many decades, researchers have sought to understand the causes of yearly fluctuations in the recruitment of benthic invertebrates (Thorson, 1950), particularly the roles of competition and predation on adults (Connell, 1961, 1985). More recently the focus has shifted to the role played by larval supply, as affected both by shifts in water current patterns (Young, 1991) and by responses of larval stages to chemical and physical conditions (reviewed by Pechenik, 1987). It is now becoming apparent that the fate of juveniles after metamorphosis may be determined to a large degree by factors experienced during the larval stage; i.e. that there is a link between larval experience and the physiological quality and performance capacity of juveniles. In particular, juvenile survival, growth rates, or rates of development have been shown to be compromised in some species if individuals have experienced food limitation during development or if they have delayed their metamorphosis (Pechenik, 1985; Woollacott et al., 1989; Pechenik and Cerulli, 1991; Qian and Chia, 1991, 1993; Pechenik et al., 1996a,b). This connection between larval experience and juvenile performance was not considered in Thorson's classic discussion of mortality sources during invertebrate development (Thorson, 1950) or in models of life cycle evolution of marine invertebrates (e.g. Vance, 1973a,b). The relative sensitivity of different life history stages to environmental stresses, and the extent to which larval exposure to those stresses influences post-metamorphic fitness, have not been studied.

Hydroides elegans (Haswell) is a widely distributed, tropical and subtropical serpulid polychaete and a major fouler of seawater pipe systems in Hong Kong waters. Recent studies have indicated that larvae of *H. elegans* can become competent within 4–6 days at 25°C and will settle and metamorphose in response to excess K⁺, the non-specific phosphodiesterase inhibitor 3-isobutyl 1-methylxanthine (IBMX), or to a crude, filtered homogenate of crushed adults (Bryan et al., 1997). In this paper, we examined the effect of delayed settlement and starvation during the larval stage on juvenile survival and growth. In the process of designing these experiments we also considered whether any of the metamorphic inducers or starvation affected the ability of larvae to respond to the different chemical cues over time.

2. Materials and methods

2.1. Culture conditions

All experiments were conducted at 25°C using seawater (~34 ppt salinity) filtered to 0.22 µm. Photoperiod was 12L:12D in all cases. Adults were collected from 1/2" diameter nylon ropes hanging off a floating pier at the V. Whale Limited fish farm in Port Shelter, Hong Kong (22°19' N, 114°16' W), and maintained in the laboratory for no more than 3 days before use. Gamete release was induced artificially in the laboratory according to the methods described in Bryan et al. (1997). Adult *H. elegans* were placed in a clean petri dish containing 20 ml of 0.22 µm filtered seawater (FSW) and the tubes were gently cracked. Gametes were generally released from reproductive individuals

within 5 min. Oocytes were transferred to a second petri dish containing 20 ml FSW and mixed with 10 μl of dilute sperm. Since fertilization is successful over a remarkably broad range of sperm concentration in this species (Pechenik and Qian, 1998), no attempt was made to control sperm concentrations in the present experiments. More than 95% of eggs were fertilized in all of our experiments within 15 min after eggs and sperm were mixed. Excess sperm were decanted by filtering the sperm through a 35- μm mesh; fertilized eggs were retained on the screen. The fertilized eggs were then transferred to 350-ml glass culture beakers containing 0.22 μm FSW. Larvae were always reared on the flagellate *Isochrysis galbana* (Tahitian strain) at a concentration of $\sim 2.0 \times 10^5$ cells ml^{-1} in batch culture for 3–4 days in 200–250 ml of phytoplankton suspension. Larvae were removed by filtering or pipetting and transferred to clean glass and freshly-prepared medium daily.

2.2. Preparation of adult worm homogenates

Adult homogenates were prepared by following the methods described in Bryan et al. (1997). Adult worms were gently removed from their tubes and dipped in FSW then blotted dry on a paper towel and weighed to the nearest 1.0 mg. Homogenates were prepared by crushing and sonicating the adults in deionized water at a ratio of 0.2 g worm ml^{-1} water. The homogenate was centrifuged at $12\,660 \times g$ for 10 min, and the supernatant was stored at -20°C until use. In all experiments, we tested larvae using supernatant at 0.2 mg supernatant ml^{-1} seawater ($1 \times$ homogenate concentration); this concentration previously induced the highest percentage of metamorphosis in *H. elegans* (Bryan et al., 1997).

2.3. Testing for metamorphic competence

Beginning 3–4 days after fertilization, competence was assessed by pipetting a subsample of larvae into a solution of 10^{-4} M IBMX in seawater. Replicates consisted of ~ 12 larvae placed in six-cell tissue culture wells containing 5 ml of treatment solution and incubated at 22°C on a 15 h light:9 h dark photoperiod. The status of larvae in experimental dishes was determined using a dissecting microscope ($25 \times$ magnification) at 24 and 48 h after initiation of an assay, unless otherwise noted. Larvae that had attached to the culture well, produced a tube, and grown tentacles, were considered to have undergone normal metamorphosis. Larvae were not fed during the experiment and the testing solution was not changed. Larvae that were unattached and swimming or crawling were considered to be unmetamorphosed. There were five replicates of each treatment or control, except where otherwise noted.

Once at least 90% of subsampled larvae became competent to metamorphose in response to IBMX, remaining larvae were transferred to four clean glass beakers; two beakers contained 250 ml of phytoplankton suspension of *Isochrysis galbana* at $\sim 20 \times 10^4$ cells ml^{-1} each (fed group) and another two beakers contained 0.22 μm FSW only (starved group). Larvae in these treatments were filtered or pipetted into clean glassware with freshly-prepared medium daily, to avoid biofilm build-up on beaker surfaces.

2.4. Effects of artificial inducers on larval settlement and juvenile growth of *Hydroides elegans*

These experiments were undertaken to determine whether certain inducers might affect growth or survival of postmetamorphic juveniles, and whether competent larvae might remain responsive to some inducers longer than others. All experiments were conducted using six-well plastic tissue culture plates. Previous studies showed that the incidence of metamorphosis in such containers does not differ significantly from that on glass (Pechenik and Qian, 1998). Larvae developed from the eggs fertilized on May 31, 1996 were reared for 6 days as described above. Approximately 70 larvae taken from that culture were then tested for their competence to metamorphose by exposing them to 10^{-4} M IBMX according to the method described in Section 2.3; >90% of tested individuals metamorphosed within 24 h. The remaining larvae were then transferred to filtered seawater (controls), seawater whose K^+ concentration was increased by 30 mM using KCl, 10^{-4} M IBMX, $1 \times$ adult homogenate, or $3 \times$ adult homogenate. Five replicates with 10–12 larvae each were used for each treatment. Larvae were pipetted into a bath of test solution before being distributed among wells, to avoid diluting test solutions with $0.22 \mu\text{m}$ FSW. The percentage of individuals that metamorphosed was assessed after 24 and 48 h. Arcsin-transformed percentage data were compared by Kruskal-Wallis non-parametric ANOVA test and non-parametric multiple comparison tests.

To measure juvenile growth, all individuals that had lost their ciliary bands and were developing anterior tentacles were then transferred the same day to a glass beaker containing 200 ml phytoplankton suspension ($\sim 2.0 \times 10^5$ cells ml^{-1}). Newly-metamorphosed individuals can easily be dislodged by blowing them off the substratum with a pipette. On the following day, we videotaped all individuals that were far enough from the sides of the glass beakers to be clearly visible to determine initial size; this was done using a videocamera coupled to a dissecting microscope. The same individuals were videotaped again every 2 days for 8 days so that increases in tube length of individual worms could be determined. The image of each tube was digitized and the length of each worm tube was determined by using a Leica Image Analysis system. Growth rates were determined as the increase (mm) in tube length per day for each 2-day interval. The data on tube length or growth rate (increase in tube length per day) were compared for each sampling interval by using Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparison tests.

2.5. Relationship between tube length and biomass of juveniles

The relationships between tube length, and total dry weight, and ash-free dry weight were determined after videotaping specimens on day 9. Each worm was carefully dislodged from the glass substrate and then its tube (including any broken pieces of the tube) were sucked gently into a fine pipette, rinsed quickly with distilled water to remove adhering salts, and transferred to a preweighed aluminum foil pan (8–18 μg empty weight). Samples were dried for 12 h at 50°C and weighed to the nearest μg

using a Perkin-Elmer Model AD-6 electronic balance; a dish of desiccant (indicating CaSO_4 ; Drierite) was placed in the weighing chamber of the balance to prevent specimen rehydration. Samples were then combusted for 6 h at 500°C and reweighed, to assess individual organic content by weight loss (Paine, 1964). As a check on our methodology, five of the samples were recombusted for an additional 6 h at 525°C and reweighed; the additional combustion caused no further weight loss. In total, 52 individuals were weighed in this experiment. The relationships between tube length and total dry weight or ash-free dry weight of each worm were determined by linear regression.

2.6. Effect of starvation and delayed metamorphosis on maintenance of metamorphic competence, juvenile survival, and juvenile growth

Larvae developed from eggs fertilized on June 19 were reared for 6 days as described above. Metamorphic competence was assessed on day 6 (day 0 of delayed metamorphosis) according to the method described in Section 2.3. Over 95% of larvae tested on day 0 attached and metamorphosed within 24 h, so that nearly all larvae were clearly delaying their metamorphosis as the experiment continued. In the rest of the experiments, both 10^{-4} M IBMX and the adult homogenate were used to test the effect of starvation and delayed metamorphosis on the maintenance of larval metamorphic competence, and on juvenile survival and growth. About 1500 larvae were transferred from the larval stock culture to a glass beaker holding 250 ml of $0.22 \mu\text{m}$ FSW while another ~ 1500 larvae from the stock culture were transferred to a glass beaker holding 250 ml of phytoplankton suspension ($\sim 2.0 \times 10^5$ cells ml^{-1}). On days 3, 6, and 9 after testing the initial control group on day 0, 240 starving larvae and 240 fed larvae were taken from appropriate beakers. Sixty starving larvae and 60 fed larvae were tested for metamorphic competence in IBMX; 60 starving larvae and 60 fed larvae were tested for metamorphic competence in $1 \times$ adult homogenate; 60 starving larvae and 60 larvae were tested for metamorphic competence in FSW (negative controls for IBMX testing) while the remaining 60 starving larvae and 60 larvae were tested for metamorphic competence in FSW (negative controls for adult homogenate testing). Two sets of negative controls were required because data of metamorphosis for IBMX were mainly collected after 24 h (larvae settled in response to IBMX much faster than to homogenates, see Pechenik and Qian, 1998) while the data for adult homogenate were collected for both 24 h and 48 h. Each group of 60 larvae were equally divided into five replicate cell-wells, resulting in 12 larvae in each well. A sample of 50–60 larvae from each stock culture were also tested for their ability to metamorphose in response to IBMX and adult homogenate on days 11, 12, and 13. Percentage of larval metamorphosis in response to IBMX and $1 \times$ adult homogenate were recorded for each test. Arcsin-transformed percentage data were first compared by Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparison tests for each test to determine the effect of larval starvation on responsiveness to IBMX or adult homogenate. To determine the effect of delayed metamorphosis on maintenance of larval competence to metamorphose in response to IBMX and adult homogenate, arcsin-

transformed percentages for both fed and starved larvae were compared by Kruskal-Wallis non-parametric ANOVA followed by Dunn's multiple comparison tests.

All attached and metamorphosing individuals tested on day 0 were transferred to a glass beaker containing 200 ml phytoplankton suspension. Care was taken to provide the same food concentration (2.0×10^5 cells ml^{-1}) to juveniles in each beaker. Juveniles were reared for 8 days at 25°C, with phytoplankton suspension being changed daily. These juveniles served as the controls (day 0 of delayed metamorphosis). Since few fed or starved larvae tested on days 3, 6, 9 attached and metamorphosed within 24 h in $1 \times$ adult homogenate or FSW (controls), only the individuals metamorphosing within 24 h in response to 10^{-4} M IBMX were used to assess the effects on juvenile growth rate. The attached and metamorphosing individuals from five testing wells were pooled and then evenly distributed between two glass beakers. These juveniles were then reared for 8 days starting from the day after inducing metamorphosis. All remaining juveniles in each beaker were counted on day 9 to determine percent survival for the juveniles in both starved and fed groups. The survival of juveniles in the fed and starved groups plotted over the time of delayed metamorphosis was compared using analysis of covariance (ANCOVA) to determine difference in slopes and intercepts.

To determine effects of starvation and delayed metamorphosis on rates of biomass accumulation, both dry tissue weight and ash-free dry weight were determined for each individual that survived through 8 days of the growth experiment. Dry weight and ash-free dry weight were determined as described above and compared with one-way ANOVA and Tukey-B multiple comparison tests to determine the effect of delayed metamorphosis on rates of biomass accumulation. To determine the effect of larval starvation on juvenile growth, dry weight and ash-free dry weight of individuals in the fed group were compared with comparable data for those in the starved group, using Student *t*-tests.

3. Results

3.1. Larval metamorphosis in response to different artificial inducers

On day 6 after fertilization, while only about 12% of larvae metamorphosed in response to excess K^+ over 48 h, 100% metamorphosed in 10^{-4} M IBMX and about 80–87% of the larvae metamorphosed in both $1 \times$ homogenate and $3 \times$ homogenate (Fig. 1). Less than 5% of larvae metamorphosed in control FSW (Fig. 1).

3.2. Effect of different artificial metamorphose inducers on juvenile growth rate

The different inducers had significantly different effects on juvenile growth rates (Fig. 2). For the first 2 days after attachment and metamorphosis, the tube length for juveniles induced using different chemical cues can be ranked as follows: $\text{K}^+ < \text{IBMX} = 1 \times \text{homogenate} = 3 \times \text{homogenate} < \text{controls}$ (Fig. 2). By day 4 after attachment, juvenile tube lengths can be ranked as follows: $\text{K}^+ < \text{IBMX} = 1 \times \text{homogenate} = 3 \times \text{homogenate} = \text{controls}$ (Fig. 2). By day 6 after attachment, juvenile tube lengths can be

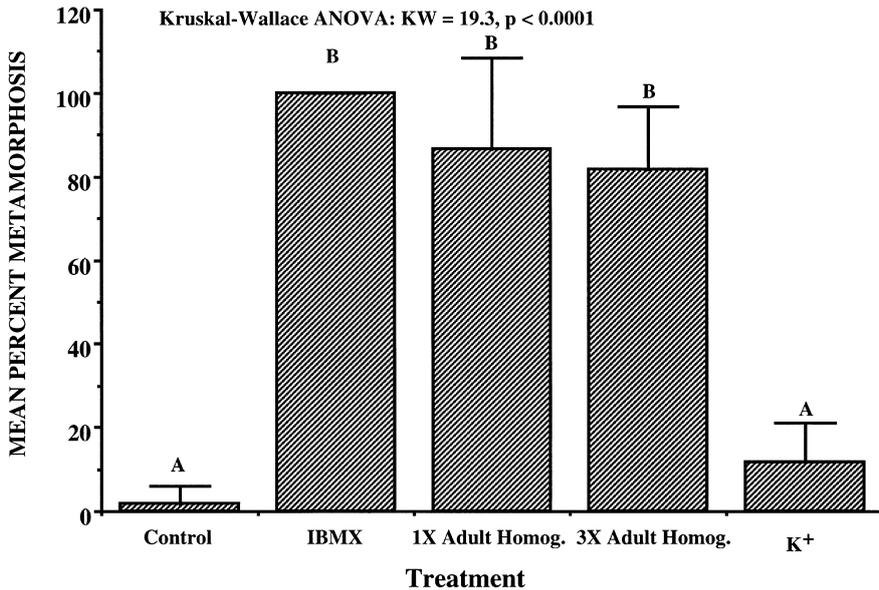


Fig. 1. Forty-eight h responses of 6-day old larvae of *Hydroides elegans* to three artificial inducers. Each point represents the mean (\pm S.D.) of five replicates with 12 larvae in each replicate. Letters on the top of each column indicate the results of Dunn's test following Kruskal-Wallis non-parametric ANOVA; columns with the same letter represent means that are not statistically different ($P > 0.1$).

ranked as follows: $K^+ < 3 \times$ homogenate = $1 \times$ homogenate $<$ IBMX = controls (Fig. 2). Even by the end of the 8-day growth period, juvenile tubes in the K^+ -induced group were only about 40% as long as those of control individuals; juvenile tubes for individuals induced using $3 \times$ homogenate were the second shortest (about 55% of control length), while juveniles from both IBMX and $1 \times$ homogenate-induced groups were similar in length and about 10% shorter than those of control individuals (Fig. 2).

Mean juvenile growth rates were significantly reduced by the different chemical treatments used to induce metamorphosis. Individuals were most sensitive to excess K^+ , with the effects already apparent by days 2–4, but mean growth rates were significantly different from controls ($P < 0.05$) for all treatments by days 6–8 (Fig. 3).

3.3. Relationship between worm tube length and biomass

A reasonably convincing relationship was established between tube length and tissue biomass, based on measurements of 52 individuals videotaped at 3-day intervals from day 1 to day 9; tube length explained $\sim 63\%$ of variation in dry weight while the dry weight explained $\sim 68\%$ of variation in ash-free dry weight (Fig. 4a and b). Variation was greater among individuals with the least biomass, with a closer fit for heavier individuals. Juvenile tube length or dry weight can therefore be used as a rough indicator of body mass in *H. elegans* in future studies, particularly for the individuals older than 9 days (larger than our largest juveniles measured).

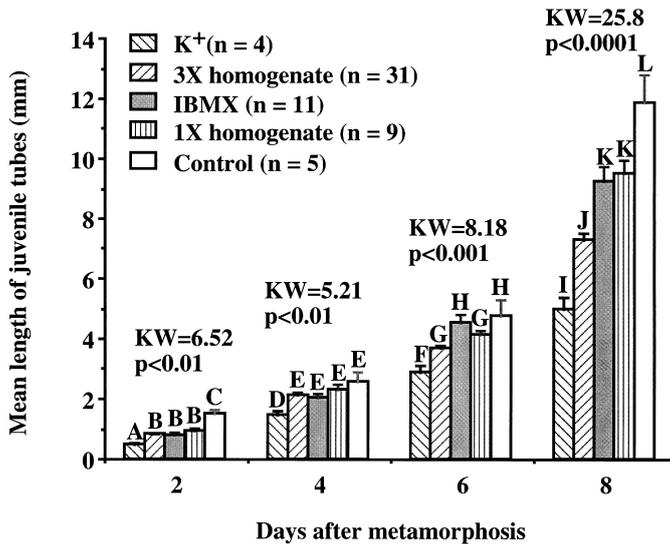


Fig. 2. Effects of artificial inducers on juvenile growth (length of juvenile tubes) of *Hydroides elegans*. Each point represents the mean length (\pm S.D.) of juvenile tubes (mm). Results of Kruskal-Wallis non-parametric ANOVA (KW-value and P value) are presented above each set of bars; Dunn's multiple comparisons were performed among the four treatments and control for each interval; bars with the same letter represent means that are not statistically different at the $P = 0.05$ level.

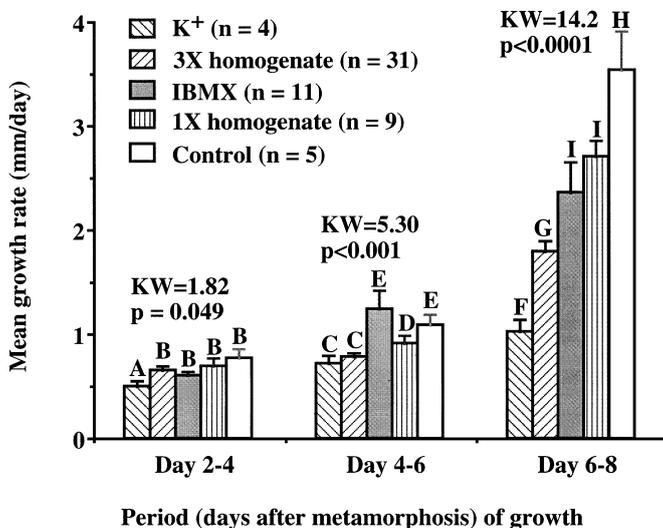


Fig. 3. Effects of artificial inducers on juvenile growth rate (increase in tube length per day) of *Hydroides elegans*. Each point represents the mean (\pm S.D.). Results of Kruskal-Wallis non-parametric ANOVA (KW-value and P value) are presented above each set of bars; Dunn's multiple comparisons were performed among the four treatments and control for each interval; bars with the same letter represent means that are not statistically different at the $P = 0.05$ level.

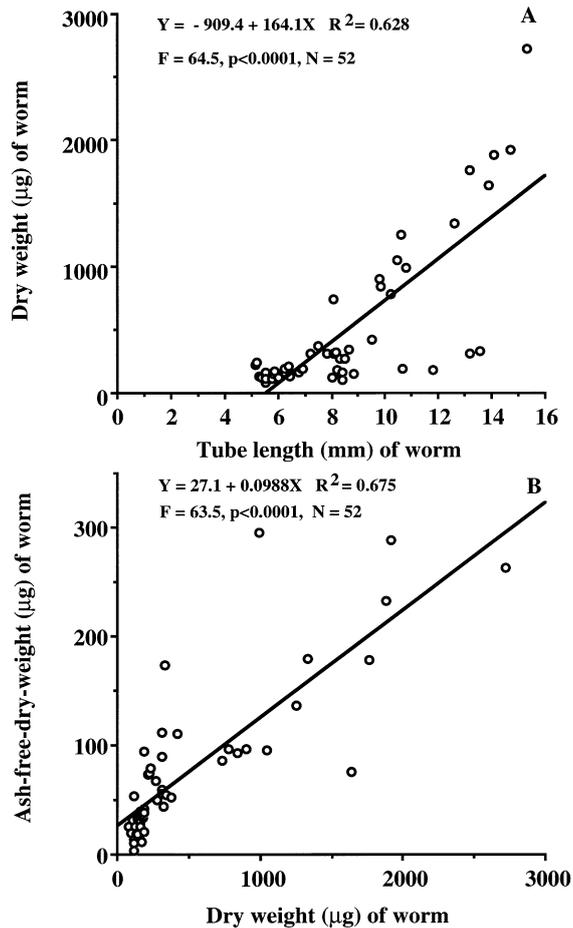


Fig. 4. Relationship between biomass (μg) and tube length (mm) in juvenile *Hydroides elegans*. (A) Total dry weight (μg) as a function of tube length (mm). (B) Ash-free dry weight (μg) as a function of dry weight (μg).

3.4. Effects of starvation and delayed metamorphosis on loss of metamorphic competence

The metamorphic response of *H. elegans* to 10^{-4} M IBMX and $1 \times$ adult homogenate was tested for larvae that were not stimulated to metamorphose for 3, 6, 9, 11, 12, and 13 days after reaching competence; some of the larvae were fed during this time while others were starved (Fig. 5). Larvae remained responsive to IBMX while delaying metamorphosis for 3, 6, 9 days, regardless of whether they were starved or fed during this time. Moreover, starvation had no significant effect ($P > 0.1$) on the percentage of larvae responding after 3 days, 6 days and 9 days of delay. After 9 days of delay, there were not enough larvae in the starved group to continue testing for larval

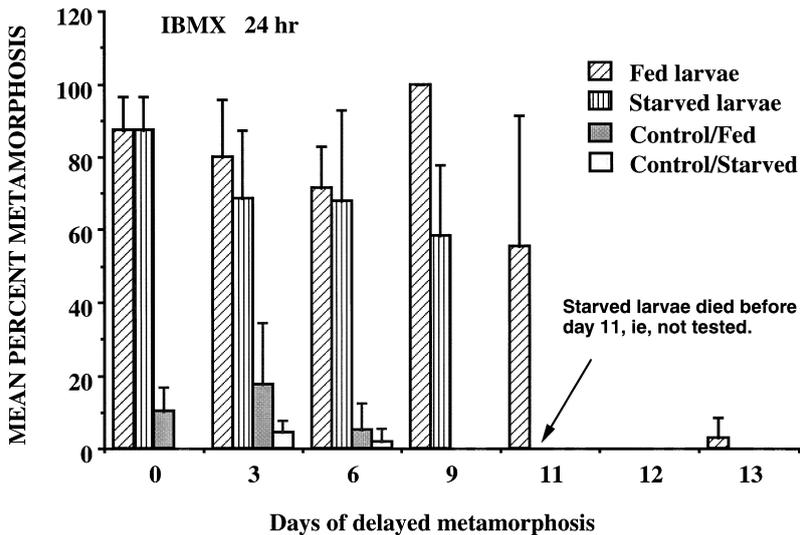


Fig. 5. Effects of starvation and delayed metamorphosis on larval competence to metamorphose in response to 10^{-4} M IBMX in *Hydroides elegans*. Larvae were 6 days old on day 0. Each point represents the mean (\pm S.D.) of five replicates with 10–12 larvae in each replicate. 'Fed' and 'Starved' larvae were exposed to IBMX for 24 h on the days indicated. 'Control/Starved' and 'Control/Fed' larvae were maintained in $0.22 \mu\text{m}$ filtered seawater throughout the testing.

competence. Fed larvae, however, remained responsive to IBMX after being delayed for 10 days but lost competence within the next few days. Larvae that were fed continuously but discouraged from metamorphosing for 12 and 13 days showed a negligible response to IBMX. Those larvae became very sluggish, moved very slowly close to the bottom of the glass beakers, and died within 4–12 h in IBMX solution. Few fed or starved larvae attached and metamorphosed in $0.22 \mu\text{m}$ FSW during the 13 days of testing. Delayed attachment did not enhance nor reduce the competence of metamorphosis in $0.22 \mu\text{m}$ FSW (Fig. 5).

There was no apparent trend in larval responsiveness to $1 \times$ homogenate (24 h assays) for either the starved or fed larvae that were delayed for 3, 6 and 9 days. In most cases, less than 40% of larvae metamorphosed within 24 h in $1 \times$ homogenate (Fig. 6a). In 48 h assays, over 80% of the larvae metamorphosed in $1 \times$ homogenate when tested on day '0' while less than 10% of larvae metamorphosed in controls ($0.22 \mu\text{m}$ filtered seawater) (Fig. 6b). Prolonging the swimming period significantly ($P < 0.05$) reduced larval responsiveness to $1 \times$ homogenates: both starved and fed larvae showed reduced metamorphosis when they were delayed for 3, 6 or 9 days in comparison to those tested on day 0 (Fig. 6b).

3.5. Effects of starvation and delayed metamorphosis on juvenile survival

Delayed metamorphosis had a strong effect on juvenile survival: the longer that metamorphosis of competent larvae was delayed, the lower the percentage of juvenile

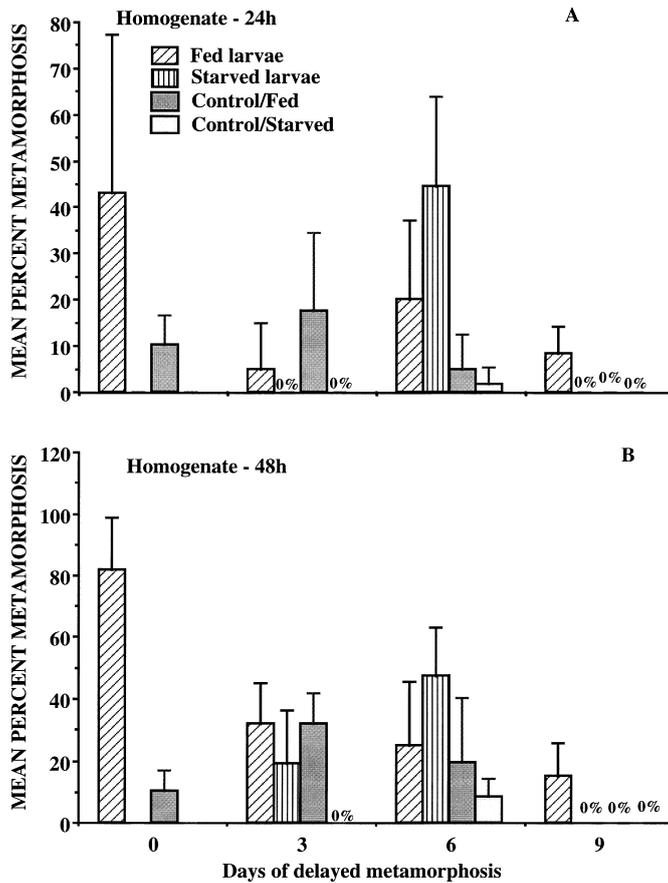


Fig. 6. Effects of starvation and delayed metamorphosis on larval responsiveness to adult homogenate in *Hydroides elegans*. (A) Percent metamorphosis within 24 h. (B) Percent metamorphosis within 48 h. Each point represents the mean (\pm S.D.) of five replicates with 10–12 larvae in each replicate. ‘Fed’ and ‘Starved’ larvae were triggered to metamorphose in adult homogenate. ‘Control/Fed’ and ‘Control/Starved’ larvae were maintained in 0.22 μ m filtered seawater throughout the testing.

survival over the subsequent 9 days of observation (Fig. 7). Starvation during the delay period had no effect on juvenile survival (Fig. 7, ANCOVA, $P > 0.05$ for both slope and intercept of the regression lines for starved and fed larvae).

3.6. Effects of starvation and delayed metamorphosis on juvenile growth

Duration of delayed metamorphosis had negative effects on juvenile growth rates, as determined from tissue dry weight measurements; 9-day old juveniles derived from the larvae that were delayed for 3, 6, and 9 days were significantly lighter than control individuals induced to metamorphose on day 0 (Fig. 8a; one-way ANOVA: $F_{6,103} = 12.71$, $P < 0.01$; Tukey-B test for dry weight; Fig. 8b; one-way ANOVA: $F_{6,103} = 3.63$,

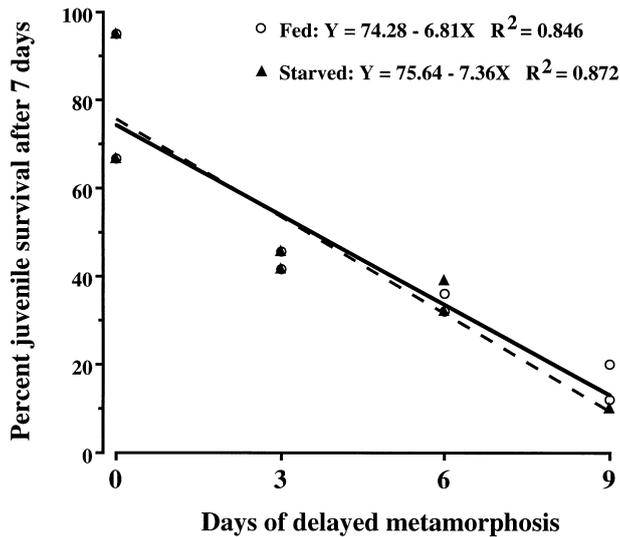


Fig. 7. Effects of starvation and delayed metamorphosis during larval development on juvenile survival in *Hydroides elegans*. Fed: juveniles developed from the larvae that were fed while delaying metamorphosis. Starved: juveniles developed from the larvae that were starved while delaying metamorphosis. Juveniles in all treatments were fed continuously after metamorphosis.

$P < 0.01$; Tukey-B test for ash-free dry weight). However, starvation had no clear effect on juvenile growth rate, as the juveniles that developed from larvae that were fed while being delayed for 3, 6, and 9 days were similar in weight to those that developed from starved larvae (see results of Student t -test given on the top of bars in Fig. 8).

4. Discussion

4.1. Impact of artificial inducers on juvenile growth

In this study, we found that juvenile growth rates differed significantly ($P < 0.05$) depending on whether the larvae metamorphosed spontaneously without added cues or were induced to metamorphose using IBMX, $1 \times$ homogenate, $3 \times$ homogenate, or excess KCl (Fig. 2 and Fig. 3). These results confirm and extend our earlier observations that juveniles grew more slowly if as larvae they were induced to metamorphose using excess K^+ for 48 h (Fig. 3). Pechenik and Qian (1998) found that larvae of *Hydroides elegans* responded far more rapidly to IBMX than to the other inducers tested; most larvae attached and metamorphosed in response to IBMX within 8 h. Larvae responded much more slowly to adult homogenate, although juveniles usually grew well and formed normal tubes. Larvae generally responded even more slowly to excess K^+ and juveniles rarely formed conspicuous, calcified tubes even though larvae lost their cilia and developed anterior tentacle buds. However, juveniles did calcify their tubes after

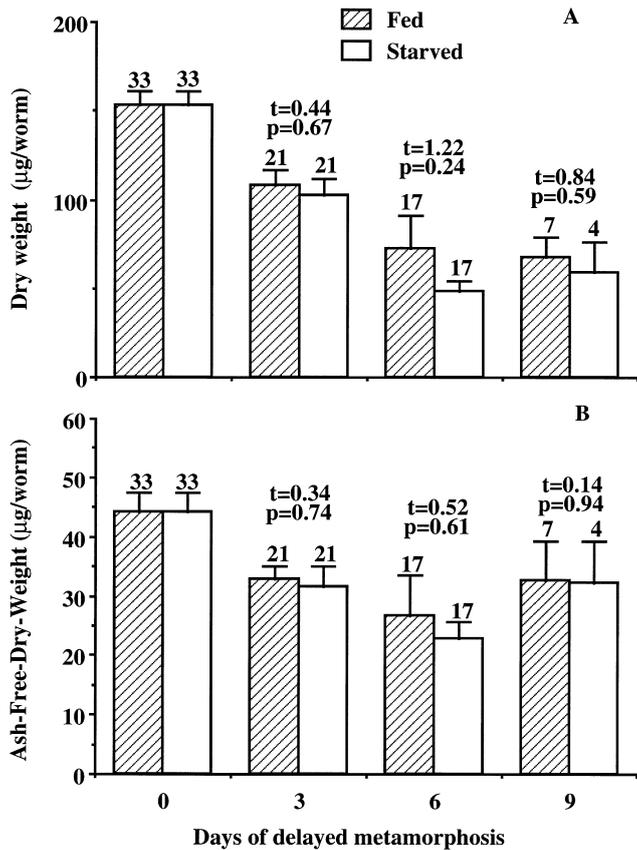


Fig. 8. Effects of starvation and delayed metamorphosis on mean juvenile growth rate in *Hydroides elegans*. (A) Total dry weight (including tube) of juveniles after 8 days of growth at 25°C. (B) Ash-free dry weight of juveniles after 8 days of growth at 25°C. Each bar represents mean (\pm S.D.) weight, with the number of juveniles indicated above each bar. Fed: juveniles developed from larvae that were fed while delaying metamorphosis. Starved: juvenile developed from larvae that were starved while delaying metamorphosis. Larvae were 6 days old on day 0. Juveniles in all treatments were fed continuously after metamorphosis. Student *t*-tests were used to compare the mean biomass of the fed and the starved groups for each interval; results (*t*-value and *P* value) are presented above each set of bars.

they were removed from excess K^+ , so K^+ did not cause a permanent inhibition of the calcification process.

Our results suggested that at least some effective artificial inducers may have detrimental effects on juvenile survival and growth. A similar phenomenon has been reported in some other marine invertebrates. For instance, juvenile abalone *Haliotis rufescens*, resulting from larvae that were induced to metamorphose by GABA (gamma aminobutyric acid), had much higher mortality and slower growth than those induced by algae (Slattery, 1992). Why do juveniles grow more slowly when metamorphosis is triggered by some artificial cues? In our experiment, animals were in the inducers only

until they metamorphosed; after that the worms were reared under control conditions. Thus, it is unlikely that the artificial cues had any direct effect on juvenile feeding behavior. This argument however, needs to be addressed in future studies.

4.2. Effects of starvation and delayed metamorphosis on responsiveness to different inducers

Pawlik and Mense (1994) found that competent larvae of the sabellariid polychaete *Phragmatopoma lapidosa* became unresponsive to the natural inducer when starved and regained sensitivity to the inducer when they were refed, suggesting at least partial degradation or inactivation of the metamorphic pathway during starvation and reconstruction or reactivation of that pathway during the period of refeeding. Whether those polychaete larvae lost and regained competence to different cues simultaneously was not examined. Pechenik et al. (1996a) found that the prosobranch gastropod *Crepidula fornicata* became competent to respond to excess K^+ even while they were being starved but they did not determine if starving larvae could also become responsive to other chemical cues. Pechenik and Qian (1998) documented an effect of starvation on the acquisition and loss of responsiveness of *Hydroides elegans* to three different cues but did not examine the effects of starvation and delayed settlement on juvenile growth performance. In the present study, we found that larvae of *H. elegans* retained their competence to metamorphose in response to IBMX up to 10 days after becoming competent at 25°C, regardless if they were starved or fed; thus, neither starvation nor delayed metamorphosis accelerated the loss of responsiveness to IBMX (Fig. 5). This suggests that the eventual deactivation of this metamorphic pathway is not due to nutritional effects. However, larvae lost their ability to respond to $1 \times$ homogenate after being prevented from metamorphosing for only 3 days (Fig. 6). This suggests that either there are different metamorphic pathways for responses to IBMX and conspecific metamorphic cues, or that IBMX simply acts further downstream in the pathway while conspecific metamorphic cues act more upstream in the pathway. Thus, only upstream parts of the pathway become inactivated or degraded during starvation or delayed metamorphosis if one pathway is involved.

Coon et al. (1990) showed that oyster larvae become competent to respond to chemical cues behaviorally before they become competent to actually metamorphose, and that some chemical cues can trigger the behavioral response without triggering metamorphosis and vice versa. In our studies, larvae did not become responsive to all effective cues at the same age (Pechenik and Qian, 1998), as also documented for the gastropods *Crepidula fornicata* (Pechenik and Gee, 1994) and *Phestilla sibogae* (Pechenik et al., 1995). However, this is the first study to demonstrate the sequential loss of responsiveness to different cues as larvae age. Clearly, competence must always be defined with respect to particular cues and particular responses. Further studies along these lines should help to determine how the pathway is constructed during development and how it functions.

In contrast to results for *Crepidula fornicata* (Pechenik et al., 1996a), starving competent larvae did not cause them to metamorphose (Fig. 5).

4.3. Effect of starvation and delayed metamorphosis on juvenile survival and growth

Researchers have been documenting larval tolerances to various environmental stresses for many decades. It is now well known that marine invertebrate larvae are typically 10–100 times more sensitive to chemical and other stresses than are the adults and juveniles of the same species (reviewed by Pechenik, 1987). Such sensitivity may contribute to yearly variation in recruitment success of particular species in the field. Factors such as nutritional stress or pollutant stress that prolong larval life may also influence larval abundance by prolonging exposure to planktonic predators (Thorson, 1950, 1966; Young and Chia, 1987). In addition, however, it is becoming increasingly clear that larval experiences may affect juvenile success in more subtle ways. We found that delayed metamorphosis of *H. elegans* has a dramatic impact on juvenile survival; the longer that larval life was prolonged, the lower the percentage of juveniles that survived through 9 days of growth after attachment (Fig. 7). These results suggest that larvae become weaker somehow as they delay their metamorphosis. Surprisingly, the effect seems not to reflect depletion of energy reserves, as juveniles from starved larvae survived as well as those developing from fed larvae (Fig. 7); thus, feeding during the period of delayed metamorphosis did not keep larvae in good condition. We have no clear explanation for this phenomenon. It appears that soon after the larvae of *H. elegans* become competent, further feeding has very little impact on juvenile survival or growth, even though feeding may prolong the larval life-span. In fact, we have noticed during the competent period, that feeding reduced larval mortality as we usually had more larvae left towards the end of our experiment when glass beakers contained food. It has long been assumed that energy reserves acquired by larvae are an important determinant of early juvenile mortality and growth (Bayne et al., 1978; Berglund, 1984; Lawrence et al., 1984; McEdward, 1986; McEdward and Carson, 1987; McEdward and Coulter, 1987; McEdward et al., 1988; George et al., 1990; Marsh et al., 1990; Miller, 1993). However, the hypothesis has not been specifically tested. For instance, in some species, feeding does not begin until days or weeks after metamorphosis (Slattery, 1992; Gosselin and Chia, 1994) and may initially be insufficient to meet the demands of early juvenile growth (Whyte et al., 1992). If larvae are forced to postpone metamorphosis due to the absence of appropriate optimal environmental cues, juveniles of some species show higher post-settlement mortality (Pechenik, 1990; Qian et al., 1990; Pechenik and Cerulli, 1991; Qian and Chia, 1993), greater sensitivity to physical stress (Highsmith and Emler, 1986), or slower rates of juvenile growth or development (Pechenik and Eyster, 1989; Woollacott et al., 1989; Qian et al., 1990; Pechenik et al., 1993, 1996a,b; Qian and Chia, 1993; Strathmann et al., 1993). Limited periods of starvation or food limitation during larval development can also seriously reduce juvenile growth rates (Qian et al., 1990; Qian and Chia, 1993; Pechenik et al., 1996a,b). In this study, we found that although starvation alone did not have clear, negative effects on larval responsiveness to artificial metamorphic inducers or on juvenile survival and growth, a short period of delayed metamorphosis could have a drastic impact on metamorphic competence and juvenile survival. Delayed metamorphosis alone had a strong, negative effect on juvenile growth, as 9-day old juveniles resulting from larvae that were delayed for 3, 6, 9 days were significantly smaller than controls triggered to metamorphose on

day 0 (Fig. 8), whether or not larvae were fed. Thus it is becoming apparent that events experienced by larvae can substantially influence juvenile performance.

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