



Effects of the duration of larval life on postlarval stages of the demosponge *Sigmadocia caerulea*

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Abstract

Variability in the duration of the free-swimming period of lecithotrophic larvae of the demosponge *Sigmadocia caerulea* (Hechtel, 1965) was assessed in the laboratory in the absence of metamorphic inducers and inhibitors. The free-swimming period in three clutches of larvae ranged from 8 to 70 h and the cumulative settlement curve was sigmoid, with settlement peaking between 20 and 28 h after release. There were significant differences among clutches in the time to reach 50% settlement. Within each clutch, small percentages of larvae (< 25%) were short-lived (settled less than 12 h after release) or long-lived (settling more than 32 h after release). Juveniles originating from short-lived larvae survived better, grew faster and were more regular in shape than those originating from long-lived larvae. In additional laboratory experiments, we examined the consequences of feeding during the early postsettlement period. Size differences between fed and starved juveniles became apparent 5 days after osculum formation, suggesting that the filter-feeding ability does not begin immediately after osculum formation. Our results are consistent with the hypothesis that long-lived larvae bring fewer reserves to the postsettlement period than do short-lived larvae, thereby experiencing nutritional stress prior to the onset of feeding that can reduce the vigor of the juveniles. Although larvae delaying metamorphosis should theoretically be important in colonizing new habitats and increasing gene flow, low postsettlement survival of such individuals may reduce the evolutionary importance of variability in the duration of the larval period. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

In benthic marine invertebrates that rely upon a planktonic larval stage for dispersal, the capacity to delay metamorphosis is generally considered to be advantageous. Extending the larval period should increase the likelihood that larvae will locate suitable habitats (Thorson, 1950; Scheltema, 1974; Pechenik, 1990), reduce the probability of intraspecific competition for limited space or other resources, spread the risk in variable environments (Strathmann, 1974), and increase gene flow among distant portions of populations. However, lecithotrophic larvae delaying metamorphosis also consume energy reserves during the delay period. Overconsumption of reserves could compromise the vigor of subsequent developmental stages where these reserves are needed. Indeed, abnormal metamorphosis, poor juvenile survival, and slow juvenile growth have been observed in lecithotrophic larvae of barnacles, bryozoans, polychaetes and crinoids that were experimentally induced to extend their swimming periods (Lucas et al., 1979; Nielsen, 1981; McEdward et al., 1988; Woollacott et al., 1989; Pechenik and Cerulli, 1991; Pechenik et al., 1993).

In most recent studies of the consequences of delaying metamorphosis, the duration of the swimming period has been manipulated experimentally using either metamorphic inducers (reviewed by Pawlik, 1992) or inhibitors (Pechenik, 1990). This approach has yielded many important insights into the energetics of larvae and juveniles, but it does not help us to understand the role of inherent (noninduced) variability in the duration of the planktonic period. The existence of such variability is well known. For example, numerous studies report small percentages of very short-lived and long-lived larvae in the absence of external settlement cues, and under apparently uniform conditions in the laboratory (e.g. Strathmann, 1978; Hadfield, 1984; Woollacott, 1984, 1990, 1993; Svane and Young, 1989; Rumrill, 1990; Bachelet et al., 1992; Maldonado and Young, 1996). The biological significance and the potential postlarval effects of this intra-clutch variability remain poorly understood. On the one hand, there is the belief that late developers within a batch of sibling lecithotrophic larvae may represent 'low quality' offspring (e.g. Woollacott, 1993). Alternatively, it has been suggested that late developers may represent an adaptation that provides flexibility needed for success in spatially and temporally variable environments (e.g. Strathmann, 1974; Gibson, 1995).

By using lecithotrophic larvae (parenchymella type) of the haplosclerid sponge *Sigmadocia caerulea* (Hechtel), we tested for the first time in the phylum Porifera the validity of the two competing hypotheses mentioned above. We assessed intra-clutch variability in the duration of the free-swimming period and determined its effects on the survival, growth rate and morphology of the juveniles. As in other lecithotrophic larvae, that portion of the yolk remaining after differentiation of the parenchymella is thought to provide energy for the metabolic demands of larval dispersal and metamorphosis. Moreover, variability in the duration of larval life is likely to have important effects on early postlarval stages if remnants of the larval yolk are also to be used to sustain the early juvenile stage. Some reports suggest that the filter-feeding system of young sponges may only become functional several days or even weeks after settlement (Boury-Esnault, 1976; Uriz, 1982; Fell, 1989). Therefore, an additional aim was to determine whether early postlarval stages of *S. caerulea* were initially sustained with

reserves transferred from the larva or whether they feed on particulate food immediately after osculum formation.

2. Materials and methods

2.1. Animal collection and culture

The haplosclerid demosponge *Sigmatocia caerulea* is common throughout the Caribbean (Zea, 1987) and on the Pacific coast of Panama (Wulff, 1996). This species incubates early embryos and releases lecithotrophic, free-swimming parenchymella larvae. During August and September of 1994, we collected ripe individuals from the Indian River Lagoon near Fort Pierce, Florida, USA. Larval release often occurred spontaneously immediately after collection, but was also triggered artificially by exposing adults to air for a few seconds (Maldonado and Young, 1996). Larvae were expelled through the oscules of the parental sponge for about 1 h, then collected from a shaded portion of the dish, where they tended to congregate. For our experiments, all larvae were considered to be 1 h old at this point. Parenchymellae of *S. caerulea* are vigorous swimmers that swim with a clockwise corkscrew motion for a relatively short planktonic period of between 1 and 3 days. When settlement approaches swimming speed gradually decreases and larvae adopt an ‘exploratory’ behavior moving close to the bottom of the containers (demersal or crawling phase). At the time of settlement, larvae position themselves perpendicular to the bottom, with the anterior pole oriented toward the substratum, and they spin with clockwise rotation around the future settlement spot (Maldonado and Young, 1996; Maldonado et al., 1997). We counted as a settler the stage at which rotation ceased and the larva was irreversibly attached to the substratum by the anterior pole to initiate metamorphosis. Settlers for the experiments were obtained by placing larvae in small polystyrene petri dishes. Each dish contained 30 ml of 0.45 μm filtered seawater (salinity = 34‰, temperature = 22–24°C) that was replaced daily.

2.2. Duration of the free-swimming period

To describe the variability in duration of the free-swimming period within a clutch, 15 batches of 10 larvae were placed into polystyrene dishes and cultured under the conditions mentioned above. Dishes were arrayed randomly on a black laboratory bench and maintained on a 18 h light–6 h dark cycle for 3 days. Larvae were monitored at 4–8 h intervals to record the cumulative percentage of settlement in each dish over time. From previous observations and preliminary experiments (Maldonado and Young, 1996; Maldonado et al., 1997), we determined that settlement of larvae within a dish did not induce or inhibit settlement of the remaining swimmers. Therefore, settlement of each individual is assumed to represent an independent event not influenced by exogenous cues. The experiment was repeated three times during August and September, using a clutch produced by a different sponge on each occasion. The three individuals we used represented only a small percentage of the many individuals brought into the laboratory,

as the majority of adults did not release larvae or released too few larvae for the experiments. Differences among clutches in the time to 50% cumulative settlement were analyzed by Kruskal–Wallis one-way analysis of variance, as data failed the normality test (Kolmogorov–Smirnov test) after transformation. A posteriori pairwise comparisons were made using the nonparametric analogous of the Student–Newman–Keuls (SNK) test (Zar, 1984).

2.3. Effects of the duration of the free-swimming period on postlarval stages

To investigate if differences in the duration of the swimming period within a clutch of larvae had an effect on early postsettlement stages, we compared survival, growth rate and body shape among sibling juveniles that developed from short-lived and long-lived larvae. We assume, as others have (e.g. Woollacott et al., 1989, Pechenik et al., 1993) that differences observed under uniform laboratory conditions reflect inherent genetic differences in the overall vigor of individuals.

Upon release, larvae were transferred to a 4-l glass bowl of 0.45 μm filtered seawater (salinity = 34‰, temperature = 22–24°C). Those larvae that initiated a crawling behavior (indicative of the onset of metamorphosis) were transferred from the bowl to a set of small polystyrene dishes for settlement. Larvae still unsettled 12 h after release were removed in order to obtain a set of juvenile sponges originating exclusively from short-lived (≤ 12 h old) larvae. Similarly, those larvae still swimming in the large bowl after a period of 32 h were transferred to a different set of small dishes to obtain sponges exclusively developed from long-lived (≥ 32 h old) larvae. All but three juveniles were removed from each dish to minimize the potential effects of crowding on survival, growth and shape. By this procedure, we obtained two sets of twenty-five culture dishes, each containing three juvenile sponges that developed from either short-lived or long-lived larvae settled without the use of metamorphic inducers or inhibitors. Dishes were then distributed at random in three aquaria (50 l) and maintained in the laboratory for 17 days on a 18 h light–6 h dark cycle. Sponges were fed daily by providing unfiltered natural seawater from their natural habitat (salinity = 33‰, temperature = 22–24°C).

Mortality was expressed as the cumulative percentage of dead sponges per dish. Differences in mortality per dish between the two groups of juveniles after 17 days in the laboratory were analyzed by the Mann–Whitney rank sum test. This nonparametric test was used because the percentage data were not normally distributed after transformation.

Sponge growth (in area) and the overall changes in shape (patch contour) were measured over time through a dissecting microscope connected to an integrated image analysis system using MicroComp software. Overall changes in patch shape were assessed by using the circularity index (C) wherein:

$$C = A_s/A_p$$

where A_s = projected area of the object and A_p = area of a circle with perimeter equivalent to that of the object (Maldonado et al., 1997). Measurements of area and

circularity were assumed to be good descriptors of growth dynamics at early life-history stages, because juvenile sponges are flat patches that experience a negligible growth in height for the first 2 months. Sponge circularity was assumed to be a useful tool to assess differences in body form between sponges that developed from long-lived and short-lived larvae. Young sponges tend to form nearly circular patches. Irregular shapes may be indicative of any type of anomalous development, as also documented for other sessile organisms (e.g. Woollacott et al., 1989). Differences in mean size and mean circularity per dish between the two groups of juvenile sponges after 17 days under laboratory conditions were analyzed by *t*-tests. Mean sponge size and mean sponge circularity per dish were calculated on unequal numbers of individuals because of mortality. Dishes where all sponges died were discarded and not included in the analyses (i.e. one dish in the set of short-lived larvae, six dishes in the set of long-lived larvae).

2.4. Postlarval feeding on yolk reserves

To determine whether early postlarval stages need particulate food immediately after osculum formation or whether they can be initially sustained with storage products transferred from the larva, we compared body size between starved and fed juveniles. For this experiment, we used only sibling juveniles that developed from larvae that swam between 20 and 32 h. Juveniles were cultured in small polystyrene dishes (5–10 sponges per dish) for 11 days after settlement. Each dish was maintained in a culture jar containing 1 l of seawater. Starved sponges were reared in artificial seawater (Instant Ocean[®]) with no added food. Fed sponges were reared in unfiltered, natural seawater collected from the sponge habitat. In both cases, water in cultures was changed daily. Sponge growth was assessed by measuring the projected area in 40 randomly selected individuals 3, 5 and 7 days after osculum formation. Each juvenile was measured only once, so size data are independent over time. Differences in size between food treatments were analyzed by a standard 2-way analysis of variance (factors: food, time) without repeated measures. A posteriori comparisons were made using the Student–Newman–Keuls (SNK) test.

3. Results

3.1. Duration of the free-swimming period

Although sibling larvae were all maintained under virtually identical laboratory conditions, the total duration of the free-swimming period ranged between 8 and 70 h. Upon release from the adult sponge, larvae swam actively near the water surface for approximately 6 to 12 h. After that time, they began to move more slowly, remaining near the bottoms of the containers. They stopped repeatedly, then resumed swimming. This demersal presettlement phase lasted from a few minutes to 40 h, until larvae finally attached and started metamorphosis. The cumulative settlement curve was sigmoid in all three clutches, with settlement rates peaking between 20 and 28 h after release (Fig. 1). Between 4 and 16% of larvae in the three clutches were short-lived (i.e. they swam for

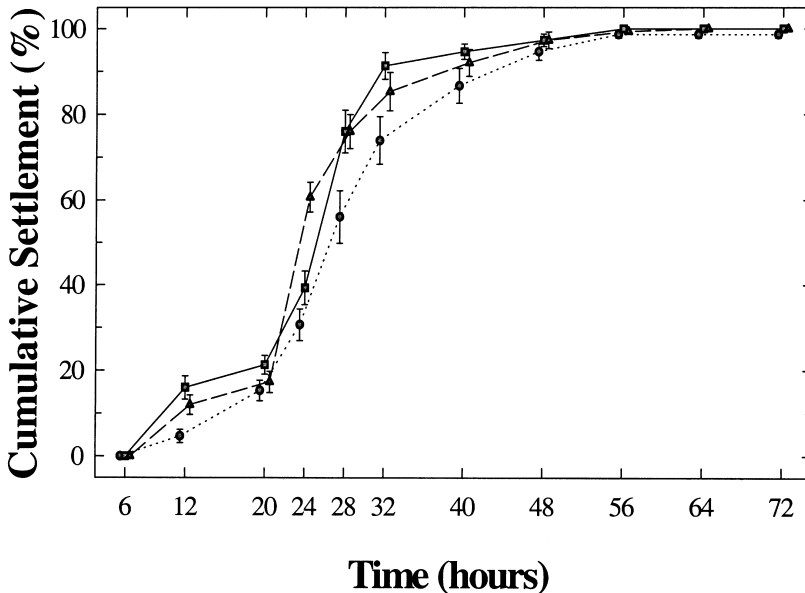


Fig. 1. Percent cumulative settlement (mean \pm S.E. per dish) over time in three clutches of larvae produced by three different individuals of *Sigmadocia caerulea*.

less than 12 h). Similarly, long-lived larvae (i.e. those swimming for more than 32 h) accounted for between 9 and 25%, depending upon clutch. The mean times (\pm S.E.) to 50% cumulative settlement per dish were 29 ± 1.2 h, 26.9 ± 0.6 h and 25.6 ± 1.0 h in the three clutches. A Kruskal–Wallis test detected significant differences among these values ($H=10.2$, $P=0.006$), with the last value being significantly lower than the other two (nonparametric analogous of the SNK test, $P<0.05$), which were not statistically different from each other.

A small number of larvae (between 0.5 and 1.3%, depending upon clutch) remained unattached after 3 days, apparently having passed the period of metamorphic competence.

3.2. Effects of the duration of the free-swimming period on postlarval stages

The duration of larval life had a pronounced effect on the survival of the juveniles ($N=25$, $T=416$, $P<0.001$). Juveniles that developed from short-lived larvae experienced very low mortality ($7.9 \pm 4\%$) during the first 17 days under laboratory conditions (Fig. 2). By contrast, juveniles that developed from larvae that delayed settlement for more than 32 h experienced higher mortality ($58.6 \pm 7\%$). The incidence of mortality became important only 4 days after settlement.

The time that larvae swam prior to settlement also had significant effects on both size ($t=11$, $P<0.001$) and shape ($t=4.39$, $P<0.001$) of the surviving juvenile sponges. After 17 days, juveniles that developed from short-lived larvae were significantly larger

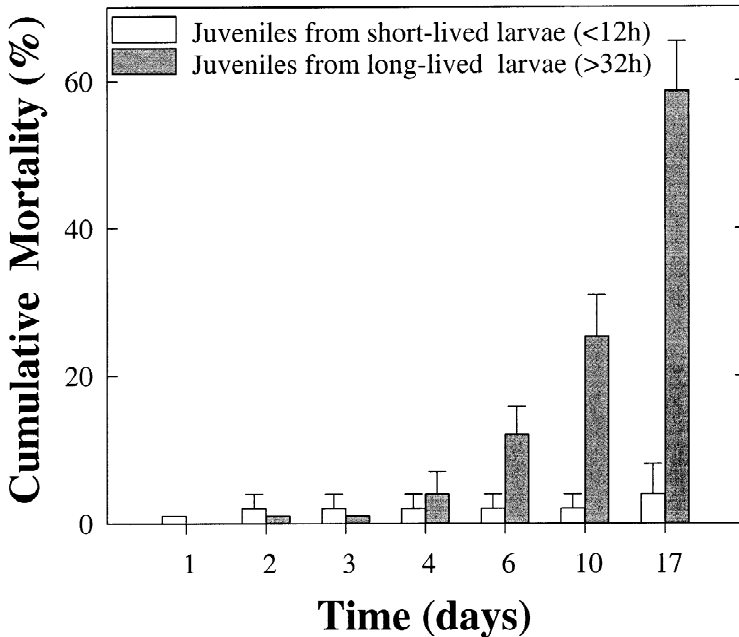


Fig. 2. Mortality incidence (cumulative percentage \pm S.E. per dish) among juvenile sponges that developed from short-lived and long-lived larvae, respectively.

(area = 2.137 ± 0.088 mm²) and more regular in shape ($C = 0.71 \pm 0.01$) than juveniles developed from long-lived larvae (area = 1.012 ± 0.101 mm², $C = 0.58 \pm 0.02$). However, the growth curves ran parallel in the two groups of sponges (Fig. 3).

Following settlement, the sponge body was a flat patch with several radial projections (Fig. 4A to C). After an initial expansion phase, the area of the juveniles decreased as the sponges became taller and began to form the spicule skeleton and the aquiferous system (Figs. 3 and 4D to E). The first osculum was formed as a finger-like structure between 100 and 124 h after release (about 2.5 days after settlement). After these changes, juveniles adopted the appearance of fully developed, functional sponges (Fig. 4F).

3.3. Postlarval feeding on yolk reserves

The first osculum appeared in *Sigmadocia caerulea* juveniles between 2 and 3 days after settlement (Fig. 4D to E), marking the end of metamorphosis. A two-way analysis of variance indicated that the presence/absence of particulate food during the first week after the formation of the osculum had a significant effect on the sizes of juvenile sponges (Table 1 Fig. 5). There was no significant difference in area between fed and starved juveniles within the first 3 days after opening of the osculum, but differences became apparent 5 days after osculum formation. There was also a significant interaction between time and food, indicating that differences in size between fed and starved

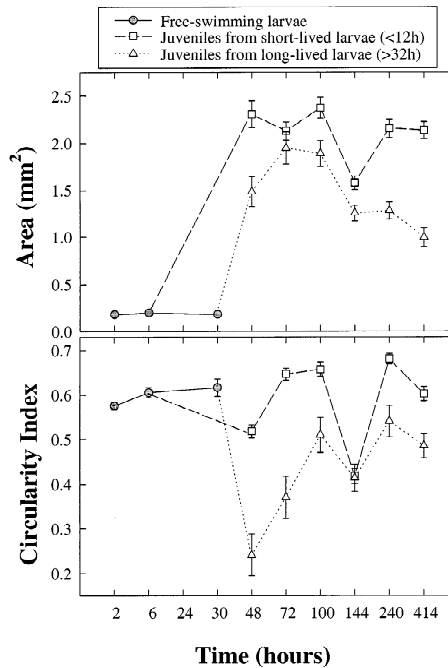


Fig. 3. Projected area and circularity (mean \pm S.E.) of larvae and settlers over time.

sponges increases with time. Continued starvation led to progressive shrinkage and ultimately death of the juveniles.

4. Discussion

In the absence of natural cues, settlement within a clutch of *Sigmatodia caerulea* larvae occurred gradually over a period of 3 days. Differences in settlement dynamics among clutches may be explained in terms of quantitative or qualitative differences in yolk provisioning. Differences in total protein content among offspring from different individuals of *S. caerulea* have been reported (Maldonado et al., 1997).

In species such as *Sigmatodia caerulea* where no apparent exogenous cues are required for metamorphosis, settlement could be triggered when energy reserves of the larva drop below some threshold level. If this were the case, the duration of the larval life would be determined by the amount of energy initially stored and by the rate of energy consumption. Short-lived and long-lived larvae would begin juvenile life with similar energy reserves, so they would experience similar rates of survival and growth. This is clearly not the case in *Sigmatodia caerulea*. Juvenile that develop from long-lived larvae experienced dramatically higher mortality and lower growth rates than juveniles from short-lived larvae.

It has often been assumed that differential mortality in the laboratory should reflect

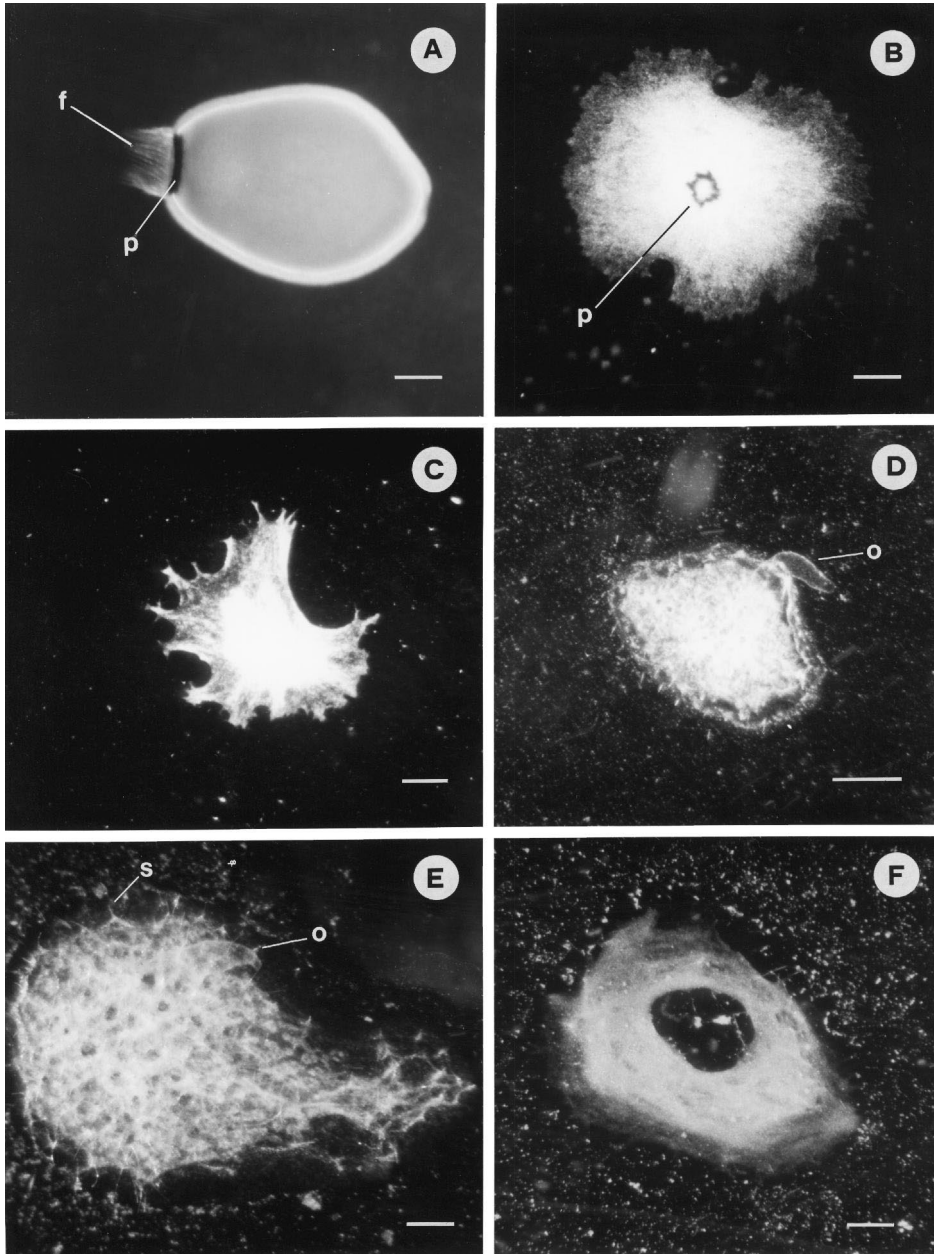


Fig. 4. *Sigmadocia caerulea*; (A) swimming parenchymella showing the posterior flagellar tuft (f) and the pigimentary ring (p). Scale bar = 100 μm ; (B) metamorphic stage 3 h after attachment, in which the pigimentary ring is still visible. Scale bar = 150 μm ; (C) metamorphic stage 30 h after attachment. Scale bar = 250 μm ; (D) 3-day old juvenile showing the oscular tube (o). Scale bar = 250 μm ; (E) 5-day old juvenile showing the oscular tube (o), hispidating spicules (s), and numerous choanocyte chambers and internal canals. Scale bar = 200 μm ; (F) 13-day old juvenile. The oscular tube has regressed and sponge tissues become denser. Scale bar = 250 μm .

Table 1

Two-way analysis of variance to test for differences in the area of juveniles as a function of time (3, 5 and 7 days after osculum formation) and the presence/absence of food^a

Source	df	MS	F	P
Time	2	0.348	1.370	0.256
Food	1	10.269	40.377	<0.001
Time*Food	2	5.340	20.992	<0.001
Error	234	0.254		

^a A posteriori comparisons are given in Fig. 5.

the ability of organisms to respond to adverse conditions in nature (e.g., Highsmith and Emler, 1986 (p. 350); Pechenik and Cerulli, 1991; Pechenik et al., 1993). Although laboratory mortality rates are measured in the absence of many natural selective pressures, they are nevertheless useful in assessing differences in the overall inherent vigor of organisms. We make no assumptions about how juveniles might respond to various sources of mortality in the field; we view our data as indicative of inherent traits of the larvae such as energy reserves.

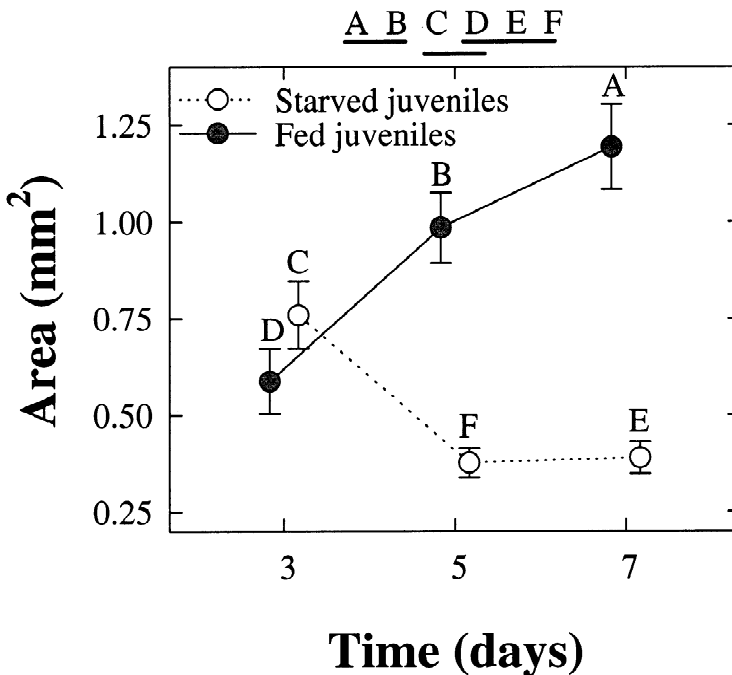


Fig. 5. Projected area (mean \pm S.E. mm²) of fed and starved juveniles at 3, 5, 7 days after osculum formation. Letters (A to F) indicate mean values arranged in descending order. Groups of underlined letters indicate nonsignificant differences between pairs of means according to a posteriori SNK tests ($P > 0.05$) following a two-way ANOVA (see Table 1).

Our results are consistent with a number of prior studies on lecithotrophic barnacles, bryozoans and polychaetes in which delayed settlement negatively affected both survival and growth rates during early postlarval stages (Lucas et al., 1979; Woollacott et al., 1989; Pechenik and Cerulli, 1991; Pechenik et al., 1993). These results contrast markedly with studies on planktotrophic polychaetes, barnacles, gastropods, nudibranchs and echinoderms in which no adverse effects were detected by experimentally prolonging the planktonic period for days or even weeks (Wilson, 1937; Kempf, 1981; Highsmith and Emler, 1986; Miller, 1988; Pechenik and Eyster, 1989). The most widely accepted explanation for this difference is that lecithotrophs experience nutritional stress during the delay period. Delay of metamorphosis causes energy reserves to fall below the level required to metamorphose successfully and reorganize tissues in early postlarval stages (Lucas et al., 1979; Woollacott et al., 1989; Pechenik et al., 1993). Some reports suggest that most of the energy provisioned in the egg are consumed during the larvae stage, leaving little for the juvenile. For example, McEdward et al. (1988) have estimated that a crinoid larva uses more than 80% of the energy contained in the egg during planktonic larval development and metamorphosis. In *Sigmadocia caerulea*, overconsumption of yolk caused by delayed settlement probably has a major influence on the success of the juvenile, as young juveniles appear to rely on storage products until their filter-feeding mechanisms are fully differentiated and functional.

It is difficult to determine when a particular individual makes the transition from lecithotrophy to filter feeding. There was no difference in size between juveniles of *S. caerulea* maintained with and without particulate food for the first 3–4 days after the osculum formed. By assuming optimal culture conditions in the laboratory, this result suggests that the filter-feeding system was not actually functional during this period, though it was apparently developed. Both sets of juveniles presumably survived during this 3-day, nonfeeding period by using storage products left over from the larval period.

We have presented laboratory evidence showing that the duration of the free-swimming period affects the survival chances of early juvenile sponges. The extent to which the duration of the free-swimming period varies in the field and how field recruitment is affected by delaying metamorphosis remains unknown. These data, although appearing laboratory-limited, are the first contribution from the phylum Porifera to the study of the variability in the duration of larval life. Our results lead to the notion that, although slow developers within a clutch of lecithotrophic larvae are theoretically important colonizers of new habitats and may maintain gene flow among populations, the importance of such individuals may be negligible because few will survive the early postsettlement stages.

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