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Depth regulation in parenchymella larvae of a demosponge: relative roles of skeletogenesis, biochemical changes and behavior

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ABSTRACT: To assess factors that influence depth regulation of sponge larvae, we documented ontogenetic changes in larval size and shape, lipid and protein content, skeletal development, and photoresponse in *Sigmadocia caerulea*, a shallow-water demosponge in the order Haplosclerida. We also measured size and biochemical differences among larvae from different parents to determine how depth regulation might vary across the population. Larvae were photonegative during the entire free-swimming period. Younger larvae swam faster than older larvae, but older larvae swam away from light for greater time and distances. Sinking rates of anesthetized larvae increased as a function of age, not because of lipid depletion or shape changes, but because addition of spicules increased density. Neither lipid nor protein changed significantly during larval life, but protein content increased abruptly just after settlement. Minor differences in length and protein content among offspring from different parents had no apparent effect on depth regulation. Both active movement and passive sinking play roles in moving late-stage larvae towards the sea floor, but increase in larval spicular mass appears to be the most important factor

KEY WORDS: Depth regulation · Larval behavior · Larval composition · Photobehavior · Sponge larvae

INTRODUCTION

Marine invertebrate larvae must generally have mechanisms for remaining suspended while dispersing and for returning to the bottom prior to settlement. Thorson (1964) proposed that ontogenetic changes in behavior, particularly photoresponses, often mediate the transition from the plankton to the benthos. However, changes in the buoyancy of larvae can also play a significant role. For example, ophiuroid larvae often undergo metamorphosis in the water column, thereafter falling to the bottom passively as heavy ossicles develop and ciliary swimming ability is lost (Emlet 1983). Anecdotal observations indicate that many larvae experience an increase in mass and a commensurate reduction in buoyancy near the end of larval life. The relative roles of active behaviors and passive sinking have been discussed for crustacean (Sulkin 1984) and echinoderm (Emlet 1983) larvae, but the proximate factors influencing sinking rate have seldom been analyzed.

Passive sinking rate of a small larva heavier than seawater is determined by the balance between the upward force of drag and the downward force of gravity pulling on the larva's mass. If we assume a Reynolds number (Re) less than 1, a spherical larva of radius a sinks at a terminal velocity U calculated as

$$U = 2a^2g(\rho - \rho_o)/9\mu$$

where μ is the dynamic viscosity of seawater, ρ is the density of the larva, ρ_o is the density of seawater and g is the gravitational constant (Vogel 1981). Drag can be increased or decreased during ontogeny by shape changes, though such differences tend to be small at low Reynolds numbers. For example, at Re = 1, a pro-

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late spheroid twice as long as its diameter has a slightly lower drag coefficient (11.17) than a sphere (11.69) of the same volume (Happel & Brenner 1973, Vogel 1981). Larvae may undergo ontogenetic changes in density by metabolizing buoyant lipids, synthesizing heavier proteins, or secreting skeletal elements.

Sponges have no integrated nervous system in either the adult or larval stage, so their behaviors should be very limited. Nevertheless, it has been reported that sponge larvae switch the signs of their phototactic and geotactic responses with age (e.g. Wilson 1935, Hartman 1958, Warburton 1966, Bergquist et al. 1970, 1979, Fell 1974, Wapstra & van Soest 1987). Forward (1988) has discussed potential laboratory artifacts associated with measurements of phototaxis, and Sulkin (1990) has encompassed the conditions under which such measurements are useful. Although the responses to light and gravity in the laboratory may not resemble the actual behaviors exhibited in the field, changes in response between stages are nevertheless indicative of physiological processes taking place within the larvae. All known sponge larvae are lecithotrophic and presumably metabolize storage products during the course of larval life, so it is also possible that density changes help larvae move toward the bottom. There are, however, no data whatsoever on the biochemical composition of larval or juvenile demosponges.

In this study, we document various changes that occur during larval life of *Sigmadocia caerulea* (Hechtel 1965), a shallow-water demonsponge in the order Haplosclerida. This species is common throughout the Caribbean and on the Pacific coast of Panama (Zea 1987). Our goal was to measure ontogenetic changes in the various parameters that could influence the ability of larvae to find the bottom at the end of larval life, namely: (1) changes in larval shape and size that might influence drag, (2) changes in photoresponse and swimming speed that might cause larvae to move away from the surface, and (3) changes in biochemical composition (i.e. relative amounts of proteins and buoyant lipids) and in secreted inorganic spicules that could influence larval density. In this way, we hoped to determine which factors were most important in bringing larvae to the bottom for settlement. We also measured some of these parameters in larvae from different parents to determine how the factors influencing depth regulation might vary across a population.

MATERIALS AND METHODS

Collection and maintenance of adults and larvae. We collected ripe individuals of *Sigmadocia caerulea* from boulders and cobbles between 0.5 and 2 m deep in the Indian River Lagoon near Fort Pierce, Florida, USA, during August and September 1994 and 1995. Adult sponges in this region are light blue, usually erect and branched, and up to 30 cm tall.

Larval release often occurred spontaneously shortly after collection of ripe adults. Larval release was also triggered artificially by exposing adults to air for a few seconds (Maldonado & Young 1996). Larvae were cultured at room temperature in glass or polystyrene bowls of 0.45 µm filtered seawater that was replaced daily (salinity = 34‰, temperature = 22 to 24°C).

Larval size and shape. All experimental data were checked for normality (Kolmogorov-Smirnov test) and homoscedasticity (Levene median test) prior to analysis. When, after appropriate transformation, data failed either normality or homoscedasticity tests, they were analyzed by appropriate non-parametric methods.

Changes in larval length and larval shape over time were determined by digitizing the images of live larvae with a Microcomp image analysis system linked to a Zeiss dissecting microscope.

Lengths of 413 larvae were used to estimate the larval size distribution at the population level. Differences in length among 5 age groups of larvae (2, 6, 12, 24, and 30 h old larvae; N = 50, 25, 50, 87, and 76, respectively) from a single parent were tested by 1-way analysis of variance. Differences in the lengths of 6 h old larvae obtained from 5 different parents collected from the same population were also examined by 1-way analysis of variance (N = 25).

Overall changes in larval shape over time were assessed by use of the circularity index (C) of Turón & Becerro (1992): $C = A_s/A_p$, where A_s is the area of the object, and A_p is the area of a circle with a perimeter equivalent to that of the object. Areas and perimeters projected by larvae on the bottoms of the containers were used to calculate the larval circularity index. Differences in the circularity index among 5 age groups (2, 6, 12, 24, and 30 h old larvae; N = 50, 27, 50, 51, and 26, respectively) were tested by 1-way analysis of variance.

Larval swimming, orientation, and sinking. The length of the swimming period under laboratory conditions was determined by monitoring larvae held in 15 small polystyrene petri dishes over a period of 17.5 d. Each dish contained 10 larvae in 30 ml of 0.45 µm filtered seawater (salinity = 34‰, temperature = 22 to 24°C).

Phototactic and photokinetic responses were investigated by recording movements of 6 and 30 h old larvae (N = 25 at each age) in the presence of a bright unidirectional light. As we were only interested in the intrinsic ability of larvae to respond to light, we made no attempt to mimic the angular light distributions in the field (Forward 1988). Instead, we used a standard-

ized light source for all tests. The light source was a 150 W, cold fiber-optic incandescent light filtered through a neutral density diffuser. We determined the horizontal swimming speeds of individual phototactic larvae by timing their movements along the axis of a plexiglas aquarium 75 cm long × 2 cm wide × 3 cm deep with light shining at one end. We tested for age-specific differences in swimming speed with the Mann-Whitney rank sum test.

The ability of larvae to respond to light at various stages was also investigated by monitoring the depth distributions of larvae incubated in either complete darkness or above diffuse white light. Sixteen batches of 6 h old larvae (N = 10 per batch) were placed in test tubes (2 cm diameter × 18 cm tall) of filtered seawater, and each was assigned randomly to one of the 2 light treatments. The positions of all larvae were measured after 4, 8, and 12 h (i.e. 10, 14, and 18 h old larvae). Data were analyzed by a 2-factor analysis of variance (factors: depth, time), with repeated measures on the time factor (Winer 1971), where the dependent variable was the mean depth of larvae in each tube and the replicates were the different test tubes. When significant interactions were obtained in the factorial analysis, *a posteriori* pairwise comparisons were done within levels of each factor using the Student-Newman-Keuls (SNK) test. To determine if phototactic responses persisted in larvae reared with light from below, we chose 25 (18 h old) larvae at random from the illuminated treatment and measured their horizontal speed and displacement in the presence of a horizontal light source, as explained above.

Sinking rates of larvae were measured at 3 different larval ages: 2 h (N = 48), 12 h (N = 46) and 24 h (N = 28). Ciliary activity was arrested by bubbling cigarette smoke through the water of the culture containers. Fall velocities of non-swimming larvae were then measured by timing their passage through the center of a plexiglas column 7 cm diameter × 20 cm tall. Larvae were not timed during the first 5 cm, which was the distance empirically determined to ensure that they had reached terminal velocity (Chia et al. 1983). The diameter of the column was wide enough to minimize 'wall effects' (Winet 1973). To reduce the potential effects of convection currents (Chia et al. 1983), experiments were run in a temperature-controlled room using filtered seawater at constant temperature (22.5°C) and salinity (34‰).

Body composition. Organic dry weight (ODW) and ash content (AC) were determined for swimming larvae 6 and 24 h old, as well as for early-metamorphic stages from 2 to 4 h after settlement (i.e. 30 h old non-feeding settlers). Each assay was based on 6 replicates, with 20 individuals (larvae or settlers) combined per replicate. Groups of larvae or settlers were rinsed in 3.4% ammonium formate, mounted on tared aluminum boats and dried to constant weight at 80°C. After weighing on a Mettler Analytical balance, each sample was burned at 450°C for 4.5 h then weighed again. Differences in ODW, AC and AC/ODW ratio per individual among the 3 age groups were determined by using the Kruskal-Wallis non-parametric analysis of variance.

The total protein content and lipid content of 6 and 30 h old swimming larvae and metamorphic stages 27 h after settlement (i.e. 72 h old non-feeding settlers) were estimated from 5 samples (20 larvae or settlers per sample) for each age group. The method of Lowry et al. (1951) was used to determine total protein content. Lipids were extracted with chloroform and methanol (Bligh & Dyer 1959). A hydrophobic teflon membrane (0.45 µm PTFE membrane Centrex filter) separated the organic phase containing lipids from the aqueous phase (Reisenbichler & Bailey 1991). Total lipids were measured with the colorimetric sulfophosphovanillin reaction (Barnes & Blackstock 1973). Mean differences in total lipid and protein content among the 3 age groups were determined by 1-way analysis of variance. Differences in total lipid and protein content among 6 h old larvae brooded by 5 different parents were also determined by 1-way analysis of variance. *A posteriori* pairwise comparisons were done using the SNK test.

RESULTS

Larval size and shape

Parenchymella larvae of *Sigmadocia caerulea* are elongate spheroids, whitish gray, and uniformly covered by cilia, except at the posterior end, which is bare. Cilia are short over most of the body, but the posterior end is ringed by a tuft of longer cilia (about 80 ± 5 µm in length). The cells which bear these elongate cilia are brownish, contrasting with the whitish color of the remaining larval body. Spicules are present in larvae at hatching. Although 2 different types of spicules, oxeas (rod-like spicules pointed at both ends) and sigmata (C-shaped spicules), are found in the adult, only oxeas were observed in larvae. Larval oxeas were slightly curved at the middle as in the adult, but they were shorter and thinner (102–145 × 2–3.5 µm). Larval spicules were arranged parallel to each other and densely packed in thick bundles situated near the posterior pole of larvae.

The body-length distribution of a population of larvae pooled from a mixture of several different parents had a mean of 629.2 µm (±95.8) and ranged from 406.3 to 888.9 µm (Fig. 1). Further analysis revealed that

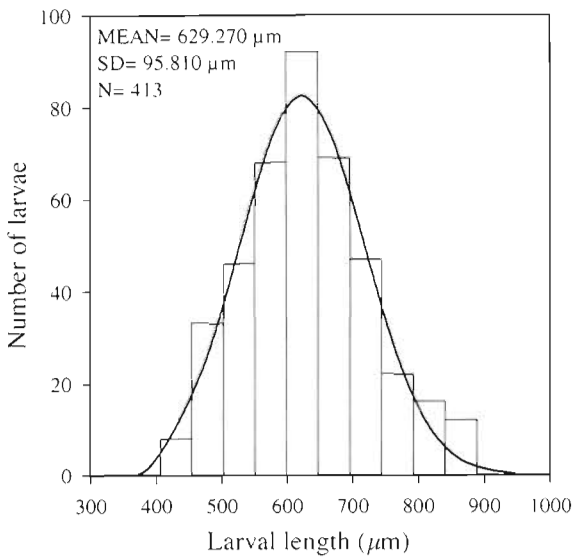

Fig. 1. *Sigmadocia caerulea*. Body-length distribution of larvae. Measurements were obtained by sampling over time and without replacement from a pool of larvae released by 5 different individuals from the same population

there were significant differences ($p < 0.001$) in mean length among 6 h old larvae obtained from different parents (Fig. 2a, Table 1). By sampling without replacement from a pool of larvae obtained from only 1 individual, we also detected significant changes in larval length with age ($p < 0.001$; Fig. 2b, Table 1). However, these changes did not result from progressive increases or decreases in length, but rather from reversible and temporary shortening and lengthening. There was no significant variation in circularity index among larvae from different age groups (Fig. 2c, Table 2). Microscope observations confirmed that these larvae are capable of temporary shape changes.

Larval swimming, orientation, and sinking

Upon release from the adult colony, larvae swam with a clockwise corkscrew motion and with the flagellar tuft directed backwards. For approximately the first

Table 1. One-way analysis of variance showing differences in larval length as a function of parental origin and of larval age

Source	df	MS	*F*	p
Parents	4	117090.370	21.965	<0.001
Error	120	5330.650		
Larval age	4	226564.033	37.428	<0.001
Error	283	6053.397		

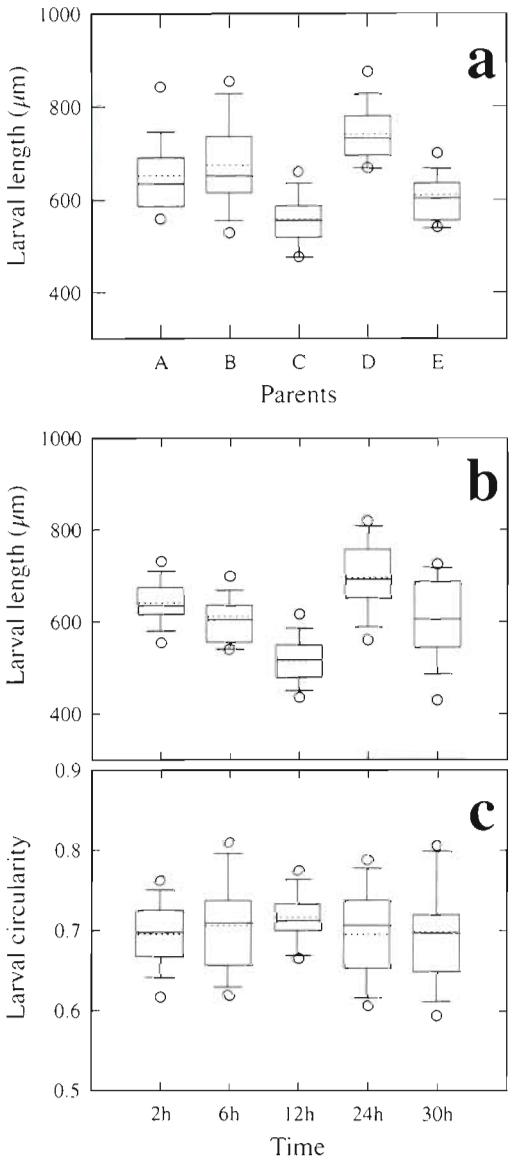

Fig. 2. *Sigmadocia caerulea*. Box plots showing (a) body length of 6 h old larvae released by 5 different individuals, coded as A, B, C, D and E; (b) body length of 5 age groups of sibling larvae (data were obtained by sampling over time without replacement); (c) circularity values of sibling larvae over time (data obtained as in 'b'). In these and subsequent box plots, lower and upper boundaries of boxes indicate 25th and 75th percentiles, respectively, vertical lines above and below boxes indicate 10th and 90th percentiles, and the 5th and 95th percentiles are shown as points below and above the caps of the vertical lines. Solid and dashed lines within the box indicate median and mean, respectively

12 to 24 h, they swam actively near the water surface. After that time, they began to move more slowly and remained near the bottoms of the containers, repeatedly stopping until they found a suitable site for settlement. The total duration of the swimming phase

Table 2. One-way analysis of variance showing no significant difference in the circularity index among 5 age groups of larvae (2, 6, 12, 24 and 30 h old larvae)

Source	df	MS	*F*	p
Larval age	4	0.004	1.439	0.222
Error	199	0.003		

ranged from 12 to 36 h (Fig. 3). About 50% of the larvae settled between 24 and 28 h after being released.

Larvae were photonegative during the whole pelagic period. The speeds of photonegative larvae swimming away from a light source varied with age (Mann-Whitney's $T = 948$, N = 25, $p < 0.001$), with 6 h old larvae swimming faster (0.4 ± 0.1 cm s^{-1}) than 30 h old larvae (0.092 ± 0.028 cm s^{-1}) (Fig. 4). The times and distances that young and old larvae swam in response to light stimulation were also significantly different ($T = 345$, N = 5, $p < 0.001$; $T = 385.5$, N = 25, $p < 0.001$, respectively). Most 30 h old larvae only stopped swimming when they reached the dark side of the tank. Larvae from both age categories swam directly away from the light, but young larvae invariably swam close to the water surface while maintaining their body in a horizontal position, whereas older larvae swam near the bottom of the aquarium with their posterior end downward.

The distribution of larvae in the water column was affected by time and light conditions, with a significant interaction between these 2 factors (Table 3, Fig. 5). The mean depth of larval distribution moved down-

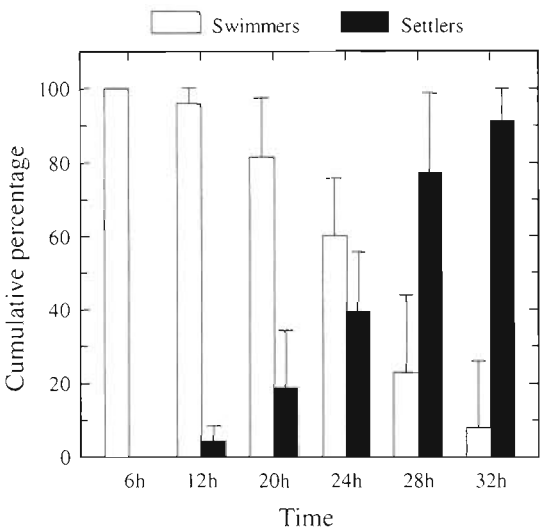

Fig. 3. *Sigmadocia caerulea*. Cumulative percentages of swimmers and settlers over time

Table 3. Analysis of variance of depth distribution of larvae in water columns as a function of illumination conditions and time. There are 2 fixed factors, light conditions (bottom illumination vs dark) and time (4, 8 and 12 h in treatment), with repeated measures on this latter factor. For *a posteriori* pairwise comparisons see Table 4. Larval age = time in treatment + 6 h

Source	df	MS	*F*	p
Between tubes				
Light	1	116.251	26.820	<0.001
Error (Light)	14	4.334		
Within tubes				
Time	2	384.031	145.600	<0.001
Time × Light	2	32.956	12.495	<0.001
Error (Time)	28	2.637		

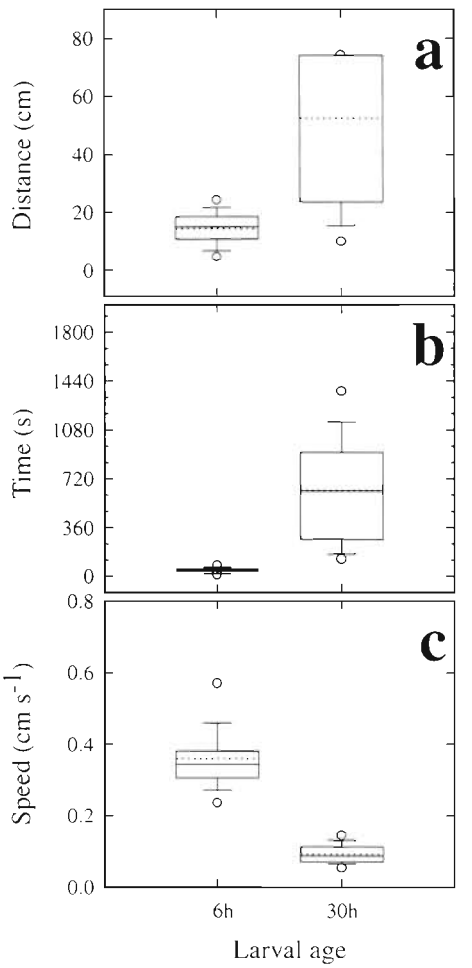

Fig. 4. *Sigmadocia caerulea*. Box plots showing (a) distances swum by 6 and 30 h old larvae as response to an artificial light source, (b) time values that 6 and 30 h old larvae swam in response to an artificial light source, (c) speed values of 6 and 30 h old photonegative larvae while swimming away from an artificial light source

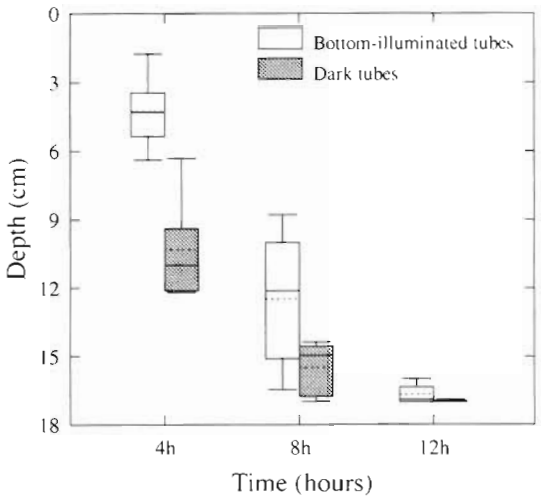

Fig. 5. *Sigmadocia caerulea*. Box plot showing the depth distribution of larvae over time in 2 different experimental light treatments: bottom-illuminated and dark test tubes. Larval age = time in treatment + 6 h

ward with increasing time. Larvae swimming in tubes illuminated from below were found, on average, at a significantly shallower depth than those in dark tubes.

Table 4. Multiple pairwise comparisons of mean differences between all levels of light treatment (BL: bottom illumination; D: dark) and time (4, 8, 12 h in treatment) and their interactions (see Table 3). Multiple comparisons were done using Student-Newman-Keuls (SNK) tests. MD: mean difference; q: q test statistic; P: number of means spanned in the comparison. Larval age = time in treatment + 6 h

Factor pairs	MD	SE	df	P	q	p
Pairwise comparisons on factor Light						
D vs BL	3.113	0.118	14	2	37.435	<0.05
Pairwise comparisons on factor Time						
12h vs 4h	9.544	0.112	28	3	99.999	<0.05
12h vs 8h	2.850	0.112	28	2	35.877	<0.05
8h vs 4h	6.694	0.112	28	2	84.264	<0.05
Pairwise comparions on Light × Time						
D:12h vs BL:4h	12.713	0.175	28	6	99.999	<0.05
D:12h vs D:4h	6.675	0.159	28	5	59.417	<0.05
D:12h vs BL:8h	4.500	0.175	28	4	36.349	<0.05
D:12h vs D:8h	1.500	0.159	28	3	13.352	<0.05
D:12h vs BL:12h	0.300	0.175	28	2	2.423	>0.05
BL:12h vs BL:4h	12.413	0.159	28	5	99.999	<0.05
BL:12h vs D:4h	6.375	0.175	28	4	51.494	<0.05
BL:12h vs BL:8h	4.200	0.159	28	3	37.386	<0.05
BL:12h vs D:8h	1.200	0.175	28	2	9.693	<0.05
D:8h vs BL:4h	11.213	0.175	28	4	90.568	<0.05
D:8h vs D:4h	5.175	0.159	28	3	46.065	<0.05
D:8h vs BL:8h	3.000	0.175	28	2	24.232	<0.05
BL:8h vs BL:4h	8.212	0.159	28	3	73.103	<0.05
BL:8h vs D:4h	2.175	0.175	28	2	15.568	<0.05
D:4h vs BL:4h	6.037	0.175	28	2	48.768	<0.05

Table 5. One-way analysis of variance showing significant differences in sinking rate of larvae with age. See also Fig. 6

Source	df	MS	F	p
Age	2	0.025	0.055	0.001
Error	119	0.003		

Pairwise comparisons showed that this phenomenon remained consistent within time groups, except at the 12 h stage (Table 4). At this age (i.e. 18 h old), larvae were located within 1 cm of the bottom in both light treatments. They were either creeping on the bottom (a sign of imminent settlement) or demonstrating an apparent exploratory behavior in which they swam near the bottom while touching the bottom periodically.

Because illumination from below did not inhibit downward movement of larvae 18 h or older, we suspected that the photonegative response had been entirely lost by this stage. We tested this hypothesis by exposing older larvae to horizontally directed light. Twenty-eight 18 h old larvae selected at random proved to be strongly photonegative, most of them swimming the full 75 cm distance to the unlit side of the aquarium (larvae swam on average 68.3 ± 15.8 cm in 749.92 ± 145.38 s).

When we tested for age-related differences in larval density, we found that density increased significantly with age; 2 h old larvae sank more slowly than either 12 or 24 h old larvae (Table 5, Fig. 6).

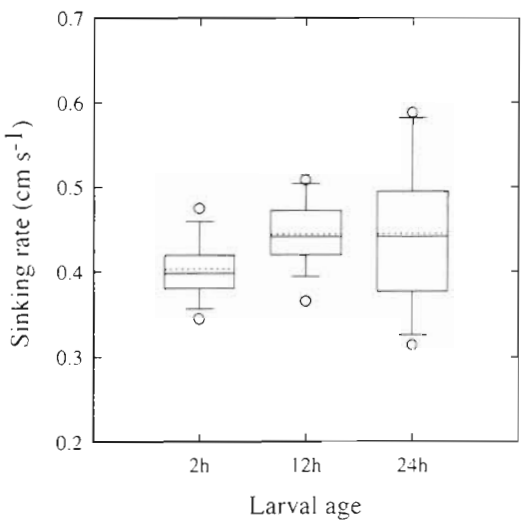

Fig. 6. *Sigmadocia caerulea*. Box plot showing the sinking rates of 2, 12, and 24 h old larvae whose ciliary activity was arrested

When larvae were ready for settlement, they swam near the bottom for a variable period of time ranging from a few minutes to 24 h. Ultimately, they positioned themselves perpendicular to the bottom, spinning with the anterior end down. Spinning ceased prior to attachment. Larvae always attached by the anterior end, then spread themselves progressively around the initial attachment point. The flagellar tuft continued to beat for between 15 and 40 min after settlement before being shed. A dark ring of cells around the posterior end was recognizable for about 6 h after settlement, then dispersed.

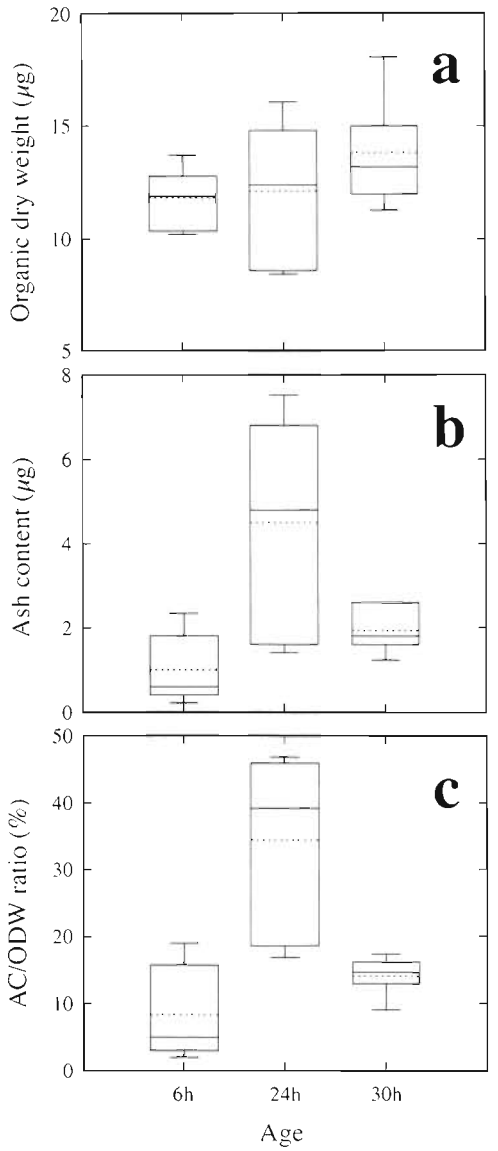

Fig. 7 *Sigmadocia caerulea*. Box plots showing values of organic dry weight (ODW), ash content (AC) and AC/ODW ratio per individual in 6 and 24 h old larvae and 30 h old non-feeding settlers

Table 6. Kruskal-Wallis 1-way analysis of variance of the ash content/organic dry weight ratio (AC/ODW) per individual among 6 and 24 h old larvae and 30 h old non-feeding settlers

Source	df	MS	*F*	p
AC/ODW (%)	2	1123.817	13.628	<0.001
Error	15	82.463		

Body composition

There was no significant difference in organic dry weight (ODW) among 6 h old larvae, 24 h old larvae and 30 h old non-feeding settlers (Fig. 7a). There were, however, significant differences in the ash content among groups ($p = 0.005$; Fig 7b), as well as in the percentage that ash content contributed to the total ODW ($p < 0.001$; Fig. 7c, Table 6). On average, ash content was equal to about 8% of the ODW in 6 h old larvae and 37.3% in 24 h old larvae. It can be assumed that most of the ash content is derived from the presence of siliceous spicules. The ratio of ash to ODW was higher in older larvae than in either young larvae or settlers, indicating that the larval stage is a period of particularly active silicification. Ash content was lower after settlement than before, apparently because most larval spicules were not actually incorporated into the skeleton of the juveniles: they protruded from the body during the reorganization of shape that occurred early in metamorphosis and were found 1 d after metamorphosis surrounding the young sponges on the bottoms of the culture dishes.

There was no significant difference in lipid content of 6 h old larvae, 30 h old larvae and 72 h old non-feeding settlers (Table 7, Fig. 8). However, protein content was significantly higher in non-feeding settlers than in

Table 7 One-way analysis of variance of total lipids and total protein as function of age and parental origin

Source	df	MS	*F*	p
Total lipids				
Age	2	51.9	0.432	0.659
Error	12	120.3		
Parents	4	45.3	2.720	0.058
Error	20	16.7		
Total protein				
Age	2	1.574	5.190	0.023
Error	12	0.303		
Parents	4	1.683	10.3	<0.001
Error	19[a]	0.164		

[a] Missing datum in parent B

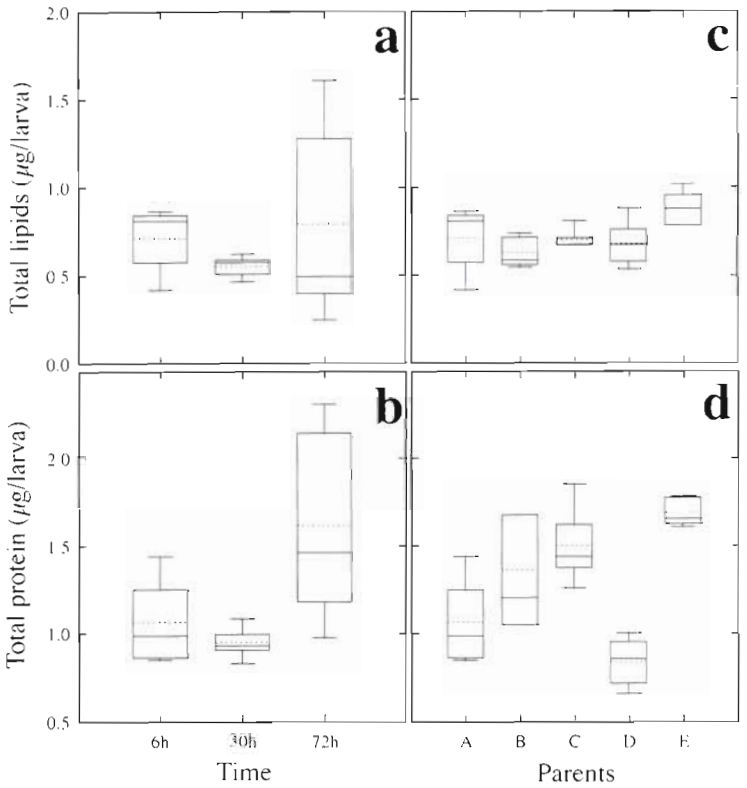

Fig. 8. *Sigmadocia caerulea*. Box plots showing (a, b) total lipid and protein contents per individual, calculated for 6 h old larvae, 30 h old swimming larvae and 72 h old non-feeding settlers (metamorphic stages); (c, d) total lipid and protein contents per larva in 6 h old larvae obtained from 5 different parents (A, B, C, D, and E)

the larval stages. Protein content varied significantly among 6 h old larvae from different parents, but total lipids did not vary (Table 7, Fig. 8). Mean values of total lipids and proteins per larvae were not correlated with each other.

DISCUSSION

Both behavioral and compositional factors were important in moving late-stage parenchymella larvae from the water column toward the bottom. Larvae became more dense relative to seawater with advancing age, causing them to sink faster. This density increase was not caused by depletion of buoyant lipids, but rather by increased spicule secretion. Moreover, most of the spicules secreted late in larval life are lost during metamorphosis; as they are only present in the late larvae, their role in facilitating sinking may be functional rather than incidental. Later larvae actually showed a slight decrease in protein content, so proteins (which are heavier than lipids) did not play a role in increasing sinking rate. However, protein did increase significantly just after metamorphosis. Although the source of the nitrogen required for this synthesis remains enigmatic, 2 origins can tentatively be suggested. An uptake of dissolved organic material cannot be discarded as a potential source of nitrogen at early-metamorphic stages, since sponge larvae have been demonstrated to efficiently incorporate the amino acid alanine which is quite common in seawater (Jaeckle 1995). Besides, ammonium is also known to be efficiently incorporated by sponges, due to the presence of symbiotic bacteria (Corredor et al. 1988). Symbiotic bacteria are likely to be present in early settlers, as there is usually a direct transfer from adults to juveniles through the larva (e.g. Lévi & Lévi 1976, Kaye & Reiswig 1991, Woollacott 1993).

In theory, larvae of a given volume could reduce drag slightly, thereby increasing sinking rate, by elongation (Vogel 1981). Our analysis of circularity index indicated that no ontogenetic shape change occurred. Individual larvae were able to change shape temporarily, apparently by flattening their body, but there was no progressive elongation as settlement approached. Our observations agree with previous studies showing that sponge larvae are quite plastic in shape during the swimming period (e.g. Meewis 1938, 1939, Bergquist et al. 1970, Fry 1971, Uriz 1982, Fell 1989, Woollacott 1990, 1993, Maldonado & Young 1996).

Some of these morphological and compositional larval parameters may also show important individual variability, as detected in the comparative analyses of larval length and protein content among larvae obtained from different parents. This individual variability does not appear to affect the passive mechanisms of depth control in larvae. However, it might be related to the variability in the duration of the larval swimming period reported in many sponge species (e.g Woollacott 1993, Maldonado & Young 1996).

Larvae of *Sigmadocia caerulea* were photonegative during their whole life, but there were subtle age-related differences in the expression of the photoresponse. Specifically, young larvae swam directly away from light at a higher speed than old larvae, but they stopped swimming after only a relatively short distance (15 cm on average), whereas the older larvae continued swimming until they had attained a mean distance of 52 cm from the light source. In clear water, light attenuates approximately as an inverse square function of distance. Thus, late-stage larvae cease

swimming at a light intensity about 2 orders of magnitude lower than that required for cessation of swimming by early-stage larvae. This suggests that larvae become much more photosensitive with age. These observations also suggest that sponge larvae use a combination of phototactic and photokinetic behaviors. The direct swimming paths away from a horizontally directed light source are definitely indicative of directional (phototactic) behaviors, but the fact that larvae stop after a certain distance away from the light suggests that a minimal intensity is required to release swimming behavior (i.e. photokinesis).

Once larvae are older than 18 h, photoresponse does not seem to be important in depth regulation. At this time, they become demersal, remaining on or near the bottom even if strong light is shone from below. It is likely that the low photokinetic response is used for selecting dark microhabitats just before settlement. Swimming ceases when a certain minimum intensity is encountered, probably increasing the likelihood that larvae will be in a dark place when metamorphosis is initiated. Thus, depth regulation in the terminal stages appears to be largely a function of larval density rather than behavior

Several authors have invoked positive geotaxis as an explanation for the demersal distribution of late-stage parenchymellae (e.g see reviews by Bergquist et al. 1970, 1979, Fell 1974, 1989). Although we have not definitively eliminated the possibility of positive geotaxis, such behavior appears unlikely because sponge larvae have no statocysts or other means for detecting the direction of gravitational pull. A geotaxis-like effect can be achieved by having a low center of gravity and using the entire body as an orientation mechanism (Pennington & Strathmann 1990; reviewed by Young 1995). In sponges, such an unequal distribution of body mass results from localized spicule production. It has long been known that spicules and sclerocytes are present in the parenchymellae of most demosponges, but the significance of these structures has been regarded as enigmatic. Woollacott (1993) has recently proposed that larval spicules might be involved in a passive orientation to gravity. Our results do not contradict Woollacott's hypothesis; indeed, we noted that the larval spicules were aligned in parallel arrays and located mostly near the posterior end of the larva. This probably explains why older larvae of *Sigmadocia caerulea* always swim with the posterior end downward. Bergquist & Sinclair (1973) reported a similar swimming behavior in late-stage *Hymeniacidon perleve* larvae. An alternative hypothesis to explain the presence of larval spicules, proposed by Efremova & Efremov (1979), is that spicules and sclerocytes have no function in the swimming larva, but are produced in the larval stage for use by the early juveniles. By accelerating the production of early spicules, juveniles may be able to concentrate their energetic resources on other critical needs in the hours and days after settlement. Our results do not support this hypothesis, as most spicules were extruded during metamorphosis rather than being incorporated into the juvenile body.

In summary, this study shows that the parenchymella larva can experience significant behavioral and compositional changes during its short swimming life. Ontogenetic changes in behavior and skeletal mass are particularly important in moving larvae toward the bottom for settlement.

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