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Influence of delayed metamorphosis on postsettlement survival and growth in the sipunculan *Apionsoma misakianum*

Jan A. Pechenik[1,a] and Mary E. Rice[2]

[1]Department of Biology, Tufts University, Medford, MA 02155
[2]Smithsonian Marine Station at Fort Pierce, 701 Seaway Drive, Fort Pierce, FL 34949, USA

Abstract. Certain stresses experienced by marine larvae from many groups can dramatically reduce aspects of juvenile performance. This study reports the effects of delayed metamorphosis and nutritional stress on survival and growth of the deposit-feeding sipunculan *Apionsoma* (= *Golfingia*) *misakianum*. Approximately 600 larvae collected from the Florida Current plankton were distributed among 3 treatment groups. Ninety larvae (controls) were offered sediment and adult-conditioned seawater 4 d after collection, to induce metamorphosis; larvae of this species could not be induced to metamorphose by increasing the K^+ concentration of seawater. The remaining 500 larvae were kept swimming for either 2 or 4 weeks, with or without phytoplankton (clone T-ISO). At the end of the periods of prolonged larval swimming, subsampled larvae (360) were induced to metamorphose as in the controls. Surviving individuals were retrieved 6 weeks after the addition of excess sediment in all treatments, and weighed to document growth. Neither delayed metamorphosis nor starvation influenced juvenile survival. However, starving larvae for 2 weeks significantly reduced mean juvenile growth rates relative to the mean growth rate of control individuals ($p<0.0001$), while prolonging larval life by 4 weeks significantly reduced mean juvenile growth rates ($p<0.05$) whether or not larvae were fed. Reduced juvenile growth rates may have been caused by nutritional stress experienced by larvae in both the starved and fed treatments. The rapid response of freshly collected larvae to sediment indicates that competent larvae of this species routinely delay metamorphosis in the field. The extent to which they also experience food limitation is not yet clear. If competent larvae are food limited while delaying metamorphosis in the field, our results suggest that juveniles will grow more slowly and may thus exhibit reduced fitness.

Additional key words: KCl, larvae, metamorphosis, potassium, sipunculans

Competent larvae of many benthic marine invertebrate species postpone metamorphosis in the absence of environmental cues signaling habitats appropriate for the juvenile stage (e.g., Brumbaugh & McConaugha 1995; Lasker & Kim 1996; Stoner et al. 1996; Weber & Epifanio 1996; Zaslow & Benayahu 1996; Avila 1998; Boettcher & Targett 1998; Bryan et al. 1998; older literature reviewed by Pechenik, 1990). The larvae of many species become less selective with time as development is prolonged (e.g., Knight-Jones 1953; Scheltema 1961; Fitt & Hoffman 1985; Crisp 1988; Rumrill 1989; Coon et al. 1990; reviewed by Crisp 1974, Pechenik 1990), and may eventually metamorphose "spontaneously," without added stimuli, in frequently cleaned glassware (e.g. Knight-Jones 1953; Pechenik 1980, 1984; Pawlik 1988; Butman et al. 1988; Zimmerman & Pechenik 1991; Coon et al. 1990; Zaslow & Benayahu 1996). Whether prolonging life in the plankton until an optimal habitat is encountered is more advantageous than eventually metamorphosing into suboptimal habitat depends on the probabilities of encountering particular habitat types and surviving to reproduce in each of those habitats against the likelihood of dying in the plankton while postponing metamorphosis (Doyle 1975). The benefits of becoming less choosy with time and eventually metamorphosing into suboptimal habitat will be increased if postmetamorphic fitness declines as the larval stage is prolonged (Pechenik 1990).

To date, reduced juvenile or adult performance (decreased survival, decreased juvenile growth rates, reduced fecundity) associated with prolonged larval life has been documented in the laboratory primarily for

[a] Author for correspondence. E-mail: Jan.Pechenik@tufts.edu

species with non-feeding larvae: the bryozoans *Bugula stolonifera* (Woollacott et al. 1989), *B. neritina* (Wendt 1996), *Celleporella hyalina* (Orellana & Cancino 1991), and *"Hippodiplosia" insculpta* (Nielson 1981); the polychaete *Capitella* sp. I (Pechenik & Cerulli 1991); the barnacle *Balanus amphitrite* (Pechenik et al. 1993); and the sponge *Sigmadocia caerulea* (Maldonado & Young 1999). In contrast, the metamorphosis of feeding larvae can generally be delayed for long periods without measurable adverse effects (Pechenik & Eyster 1989; Rumrill 1989; Miller 1993). It is difficult to generalize, however, as periods of starvation or dramatic declines in phytoplankton concentration during larval life can also compromise postmetamorphic growth rates for some planktotrophic species (Qian et al. 1990; Pechenik et al. 1996a, b). Similarly, the reduction in postmetamorphic fitness caused by prolonging larval life in the crab *Chasmagnathus granulata,* is thought to reflect a decline in feeding activity by late-stage megalopae (Gebauer et al. 1999). On the other hand, delaying metamorphosis of the serpulid polychaete *Hydroides elegans* reduced juvenile survival and growth rates to the same extent whether or not the larvae were fed during the delay period (Qian & Pechenik 1998).

This paper reports the effects of prolonging larval life on the postsettlement survival and growth of the sipunculan *Apionsoma* (= *Golfingia*) *misakianum,* distinguishing between effects on individuals that were fed in the laboratory and those that were not. Because the larvae of *A. misakianum* are found throughout the Gulf Stream, they may routinely spend long periods of time exposed to the low food concentrations characterizing the open ocean (Scheltema & Hall 1975). If natural food concentrations are insufficient to support basic metabolic requirements, larvae should stop growing (and lose weight) as they continue to delay their metamorphosis in the field. We therefore include preliminary data on metabolic rates and on the ability of field-collected larvae to grow when fed excess food in the laboratory.

Experiments concerning the consequences of delayed metamorphosis require the use of larvae that can be readily induced to metamorphose. Larvae of *A. misakianum* meet this requirement, as they readily metamorphose in response to adult-conditioned seawater in the presence of sediment (Rice 1986). However, because the stimulatory factors in adult-conditioned water are unknown, it is not possible to provide identical concentrations in sequential experiments. To search for a more quantifiable metamorphic inducer, we therefore investigated the ability of elevated external K^+ concentrations to induce metamorphosis in this species. Such treatment has induced metamorphosis of at least some gastropod, polychaete, hydrozoan, bryozoan, and echinoderm larvae, but has not proved effective for larvae of bivalves, barnacles, or corals (reviewed by Pearce & Scheibling 1994; Woollacott & Hadfield 1996). This study constitutes the first test of responsiveness of sipunculan larvae to excess K^+.

Apionsoma misakianum is well suited to these studies: competent larvae can be reliably obtained in large numbers from the Florida Current and Gulf Stream at certain times of year (Hall & Scheltema 1975; Rice 1978, 1981, 1986), they will settle and metamorphose in response to sediment and adult-conditioned seawater (Rice 1981, 1986), they tend not to metamorphose in the absence of sediment (Rice 1986), and they can survive without feeding for many weeks in the laboratory without losing the ability to metamorphose. Rice (1978) presents a detailed description of the morphological changes accompanying metamorphosis in this species.

Methods

Larval response to elevated K^+

Larvae were collected from the Florida Current off the east coast of Florida over bottom depths of ~100 m. To test the effectiveness of elevated external K^+ in inducing metamorphosis in individuals of *A. misakianum,* we raised the concentration of this ion by adding KCl either to MBL artificial seawater (Cavanaugh 1956), adult conditioned MBL seawater, or to filtered natural seawater with or without sediment present; K^+ concentrations were raised between 3 and 40 mM. Individuals were examined 3–4 d later for the loss of metatrochal cilia, a criterion for the onset of metamorphosis (Rice 1986). One experiment was conducted in 1987 using 20 larvae per replicate with 3 replicates per treatment, and a second experiment was conducted in 1993 using 10 larvae per replicate with 3 replicates per treatment.

Effects of delayed metamorphosis on survival and growth

Approximately 1,000 larvae, tentatively identified as *Apionsoma misakianum,* were collected in plankton samples taken on December 31, 1991 in the Florida Current ~33 km east of the Fort Pierce Inlet over bottom depths of ~100 m, as described elsewhere (Rice 1986). Four days after larvae were sorted, ~600 of them were distributed among three treatment groups. Larvae in the first treatment group (control) were immediately distributed among 6 bowls containing sediment and adult-conditioned seawater (15 larvae in 80 ml of seawater per bowl, see below), to induce meta-

morphosis. Larvae in the other 2 treatment groups were maintained at room temperature (~25°C) in glass bowls (10 groups of larvae, with 50 larvae and 160 ml seawater per bowl) for either 2 or 4 additional weeks. Half of these larvae were maintained in seawater filtered to 0.22 μm, while the other larvae were fed the naked flagellate *Isochrysis galbana* (clone T-ISO) at about 1.5×10^4 cells ml^{-1}. Larvae were moved to clean glassware with fresh seawater or algal suspensions every other day until metamorphosis was induced. At 2 weeks, 90 larvae were subsampled from each of the 2 treatment groups and induced to metamorphose (6 replicates, 15 larvae per replicate) using adult-conditioned seawater and sediment. Two weeks later, an additional 90 larvae were subsampled from each of the 2 treatment groups and induced to metamorphose, again with 6 replicates and 15 larvae per replicate.

Adult-conditioned seawater used to stimulate metamorphosis was prepared by placing 20 adults in 500 ml for 24 h before use. Sieved sediment of less than 102 μm particle diameter was then added to the seawater before adding larvae. The methods for inducing metamorphosis have been described by Rice (1986). Dishes containing metamorphosed juveniles and abundant sediment were then maintained in tables of flowing seawater to allow for juvenile growth.

Six weeks after larvae were exposed to adult-conditioned seawater and sediment, all juveniles were gently sieved from the sediment and preserved in 10% formalin buffered to a pH of ~8.0 with sodium borate. These individuals were later rinsed briefly in distilled water, transferred to preweighed foil pans, dried for 8 h at 75°C, and weighed to the nearest μg using a Cahn Model 21 electrobalance. Indicating anhydrous $CaSO_4$ (Drierite) was placed in the weighing chamber to prevent specimen rehydration. All individuals retrieved from a replicate were weighed together, and the average juvenile weight was determined for each of the six replicates per treatment. Mean juvenile weights (i.e., growth rates) were compared by one-way analysis of variance, followed by a Tukey-Kramer or Dunnett's Multiple Comparisons test where appropriate. All data met the criterion of homogeneity of variances (Bartlett's test, $p > 0.10$), justifying the use of parametric statistics for data analysis.

Larval respiration rates

Larval respiration rates were estimated in order to predict rates of weight loss in the absence of food. A single measurement was made in 1980 using all-glass microrespirometry (Grunbaum et al. 1955), with 25 freshly collected larvae swimming in 0.5 ml of Florida Current seawater at 25°C. Readings were taken every 30 min for 3 h. Larvae were then preserved in 10% formalin buffered with sodium borate and later weighed in a single group as described above for juveniles.

Stimulating growth in field-collected larvae

To determine if field-collected larvae of *A. misakianum* could be stimulated to grow in the laboratory, 50 larvae were collected from plankton tows made at 38°57.2′ N and 61°13.2′ W in the Gulf Stream on June 28, 1993 (25.2°C water temperature). Twenty-five larvae were preserved immediately in 10% formalin (buffered in seawater with sodium borate to maintain a pH of about 8) for later determination of initial mean weight. The remaining 25 larvae were held at 25°C in the laboratory for an additional three weeks. These larvae were transferred every second or third day to new algal suspension (*Isochrysis galbana,* clone T-ISO, at 18×10^4 cells ml^{-1}) and preserved in buffered formalin on July 20. For weight determinations, preserved larvae were quickly rinsed free of formalin and seawater in 3 baths of distilled water and then transferred on the tip of a pin to preweighed foil pans, 25 larvae being transferred to each of the two pans. Specimens were dried for 48 h at 70°C and weighed to the nearest 1 μg using a Cahn Model 21 electrobalance, with desiccant placed in the weighing chamber to prevent specimen rehydration during weighing.

Results

In a pilot study with 10 larvae per treatment and no replication, all larvae exposed to 20–40 mM excess K^+ in seawater without sediment were still actively swimming 10 h later. In the 2 subsequent, more detailed studies, excess K^+ did not significantly ($p>0.10$) increase the percentage of larvae metamorphosing in seawater or in response to adult-conditioned seawater and did not inhibit larval responsiveness to the combination of sediment and adult-conditioned seawater (Fig. 1 & Fig. 2). Larval mortality never exceeded 10% in any treatment, and there was no indication of greater mortality at higher concentrations of excess K^+; all individuals were recovered from most replicates of all treatments. Because excess K^+ did not induce metamorphosis in *A. misakianum,* adult-conditioned seawater was used to induce metamorphosis in all experiments testing the effects of delayed metamorphosis on postmetamorphic survival and growth.

When exposed to adult-conditioned seawater and sediment, all larvae disappeared into the sediment within a few minutes to 24 h, regardless of how long

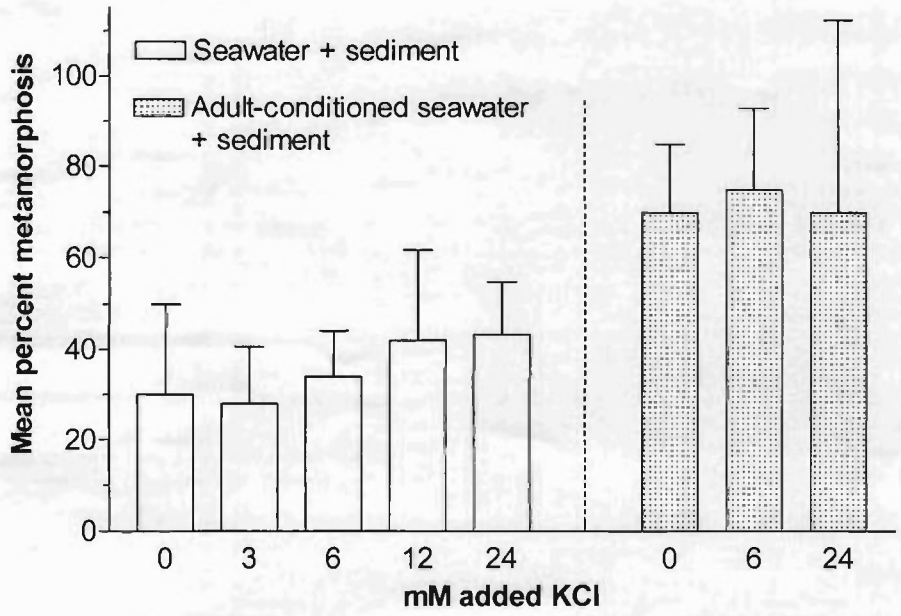

Fig. 1. Influence of excess K^+ (added as KCl) on metamorphosis of the sipunculan *Apionsoma misakianum*. Larvae were collected from the Florida Current on Feb. 25, 1987, exposed to excess K^+ in artificial seawater with sediment (open bars) or adult-conditioned seawater (20 adults incubated in 500 ml for 24 h, speckled bars) with sediment on March 6, and examined for metamorphosis 3 d later. Each bar represents the mean + standard deviation of 3 replicates, with 20 larvae per replicate. Adult-conditioned seawater increased the percentage of larvae that metamorphosed (one-way ANOVA, $F = 3.07$; d.f. = 7,15; $p = 0.033$), but excess K^+ neither stimulated nor inhibited metamorphosis.

they had been kept swimming. Between 60% (4 week delay, fed) and 85% (2 week delay, fed) of juveniles were recovered 6 weeks later. Differences between mean percent recovery (i.e., survival) were not statistically significant (one-way analysis of variance: $F = 1.7$; d.f. = 4, 25; $p = 0.18$), so that neither starvation nor length of larval life affected post-settlement survival.

However, larval feeding regime influenced mean juvenile growth rate when larvae were kept swimming for 2 weeks (Fig. 3); mean juvenile growth rate was significantly lower ($p<0.01$) than that of control individuals if larvae had been starved, but not if they had been fed. Mean juvenile growth rates were also significantly reduced relative to that of control individuals ($p<0.01$) for larvae whose metamorphosis was delayed for the longest period of time, whether or not the larvae had been fed phytoplankton (Fig. 3). Growth rates of individuals held in the laboratory as larvae for 4 weeks were significantly lower than those of individuals held in the laboratory for 2 weeks ($p<0.01$).

Mean larval respiration at 25°C was 4.74×10^{-2} µl oxygen consumed/h/larva. Mean dry tissue weight was

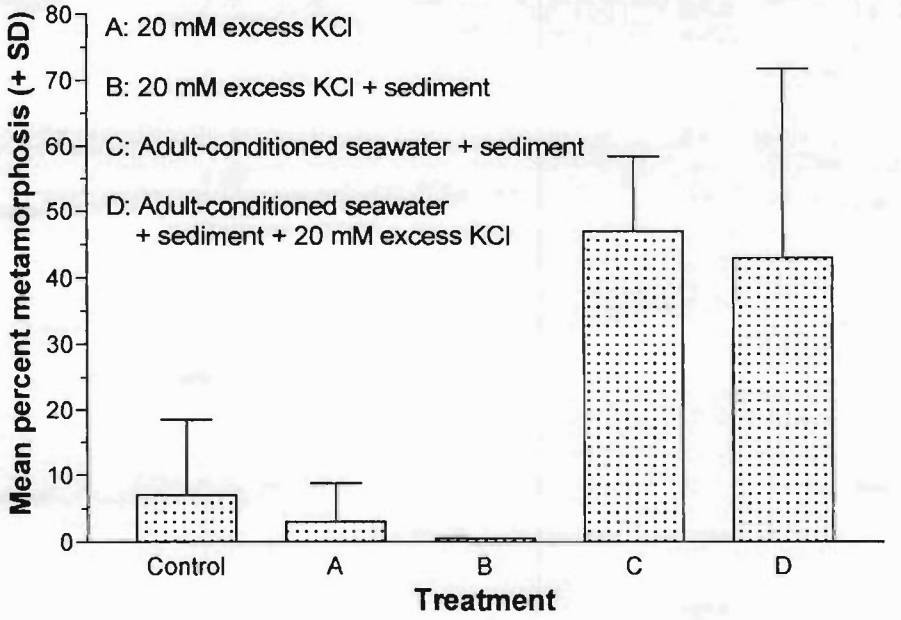

Fig. 2. Response of *Apionsoma misakianum* larvae to 20 mM excess K^+ (added as KCl) in the presence or absence of sediment. Three replicates of 10 larvae each were tested for each treatment. Larvae were collected in plankton tows on June 28, 1993. Sediment was collected on April 21, 1993 20 miles E. of Fort Pierce Inlet. Larvae were tested beginning July 6, 1993 and examined for metamorphosis 3 days later.

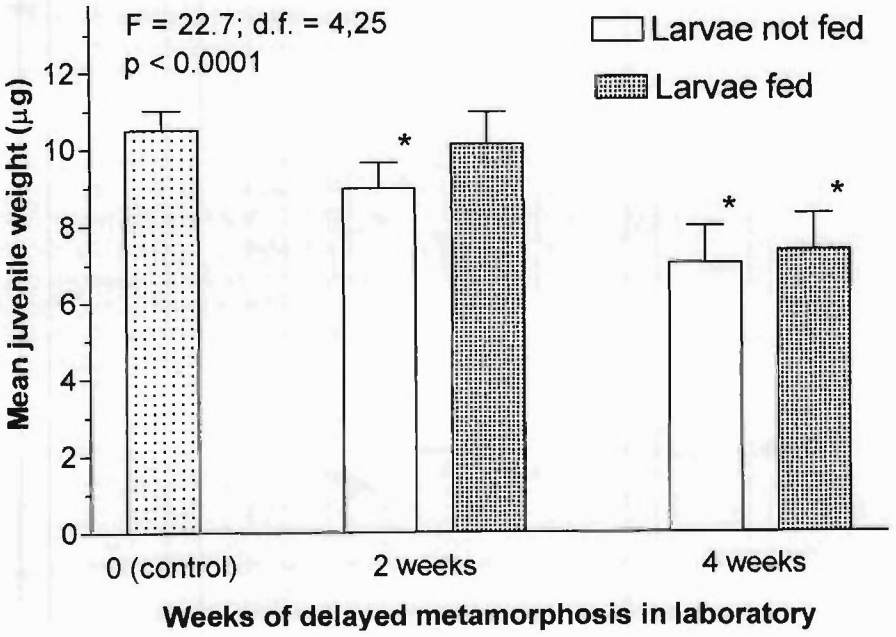

Fig. 3. Influence of delayed metamorphosis on post-metamorphic growth rate in the sipunculan *Apionsoma misakianum*. Each bar represents the mean (+ standard deviation) of 6 replicates, with 20 larvae per replicate. The larvae were collected from the Florida Current on December 31, 1991. Control larvae were induced to metamorphose 4 d later. In all treatments, juveniles were harvested for weighing 6 weeks after being exposed to adult-conditioned seawater in the presence of sediment. One-way Analysis of Variance results are indicated on the figure. The * signifies means that differ significantly from the control mean (Dunnett's Multiple Comparisons test).

7.29 μg per larva, so weight-specific oxygen consumption was about 6.5×10^{-3} μl oxygen consumed/h/μg tissue.

Larvae did not increase in biomass during 3 weeks of laboratory culture with excess phytoplankton; indeed, average larval weight declined in the laboratory from 13.5 to 10.5 μg per individual.

Discussion

Baloun & Morse (1984) found that increasing the K^+ concentration of seawater induced metamorphosis in the gastropod mollusc *Haliotis rufescens*. Excess K^+ has subsequently been found to stimulate metamorphosis in a number of other species, including other gastropods, bryozoans, echinoids, sponges, hydrozoans, and polychaetes (Avila 1998; Boettcher & Targett 1998; Pechenik & Qian 1998; Biggers & Laufer 1999; older literature reviewed by Pearce & Scheibling 1994; Woollacott & Hadfield 1996). Its widespread effectiveness suggested that excess K^+ stimulates metamorphosis by depolarizing external receptor cells (Baloun & Morse 1984; Yool et al. 1986; Baxter & Morse 1987; Chevolot et al. 1991; Ilan et al. 1993). If that is a singular and universal effect, we would expect all competent marine invertebrate larvae to metamorphose in response to excess K^+, regardless of what chemical cues trigger metamorphosis naturally (Yool et al. 1986). Yet, larvae from a number of species in a variety of groups are not stimulated to metamorphose by excess K^+ (reviewed by Pearce & Scheibling 1994) even when they are competent to respond to natural cues, as with *A. misakianum* in this study. This suggests that potassium exerts its effects at sites other than surface receptors in at least some species, and perhaps through different pathways in different species. In support of this alternative view, larvae of the nudibranch *Phestilla sibogae* and the polychaete *Hydroides elegans* first became responsive to excess K^+ at least 24 h *after* becoming responsive to other external cues (Pechenik et al. 1995; Pechenik & Qian 1998). Moreover, excess potassium inhibited the nudibranch larvae from metamorphosing under certain conditions, while the natural cue remained effective under those same conditions (Pechenik et al. 1995). Excess K^+ also inhibited metamorphosis of the opisthobranch gastropod *Adalaria proxima* (Todd et al. 1991) and the barnacle *Balanus amphitrite* (Rittschof et al. 1986). Excess K^+ is clearly not a universal inducer of metamorphosis. Understanding how it triggers metamorphosis in some species, why it takes longer to act in some species than others (reviewed by Pechenik & Qian 1998), why it does not trigger metamorphosis in some species, and why it inhibits the metamorphosis of still other species would greatly increase our understanding of how metamorphic pathways operate and develop.

Our data on *A. misakianum* larvae add to the growing recognition that prolonged larval life can reduce postmetamorphic fitness (reviewed by Pechenik et al. 1998). For example, postponing metamorphosis of competent *Capitella* sp. 1 larvae increased postsettlement mortality (Pechenik & Cerulli 1991), while postponing the metamorphosis of the polychaete *Polydora ligni* (Qian et al. 1990), the barnacle *Balanus amphitrite* (Pechenik et al. 1993), and several bryozoan spe-

cies (Nielsen 1981; Woollacott et al. 1989; Wendt 1996, 1998) reduced rates of juvenile growth or development. For species with non-feeding larvae, the negative effects of delayed metamorphosis are likely mediated through depletion of energy reserves or critical nutrients (Jaeckle 1994; Satuito et al. 1996; Wendt 1996; Maldonado & Young 1999). For species with feeding larvae, the source of the effect on postmetamorphic performance is less clear. Pechenik et al. (1996a, b) have shown that short periods of starvation or food limitation during larval life can lead to decreased growth rates in the prosobranch gastropod *Crepidula fornicata.* If the reduced juvenile growth rates that we have documented here for *A. misakianum* larvae were mediated solely or primarily through nutrient limitation during the delay period, the effect should have been greater for larvae that were starved before being stimulated to metamorphose. For larvae maintained in the laboratory for 2 weeks, mean juvenile growth rates were significantly below those of control juveniles if larvae had been starved, but not if the larvae had been fed; delayed metamorphosis itself had no apparent effect on juvenile growth rate. However, juvenile growth rates were reduced to the same degree for those held for 4 weeks whether larvae were fed or not; a similar response was reported for the polychaete *Hydroides elegans* (Qian & Pechenik 1998). Either the effects of 4 weeks in the laboratory on juvenile growth were caused by delayed metamorphosis per se, independent of nutritional stress, or the larvae in both treatment groups experienced equivalent food limitation. The guts of our larval sipunculans remained green throughout the 4-week laboratory culture period, indicating that they were ingesting the phytoplankton provided. Even so, the phytoplankton (T-ISO) used in this study may have lacked some micronutrients essential for sustained growth and development (reviewed by Pechenik 1987). The phytoplankton may have met all nutritional requirements for the first few weeks in the laboratory, but not for longer periods.

We can predict rate of weight loss for starving larvae of this species from respiration data, since respiration rates were determined at the same temperatures at which the larvae were reared, 25°C. Assuming an RQ of 0.8 and that 50% of ash-free dry weight is carbon (Nichols 1975), the larvae should have lost nearly 20 μg AFDW (~2× their total initial weight) over the three weeks of laboratory culture if the diet was completely inadequate and unmetabolizable. Instead, the larvae lost, on average, only 3 μg during that 3-week period, suggesting that T-ISO provided some nourishment. But the decline in average dry tissue weight of the sipunculan larvae held in laboratory culture on a diet of T-ISO certainly suggests that the diet was indeed suboptimal. It seems probable that larvae experienced some degree of nutrient limitation in both treatments of this experiment, at least by the 4th wk in laboratory culture. Nutrient limitation in larval life can lead to reduced juvenile growth rates (Pechenik et al. 1996a, b). To what extent do larvae of this species experience nutrient limitation at sea?

As phytoplankton concentrations are low in tropical waters, and patchily distributed throughout the ocean (Bienfang et al. 1984; Cowles et al. 1993), and as the larvae of this species are likely to spend 3–4 months traversing the N. Atlantic before encountering habitat that will appropriately stimulate their metamorphosis (Scheltema & Hall 1975), it seems likely that these widely dispersed individuals will experience at least some periods of food limitation in the field. Rice (1981) has previously suggested that sipunculan larvae do not grow in the open ocean. Larvae of the gastropod *Smaragdia* sp. obtained from the same open ocean samples containing *A. misakianum* individuals metamorphosed in response to eel grass only after feeding on phytoplankton in the laboratory for several days (Pechenik, unpubl. data), suggesting that they had been nutritionally stressed in the field. Determining average juvenile growth rates for individuals collected from different locations as larvae and induced to metamorphose in the laboratory, would suggest the extent to which delaying metamorphosis and/or nutrient limitation during the delay period compromise postmetamorphic growth rates in this species in the field (Jarrett & Pechenik 1997). The present study indicates that individuals of this species are open to such potential fitness costs.

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