Ontogenetic changes in phototaxis during larval life of the Ascidian *Polyandrocarpa zorritensis* (Van Name, 1931)

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Abstract

Ontogenetic changes in larval photoresponses of *Polyandrocarpa zorritensis* (Ascidiacea, Styelidae) were studied by characterizing the swimming trajectories of individual larvae exposed to white light. The same individuals were tested repeatedly from release to settlement to remove the effects of interindividual variation in behavior. Swimming speed and overall level of activity varied significantly over time, eventually decreasing near the end of larval life. The youngest larvae swam in large spirals or straight trajectories. Late-stage larvae, used short vertical hops to contact the substratum repeatedly with the anterior papillae. This observation supports the hypothesis that papillae function in selection of a site as well as in adhesion. *P. zorritensis* become photopositive at the end of larval life, a behavior which probably functions in selection of microhabitats rather than in depth regulation.

Keywords: Ascidian larvae, Phototaxis

1. Introduction

Larvae of marine invertebrates respond to physical environmental cues in ways that influence their distributions while swimming and improve their chances for surviving in all life-history stages. Orientation in the water column and selection of settlement sites may involve tactic (directional) responses, kinetic (activity level) responses, or a combination of the two (reviewed by Young, 1995). The major vector cues used for tactic orientation in shallow water are light and gravity. Thorson (1964) summarized the

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known patterns of phototactic responses in invertebrates and concluded that most subtidal larvae demonstrate positive phototaxis early in larval life, then switch the sign of phototaxis, becoming photonegative when it is time to move toward the bottom and select a settlement site. One of the few intertidal species included in Thorsonś compilation was an ascidian, *Perophora viridis*, which remained photopositive for its entire larval life (Grave and McCosh, 1923).

Laboratory studies of phototaxis have been criticised in recent years because most studies fail to use light with a spectral composition, intensity and angular distribution that resembles natural light (Forward, 1988). Thus, the responses that some larvae demonstrate to bright white light may not precisely reflect the behaviors that are displayed in the field and should not be used for modeling vertical migrations. Additional lab artifacts arise when the responses of many larvae are measured simultaneously (Harada, 1976; Kajiwara and Yoshida, 1985) and when differential heating causes convenction currents in the experimental chamber. Nevertheless, by attempting to control artifacts, by studying the swimming directions and speeds of individual larvae, and by focusing on changes that occur during ontogeny, we can obtain information that reflects real physiological changes during larval life (Sulkin, 1990).

Although Thorson (1964) concluded that most ascidian larvae show the usual ontogenetic shift from positive to negative phototaxis, subsequent studies have revealed considerable variation both within and among species (Grave, 1935; Crisp and Ghobashy, 1971; Young and Braithwaite, 1980; Young, 1982; Young and Chia, 1984; Hurlbut, 1993; reviewed by Svane and Young, 1989). These authors observed that there was variability in behavior among tadpoles of the same and different age within a given species. So phototaxis in ascidians would be best considered not as a stereotyped behaviour but as a phenomenon with a statistical distribution (reviewed by Svane and Young, 1989). With few exceptions, the data on ontogenetic changes in phototaxis are casual observations made during studies of larval release, settlement and metamorphosis (reviewed by Svane and Young, 1989). The most careful study to date remains that of Crisp and Ghobashy (1971) with the larvae of *Diplosoma listerianum*. This species remained strongly geonegative and slightly photopositive until just before settlement, when the signs of both tactic responses reversed conform to the classic generalization.

Tadpoles of the family Styelidae demonstrate phototactic orientation behaviors (Young and Chia, 1985) despite having simpler photoreceptors than other ascidian tadpoles. Whereas a typical tadpole has a complete and discrete ocellus consisting of pigmented tissues, lenses and photorreceptor cells, solitary styelids often have vestigial pigment cells in the posterior wall of the sensory vesicle, lack lenses, and have no associated sensory cells (Torrence, 1983). Colonial styelid tadpoles such as the species studied here often integrate the statocyst and the ocellus into a compound sensory structure known as the photolith (Berrill, 1947; Torrence, 1980).

In this study, we examined ontogenetic changes in larval photoresponses of *Polyandrocarpa zorritensis*, a common shallow-water polystyelid ascidian in Florida. Instead of documenting the overall distributional changes in groups of larvae, we characterized the swimming trajectories of individual larvae exposed to a standardized white light source. The same individual larvae were studied throughout their swimming period to determine the time course of phototactic change.

2. Materials and methods

All experiments were done with larvae of the colonial styelid ascidian *Polyandrocarpa zorritensis* (Van Name, 1931). This species has been reported from Perú (Van Name, 1931), Brazil (Millar, 1958), Florida (Vázquez and Young, 1996) and the western Mediterranean Sea (Brunetti, 1978; Turon and Perera, 1988), always from very shallow water. *P. zorritensis* incubates eggs in the atrial cavity and releases fully developed free-swimming larvae 1.4 mm long (Vázquez and Young, 1996).

Adult colonies were collected between April and June 1994 from a vertical concrete sea wall and from mangrove roots (*Rhizophora mangle*) at Jim Island, both near Ft. Pierce Inlet in the Indian River Lagoon, Florida. All individuals came from water less than 1 m in depth. The water temperature at this site ranged from 18.5°C in December to 32.4°C in August and the salinity ranged from 31 ppt in June to 34 ppt in April. A complete description of the Jim Island site and of the mangrove-associated epifauna is given by Bingham (1990).

Adult colonies were maintained in dark, aerated aquaria overnight, then moved outside into direct sunlight at 7:00 a.m. to trigger release of larvae. Each bowl contained a mixture of several adult colonies and we made no attempt to isolate larvae produced by specific parents. Larvae were liberated between 8:30 and 10:00 a.m. As soon as they emerged, they were collected with a pasteur pipette and transferred to darkened petri dishes containing 33 ppt sea water filtered through a 0.45 μm millepore filter. Larvae were then transported to a dark room with a constant temperature of 23°C where experiments were run. Larvae were no less than 5 min old and no more than 10 min old when the first experiments were begun.

The experimental chamber was a plexiglas aquarium 13 cm long, 3 cm high and 3.5 cm wide painted with matt black paint on all but one internal surfaces to eliminate reflections. The aquarium was placed in the center of a black wooden box which could be covered with a matt black cover to further reduce extraneous light (Fig. 1). Light was projected laterally so as to isolate phototactic responses from confounding effects of geotaxis. Incandescent light from a Wild dissecting microscope lamp was directed

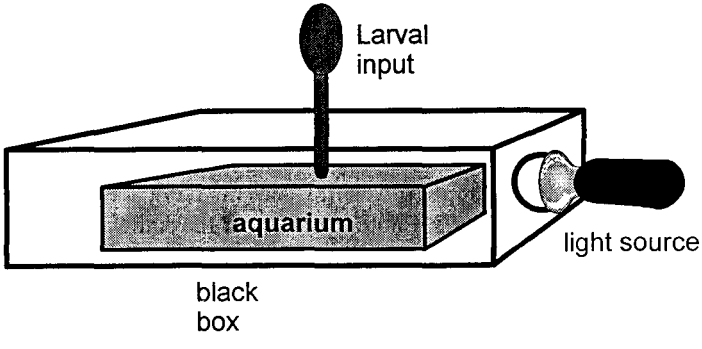

Fig. 1. Experimental chamber used in the experiments. Chamber consisted of a black plexiglas aquarium enclosed in a black box and illuminated from one end. "Larval input" indicates the point at which larvae were individually pipetted into the chamber.

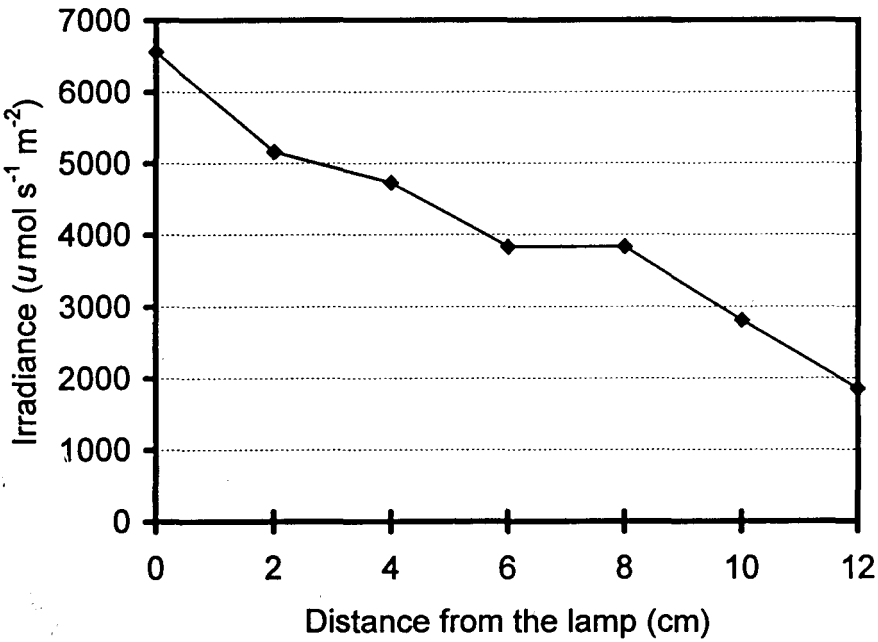

Fig. 2. Irradiance as a function of distance from the light source within the experimental chamber. Measurements were made at 2-cm intervals.

through a hole of 3 cm in diameter in the end of the black box. Irradiance was measured with a Li-cor quantum meter (Lambda Instrument Company, Model LI-185) at 20 mm increments. Attenuation over the length of the experimental chamber is shown in Fig. 2.

To avoid possible interactions among larvae, only a single individual was introduced into the experimental chamber at a time. With the light already on, the larva was injected into the center of the aquarium with a pipette. The swimming trajectory of the larva was traced on graph paper and the total duration of the inital swimming event was recorded with a stop watch. When a larva came to rest during the 1-min experimental period, its position along the light gradient (i.e., distance from the illuminated end of the aquarium was recorded). If the larva was still swimming after 60 s, its position was recorded and the run was terminated. If the larva did not swim at all during the first 60 s, the light was turned off for 30 s, then turned back on to determine if larvae were sensitive to shadows. However, the distances and directions these latter larvae swam were not used for statistical analyses. Once an observation was finished, the larva was removed and held in a dark petri dish for subsequent trials. Photoresponses of the same individual larvae were measured every 90 min (a total of five times) so that ontogenetic changes of individuals could be assessed without introducing additional variation that could arise because of parentage, collection site, day of release, etc. The number of larvae used in each run is given in Table 1. Unequal sample sizes resulted from a small amount of larval mortality and metamorphosis that occurred during the course of the experiment.

The initial direction that each larva swam upon introduction to the aquarium was

Table 1
Number of *Polyandrocarpa zorritensis* larvae that remained inactive for the first 60 s after introduction to a lighted aquarium, and the number of these same larvae that moved during a subsequent 30-s period of darkness

Larval Age (h)	Total number of larvae	Inactive larvae in presence of light		Initially inactive larvae moving after 30 s of darkness	
		number	%	number	%
0	49	7	14.29	5	71.43
1.5	38	8	21.05	6	75.00
3	38	15	39.47	13	86.67
4.5	38	15	39.47	10	66.67
6	37	14	37.84	11	78.57

recorded and the circular distributions of these instantaneous directions were analyzed for randomness using the Rayleigh test. Data were grouped into intervals of 45 degrees for the analysis and the r_c correction was applied prior to analysis (Zar, 1996).

The distributions of distances from the light after a 1-minute swimming period were compared with an uniform distribution across the aquarium using two-tailed Kolmogorov-Smirnov goodness of fit tests.

Swimming speeds were analyzed by a one-way analysis of variance. Data were analyzed with SYSTAT 5.0 for Windows. Although the data used for ANOVA violated the assumption of normality, the statistic is robust even for non-normal data (Johnson and Field, 1993). Variances were homogeneous at the 0.01 level (F_{max} test, Sokal and Rohlf, 1995). Percentage data were arcsin transformed prior to analysis.

3. Results

3.1. Swimming behavior as a function of age

Over the course of larval life, tadpoles of *Polyandrocarpa zorritensis* became progressively less active in the presence of light. At 0 h (5–10 min after being released from the colony) most of the larvae swam straight or in large circles; only 14.29% of the larvae (Table 1) stayed on the bottom without swimming, and even these flexed their tails occasionally. At 1.5 h the percentage of inactive tadpoles increased to 21.05% (Table 1), but the swimming pattern remained the same. Three-hour-old larvae swam erratically near the bottom of the illuminated aquarium. From 3 to 6 h, nearly 40% of the larvae were initially inactive (Table 1). Between 4.5 and 6 h, the larvae exhibited an apparent exploratory behavior in which the tail was held erect and the bottom was contacted repeatedly with the sensory papillae.

Only the 0 and 1.5 h sampling times had enough data to compare the swimming speed toward the light with the swimming speed away from the light (Table 2A). One-way ANOVA revealed no significant differences (0 h: $F = 0.038$, $P = 0.847$, 1,26 df.; 1.5h: $F = 0.068$, $P = 0.798$, 1, 16 d.f.), so we pooled the swimming speed data within ages and analyzed with a one-way ANOVA (Table 2B). For the analysis, speeds of 7 different

Table 2
A - Swimming speed of the larvae of *Polyandrocarpa zorritensis* moving towards or away from light as a function of age[a] B - Results of one-way analysis of variance on data in A

A

Larval Age (h)	Toward light (cm/min)±S.D.	Away from light (cm/min)±S.D.	Mean speed (cm/min)±S.D.
0	1.89±0.70	2.12±1.44	2.02±1.18
1.5	2.62±1.11*	3.03±1.49	2.77±1.41
3	1.56±0.23*	1.23±0.31*	1.43±0.30
4.5	nd	3.48±1.80	3.48±1.80
6	2.20±0.63*	nd	2.20±0.63*

B

Source of variation	SS	df	MS	*F*	*P*
Between ages	0.788	4	0.197	5.358	0.003
Among ages	0.845	23	0,03675		
Total	1.633	27			

* = $n < 5$; nd: no data.

larvae were chosen randomly for each of the five ages to avoid the interaction between age and individuals. Swimming speed varied significantly with age (Table 2B), though there was not any apparent linear trend in the data (Table 2A).

The majority of tadpoles that were shaded for 30 s after an initial period of inactivity moved by the time the light was turned on again (Table 1), indicating the presence of a shadow response.

3.2. Effects of light on swimming direction and distance

A Rayleigh test on initial swimming direction (Fig. 3), revealed no significant preference for any particular vector at 0, 1.5, 3 or 4.5 h (Fig. 3A, B, C, D). However, a significant proportion of 6-h old larvae exhibited an apparent positive phototaxis by swimming toward the light (Fig. 3E).

The distributions of larvae as a function of distance from the light were significantly different from a uniform distribution in all but the 3-h old sampling period (K.-S. tests: Fig. 4). Large peaks in the centers of all 5 distributions reflected the number of individuals that did not move from the point at which they were dropped (Fig. 4). At 0 h, larvae swam both toward and away from the light (Fig. 4) but by 1.5 h, the majority of active larvae congregated near the dark end of the tank. This apparent negative phototaxis disappeared entirely by 6 h.

4. Discussion

Ontogenetic changes in photoresponses of larvae have been documented in a very large number of marine invertebrates; indeed, such behavioral changes are among the most predictable aspects of larval life (Thorson, 1964). Most studies of phototactic

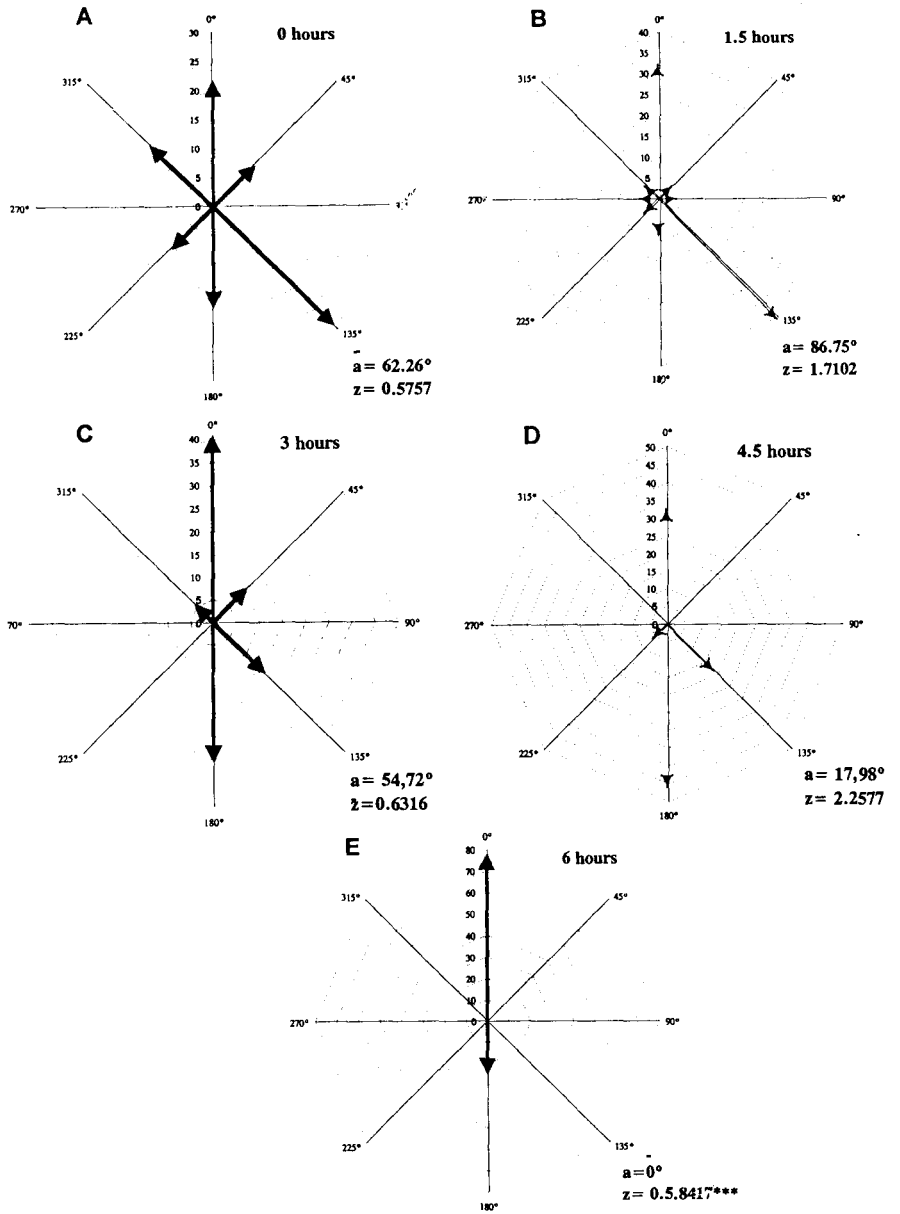

Fig. 3. Circular histogram showing the percentage of larvae at each age that initially swam at various angles relative to the light source. Directions were grouped into 45 degree increments, with the light source located at 0 degrees. Vectors indicate both the direction and the percentage of larvae. a is the mean angle of movement and Z is the Rayleigh statistic testing the significance of the mean angle. Asterisks indicate significance level: ***:$P < 0.005$.

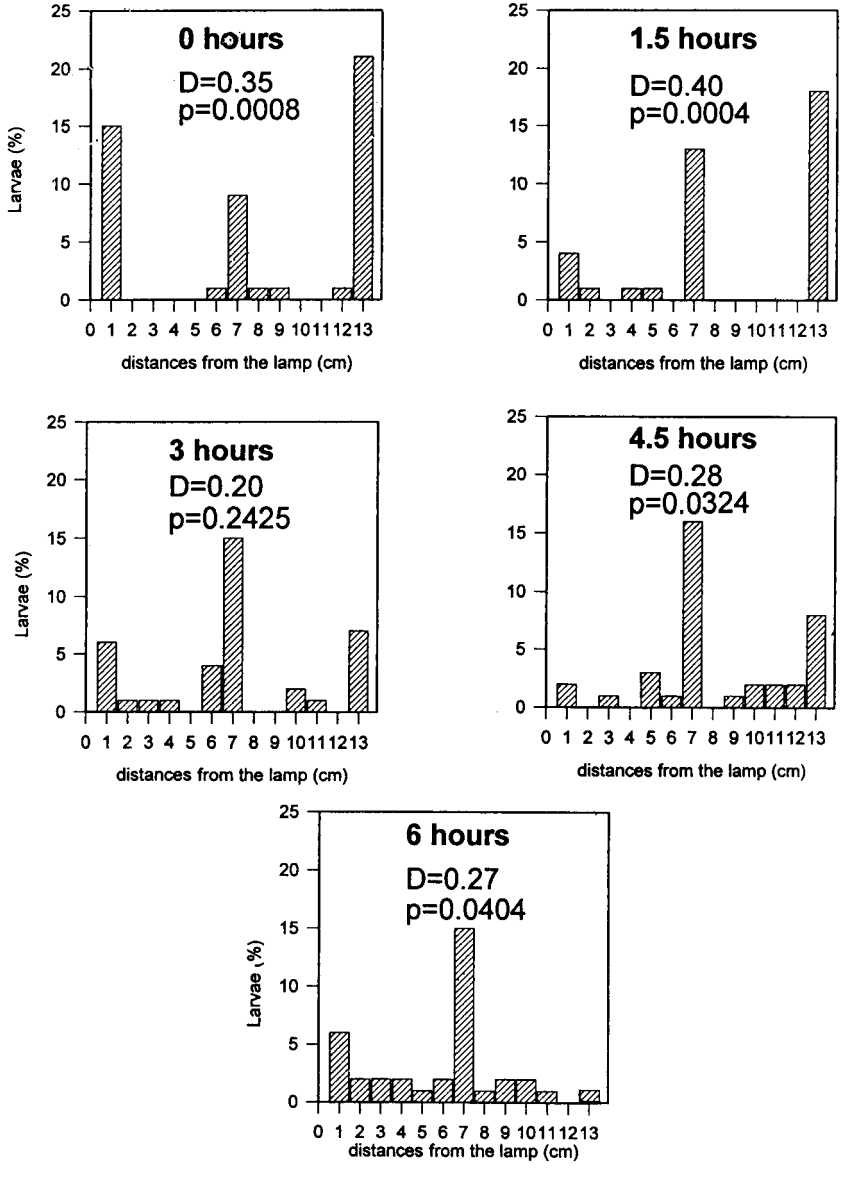

Fig. 4. Percentage of larvae that stopped swimming at various distances fom the light source. The aquarium was divided into 13 1-cm segments with 0 being nearest the light. Two-tailed Kolmogorov-Smirnov one-sample tests (D) compare observed frequency distributions with uniform distributions having the same mean.

changes are based either on groups of larvae, or on different individuals measured at each age. Our work is nearly unique in that we studied the same individual larvae from release to settlement, thereby removing the effects of interindividual variation. Our data

provide only the second example of an ascidian that becomes photopositive at the end of larval life. Both *Polyandrocarpa zorritensis* and *Perophora viridis* (Grave and McCosh, 1923), the other ascidian with similar behavior, occupy hard substrata in very shallow water. Thorson (1964) argued that this behavior increases a larva's chances of locating a settlement site at the appropriate adult depth.

Various aspects of behavior changed as a function of age in the larvae of *Polyandrocarpa zorritensis*. Swimming speed and overall level of activity varied significantly with time, but ultimately decreased by the end of larval life. The youngest larvae swam rapidly either in large loops or straight trajectories. By contrast, swimming of the late-stage larvae consisted of short vertical hops in which the substratum was contacted repeatedly with the papillae located at the anterior end of the trunk. The presence of sensory neurons in the papillae of several families of ascidians (Torrence and Cloney, 1983, Torrence and Cloney, 1988) suggest that papillae may function in site selection as well as adhesion (Cloney, 1978; Young and Braithwaite, 1980; Young, 1982; Davis, 1987; Torrence and Cloney, 1988). The apparent exploratory behavior we observed is strongly supportive of this idea. It is known that ascidians respond to many surface characteristics at settlement, including texture, light and chemistry (reviewed by Svane and Young, 1989), but additional work is needed before the interaction of exploratory behavior and neuroanatomy is completely understood.

Location of the substratum in many invertebrate larvae, including barnacle cyprids (Crisp and Ritz, 1973) and zoeae of estuarine crabs (Forward et al., 1984) is aided by a cessation of swimming in the later stages. Larvae of *Polyandrocarpa zorritensis* also demonstrate a reduced level of activity at the end of larval life. This behavior, however, is somewhat surprising since it should produce the opposite effect as positive phototaxis, which is observed during the same stage of larval life. In essence, larvae swim very little during this stage, but swim toward the light when they do become active. Clearly Thorson's simple notion of the adaptive value of these behaviors is likely to require revision. Perhaps photoresponses are not important in depth regulation, but instead function in selecting microhabitats. Some ascidians with photonegative behavior are known to select cryptic habitats where desiccation, competition and predation are less severe (Hurlbut, 1993; Young and Chia, 1984). *Polyandrocarpa zorritensis* is able to withstand considerable desiccation; it is often found on mangrove roots that are completely exposed at low tide. The tunic is strong enough to resist most predators, and the siphons are still able to open when the tunic is overgrown by algae. On the other hand, the zooids are small, perhaps too small to extend their siphons from from narrow crevices to feed, so survival probability may be better on exposed, lighted habitats.

The newly-hatched larvae of *Polyandrocarpa zorritensis* were not responsive to light, yet they swim upward when initially released from the parents. This initial upward swimming behavior is probably a negative geotaxis rather than a phototactic response. Newly hatched larvae of the solitary ascidian *Ciona savignyi* are apparently unresponsive to light because the ocellus is incompletely developed until later (Kajiwara and Yoshida, 1985).

Several intertidal or shallow subtidal ascidians, including *Diplosoma listerianum, Perophora viridis, Chelyosoma productum, Styela montereyensis and Halocynthia igaboja* demonstrated variable photoresponse at settlement that do not conform with the

ontogenetic paradigm that Thorson proposed (Svane and Young, 1989). The actual behaviors that determine settlement distributions of these species in the field remain unknown. Larvae of *P. zorritensis* are very large and live in shallow, relatively clear water, so it may be possible to obtain insights on the mechanisms of site location by following larvae in the field, as has been done successfully with many other large compound ascidian tadpoles (Olson, 1983; Young, 1986; Davis and Butler, 1991; Stoner, 1994).

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