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Effects of physical factors on larval behavior, settlement and recruitment of four tropical demosponges

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ABSTRACT: This study investigated the effects of light, water flow and water temperature on larvae and early juveniles of 4 shallow-water Caribbean demosponges, *Tedania ignis*, *Haliclona tubifera*, *Sigmadocia caerulea* and *Halichondria magniconulosa*. Larval release was not a synchronous event, either at the individual or population level in any of these species. Parenchymella larvae were photonegative during their whole life, but their swimming speed to escape from a light source varied with species and was related to the ratio of larval body length:flagellar tuft length. The presence of a water flow faster than the larval swimming speed had no effect on the settlement success of these 4 species in experimental flumes. Larvae of all 4 species preferred shaded sites at settlement in flumes. However, microhabitat irradiance in the field was correlated with abundances of adults only in 1 species, *S. caerulea*. The presence of water flow was positively associated with juvenile survivorship in the field only in the case of *T. ignis*. Water flow was also positively associated with the adult abundance of *T. ignis*, but negatively associated with the abundance of *H. tubifera*. Recruitment varied substantially among species, being extremely low in *H. tubifera* and *H. magniconulosa*. Larval activity and settlement of 2 species were strongly affected by abnormally low temperatures: swimming speed decreased, the free-swimming phase was dramatically shortened and recruitment was virtually inhibited. Low temperatures, therefore, potentially prevent larval dispersal and recruitment into cold waters and might restrict the geographic distribution of these species to tropical areas and warm shallow waters.

KEY WORDS: Parenchymella larva · Photoresponse · Sponge settlement · Sponge recruitment

INTRODUCTION

Parenchymella larvae of demosponges have often been studied morphologically, but little experimental work has been done on their behavior. It is a well-known fact that sponge larvae respond behaviorally to some physical stimuli, despite the apparent absence of sensory organs or nervous integration. Light seems to be the main cue for orientation of free-swimming larvae of shallow-water sponges (Bergquist & Sinclair 1968, Fry 1971, Wapstra & van Soest 1987, Woollacott 1993). Many shallow-water sponge larvae are either photonegative during the whole larval life or they become photonegative at settlement (Bergquist et al. 1970, Wapstra & van Soest 1987). Among sessile invertebrates, negative phototaxis is thought to facilitate settlement in crevices and on downward-facing surfaces, where juvenile survival may be enhanced because of reduced exposure to predators, bright light, silt and other factors that covary with light (e.g. Keough & Downes 1982, Young & Chia 1984, Hulburt 1993).

It has been suggested that the larval response to light can be very important in determining the final spatial distribution of adult sponges (e.g. Warburton 1966, Bergquist et al. 1970, Fell 1974). However, as the swimming ability of sponge larvae is very limited, water currents and turbulence are expected to interfere with larval photoresponse. Strong currents could even eliminate the possibility of active larval selection at settlement, leading to a spatial distribution that results exclusively from hydrodynamic factors (e.g.

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Woodin 1986, Butman 1987, Pawlik 1992, Snelgrove 1994). Hydrodynamic factors have been found to be very important in determining the spatial pattern of settlement in many filter-feeders (e.g. Warner 1977, Young & Braithwaite 1980, Dolmer & Svane 1993, Pawlik & Butman 1993). Sponges are particularly abundant in habitats characterized by the presence of a steady water flow (e.g. de Laubenfels 1950, Sará & Vacelet 1973, Wilkinson & Vacelet 1979). Thus, water flow may play an important role in either enhancing or inhibiting settlement or recruitment, but its effects on larval behavior, settlement and recruitment of sponges have never actually been investigated.

Other major abiotic factors, such as temperature, can influence the distribution of adult organisms on larger spatial scales, since they affect physiological processes during all life-history stages. For example, temperature has been considered to explain medium- and large-scale distribution patterns in sponges (Reid 1968, Sará & Vacelet 1973, Vacelet 1988). Seasonal and latitudinal changes in temperature are also known to affect the body sizes of adult sponges as well as the formation of silica and spongin skeletons (e.g. Hentschel 1929, Hartman 1958, Bergquist & Sinclair 1973, Simpson 1978, Bavastrello et al. 1993). However, the larval tolerances and behavioral responses to temperature changes remain virtually unexplored in sponges.

This study explores the role of light, water flow and water temperature on larval behavior, settlement and recruitment of 4 tropical, shallow-water demosponges.

MATERIAL AND METHODS

Habitat characteristics. We studied 4 demosponge species, *Tedania ignis* (Duchassaing & Michelotti), *Halichondria magniconulosa* Hechtel, *Haliclona tubifera* (George & Wilson) and *Sigmadocia caerulea* Hetchel. They all brood embryos and release parenchymella larvae, and all have a biogeographical distribution limited to tropical, shallow waters. These 4 species are common in a sponge-dominated community established on boulders and cobbles between 0.5 and 2 m deep in the Indian River Lagoon, near the Fort Pierce Inlet, Florida. During about half of the daylight hours, part of this community is shaded by a man-made pier. The community is also influenced by tidal currents flowing through nearby Fort Pierce Inlet. There is an onshore-offshore gradient in water flow. That portion of the community at the outer portion of the study area is subjected to currents of 10 to 50 cm s^{-1}. The inner half of the community (near shore) is affected by weaker currents (<5 cm s^{-1}) and is generally characterized by still water. Because of the interaction between currents and light conditions, we predicted the existence of 4 major microhabitats in this community (Fig. 1): (1) sites with high exposure to water movement and light; (2) sites with high exposure to water movement but low exposure to light; (3) sites with reduced water movement but high exposure to light; and (4) sites with reduced exposure to water movement and light.

In order to quantify differences in irradiance and water flow, these variables were measured at 5 sites within each of the 4 major microhabitats. Light irradiance (photons s^{-1} m^{-2}) was measured by using a LI-1000 Data Logger with a LI-192SA underwater quantum sensor. Mean daily irradiance at each site was obtained by averaging 4 measurements recorded at different times during daylight hours (10:30, 12:30, 14:30 and 16:30 h) on a cloudy and a sunny day. Net flow was measured at each site by recording weight loss of small cylinders of plaster of Paris after 24 h. This method is based on the assumption that more water movement results in a higher dissolution rate of plaster of Paris, irrespective of the direction of flow, the maximum velocity, or the fluctuation in velocity (Denny 1988). A discriminant analysis on standardized data was used to test for the existence of site groups on the basis of these light and hydrodynamic characteristics and to evaluate the goodness of fit between observed and predicted groups (Ludwig & Reynolds 1988).

Adult abundance. Adult abundance of each species in each microhabitat was estimated by counting the number of individuals contained in nine 150 × 50 cm quadrats placed at random. Population censuses were made during November 1994, after the reproductive season was over and individuals from the most recent cohort of recruits were large enough to be seen underwater. Differences in the numbers of individuals among microhabitats were tested by 2-way ANOVA (Sokal & Rohlf 1981), where light and water flow were factors.

Reproductive timing and larval release. The density of brooded larvae per adult (mean ± SD) was estimated

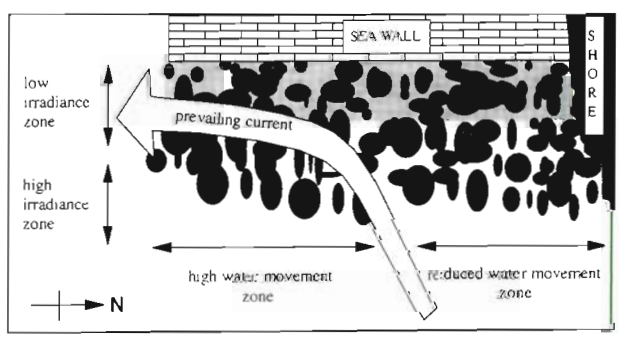

Fig. 1. Schematic diagram of the studied site, showing relationship between shaded and sunny areas and the prevailing flow

for 3 of the 4 species by counting the embryos or larvae contained in 50 $mm^2 \times 1$ mm slices of the choanosome. The samples were taken from 15 individuals per species biweekly, from March to September in 1994. Preliminary observations revealed that embryos are more or less evenly distributed throughout the whole choanosome, except in *Sigmadocia caerulea*. In this species, which is erect and branched, embryos are incubated 1 to 2 mm beneath the pynacothelium of the main exhalant channel of the branches. Given the non-uniform location of embryos in *S. caerulea*, this species was not considered in this part of the study, since the process of counting embryos would involve the destruction of specimens and significant population damage.

Larval morphology and behavior. In *Haliclona tubifera* and *Halichondria magniconulosa*, larval release was induced in the laboratory by light shock after dark adaptation of ripe individuals for 12 to 20 h. In *Sigmadocia caerulea*, larvae were released spontaneously immediately after collection of ripe adults. It was also triggered in the laboratory by exposing ripe adults to air for several seconds. Release could not be artificially induced in *Tedania ignis*. Nevertheless, larvae were sometimes spontaneously released by ripe individuals after 1 to several days in aquaria.

In order to observe larval behavior, length of the swimming period, settlement and early survivorship under a standardized set of laboratory conditions, 100 larvae per species, in batches of 5 to 10 larvae, were placed in glass dishes containing 30 to 100 ml of 0.45 µm millipore filtered sea water. These containers were maintained at room temperature (20–24°C) and monitored for 8 d.

Larval swimming speed was estimated by timing the movement of 2 to 4 h old larvae (n = 30) down the long axis of a plexiglas aquarium 3 deep × 2 wide × 72 cm long while they were responding to light. The light source was a 150 watt, cold fiber optic light passing through a neutral density plastic diffuser.

Effects of light and water flow on settlement and recruitment. To estimate recruitment in the different microhabitats of the community, we randomly deployed a total of ten 15 × 15 cm ceramic floor tiles per microhabitat. Tiles were immersed at the beginning of the seasonal peak of larval release (June 9) and checked for recruitment after 35 and 96 d. Differences in the number of recruits among microhabitats were analyzed by 2-way ANOVA, where light and water flow were factors.

The effect of current on larval choice at settlement in different light regimens was investigated in the laboratory with small circular flumes. The flumes consisted of circular glass channels 2 wide × 3 cm deep containing sea water. Water temperature and salinity ranged from 20 to 24°C and from 33 to 35‰ respectively during the experiments. A fluorescent lamp provided with two 15 w, cool white bulbs, was placed 25 cm above the channels. Light was diffused through a translucent plastic diffuser to minimize reflections by glass walls. Half of each channel was shaded by covering walls, bottom and top with an opaque black plastic. A circular water flow was created by pumping air on the water surface through 2 air jets placed at opposite sides of the channels (see Young & Braightwaite 1980). The mean speed in the center of the channel was 3 cm s^{-1}. By using larvae whose ciliary activity was arrested with 2‰ nickel sulfate, we confirmed that the shear stress created at this speed was strong enough to roll larvae along the bottom of the flumes. Additional half-shaded channels with no current served as controls. We did 2 simultaneous runs of the experiment with each species, where every run consisted of a treatment channel and a control channel, each containing 20 larvae. After 190 h, we recorded the number of surviving settlers and their distribution with regard to light conditions. Data from 2 homogeneous runs of the experiment (homogeneity tests, Sokal & Rohlf 1981) were pooled and arranged in 2 × 2 contingency tables, where the marginal totals of the variable presence/absence of flow were fixed by the experimental design at 40 larvae. Contingency tables were analyzed by the chi-squared test of independence and association was expressed by the phi coefficient (Hays 1963). When association between light and hydrodynamic conditions did not have any effect on the distribution of settlers, we examined the main effects of these variables by a chi-squared test of goodness of fit adjusted for continuity (Sokal & Rohlf 1981), where the ratio of expected values in the 2 levels of a variable was 50:50.

Temperature effects on larval behavior and settlement. The effect of water temperature on larvae of *Haliclona tubifera* and *Halichondria magniconulosa* was investigated by placing larvae in 30 ml plexiglas Petri-dishes in dark incubators at 10, 15, 20 and 25°C. Treatments were applied to batches of 5 larvae per container, up to a total of 5 containers per temperature and the percentages of swimming, settled and dead larvae were recorded over time.

RESULTS

Habitat characteristics

A discriminant analysis revealed that irradiance and water flow features of the 20 studied sampling sites allowed us to cluster them in 4 groups that show significant differences among the positions of their centroids (Wilk's lambda = 0.024, $F_{6,30} = 27.080$, $p < 0.001$;

Fig. 2). These groups correspond to 4 different community microhabitats, as predicted in Fig. 1. The magnitude of the canonical correlation coefficients (R^2_{light} = 0.947, R^2_{flow} = 0.874) indicate that light is a better discriminant factor than flow; that is, irradiance differences among microhabitats are slightly stronger than water flow differences.

Adult abundance

A total of 238 adult *Tedania ignis*, 150 *Sigmadocia caerulea*, 128 *Haliclona tubifera* and 24 *Halichondria magniconulosa* were found in the 36 quadrats. The mean densities (±SD) were 8.8 ± 6.6, 5.7 ± 5.5, 4.7 ± 6.2 and 0.8 ± 1.6 individuals m^{-2}, respectively. Analyses of the abundances of adult sponges in the 4 natural microhabitats revealed no significant interaction between light and current conditions (Table 1, Fig. 3). Light by itself had no effect on the distribution of adults of any species, except *S. caerulea*. However, the presence of water flow did influence 2 species, *T. ignis* and *H. tubifera*. Flow was positively associated with the abundance of *T. ignis* (F = 25.953, $p < 0.001$) and negatively associated with the abundance of *H. tubifera* (F = 42.771, $p < 0.001$). This effect was particularly strong for *H. tubifera*, which was virtually absent from the microhabitats with high currents. The distribution of

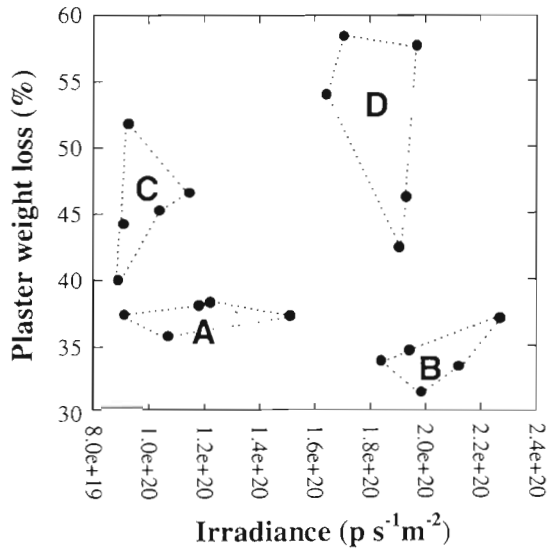

Fig. 2. Community sites clustered according to their irradiance and hydrodynamic characteristics. Water movement was measured with plaster of Paris cylinders. The statistical significance of the resulting groups was tested by discriminant analysis

H. magniconulosa did not seem to be affected by either hydrodynamics or light conditions (Table 1). The relatively small number of individuals of this species could be responsible for the observed lack of statistical significance.

Table 1 Two-way analysis of variance on the effect of flow and light on the abundance of adult sponges in the 4 major community microhabitats

Source	df	MS	*F*	p
Tedania ignis				
Flow	1	386.778	25.953	<0.001
Light	1	11.111	0.746	0.394
Flow × Light	1	1.778	0.119	0.732
Error	32	14.903		
Haliclona tubifera				
Flow	1	427.111	42.771	<0.001
Light	1	7.111	0.712	0.405
Flow × Light	1	7.111	0.712	0.405
Error	32	9.986		
Halichondria magniconulosa				
Flow	1	0.444	0.275	0.604
Light	1	0.000	0.000	1.000
Flow × Light	1	1.788	1.099	0.302
Error	32	1.618		
Sigmadocia caerulea				
Flow	1	30.028	2.593	0.117
Light	1	90.250	6.153	<0.019
Flow × Light	1	0.028	0.002	0.966
Error	32	14.667		

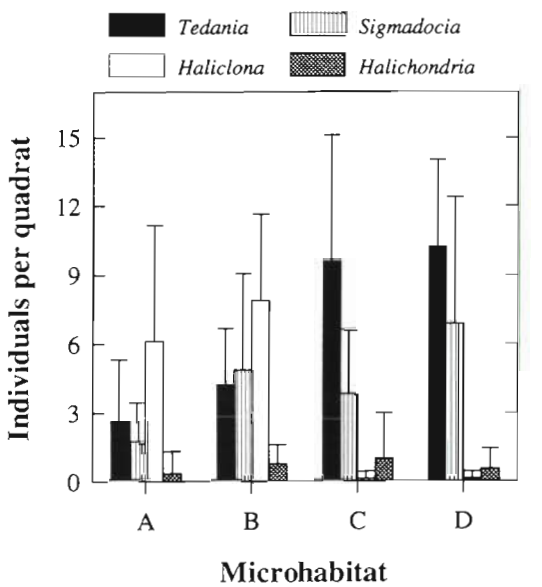

Fig. 3. Mean number of adult sponges per sampling quadrat in the major community microhabitats. Error bars are standard deviation. A: sites with low irradiance and reduced water movement; B: sites with high irradiance and reduced water movement; C: sites with low irradiance and high water movement; D: sites with high irradiance and high water movement

Reproductive timing and larval release

The larval release season of *Tedania ignis*, *Haliclona tubifera* and *Halichondria magniconulosa* started in late April, during a period of rise in water temperature (Fig. 4a). At the beginning of the reproductive season, embryos were found in 90 to 100% of the individuals. Although we did not search for male gametes, this high percentage of brooding individuals suggests that all 4 species are hermaphroditic. Densities of eggs and/or embryos were highest at the beginning of the reproductive period (Fig. 4a). The number of eggs/embryos in 0.5 $cm^2 \times 0.1$ cm sections of tissue was 10.6 ± 2.7 in *T. ignis*, 22.8 ± 5.1 in *H. tubifera* and 27.8 ± 5.5 in *H. magniconulosa*. In *H. tubifera* and *H. magniconulosa*, larval release ceased by mid July, whereas it lasted until late August in *T. ignis*. The gradual decrease of the mean number of eggs/embryos per individual over time (Fig. 4a), as well as the high standard deviation of these values from May to June (Fig. 4b), suggests that larval release in these 3 species is a relatively long and asynchronous process at the population level. Variation over time in the number of larvae contained by adults, as represented by the coefficient of variation, indicates that the dynamics of the larval release are similar in *H. tubifera* and *H. magniconulosa* (Fig. 4c). Greater asynchrony occurred in *T ignis*, especially at the end of the season. *Sigmadocia caerulea* released larvae from June until September, with a peak in August and September.

In *Haliclona tubifera*, *Halichondria magniconulosa* and *Sigmadocia caerulea*, larvae were expelled through oscula shortly after adults were illuminated and for 1 to 2 h thereafter. In *Tedania ignis*, larvae emerged through the ectosome, probably through the ostioles. *H. tubifera* and *H. magniconulosa* individuals required at least 3 massive releases, usually on successive days, to expel their entire brood of larvae. After each of the first 2 release events, the total number of larvae remaining in the adults decreased to about 50% of the original number. After a third release, most adults were virtually empty of larvae. Massive releases on consecutive days were never observed in *T ignis*, but small numbers (15 to 50) of larvae were released by adults several days before and after a massive release. It appeared that ripe individuals of *T. ignis* need at least 1 massive release along with multiple small ones to expel the entire brood.

Larval morphology and behavior

Parenchymella larvae of all 4 species are elongate spheroids, whitish in *Sigmadocia caerulea*, red-orange in *Tedania ignis*, whitish pink in *Haliclona tubifera* and yellow in *Halichondria magniconulosa*. They are uniformly covered with short cilia, except for the posterior end which is bare. This posterior end is surrounded by a ring of flagellated cells forming a distinctive tuft in *S. caerulea*, *H. tubifera* and *H. magniconulosa*, but the posterior ring of flagella is the same length as the rest of the ciliature in *T. ignis*. The mean length (±SD) of the flagellar tuft is 15 ± 3 µm in *T. ignis*, 30 ± 3 µm in *H. magniconulosa*, 50 ± 4 µm in *H. tubifera* and 80 ± 5 µm in *S. caerulea*.

Larvae can temporarily constrict the middle of their bodies, as well as extrude and protract the anterior ends. Larvae of *Halichondria magniconulosa* are capable of remarkable shape changes over relatively short times. In general, 2 to 3 h old larvae are oval, with

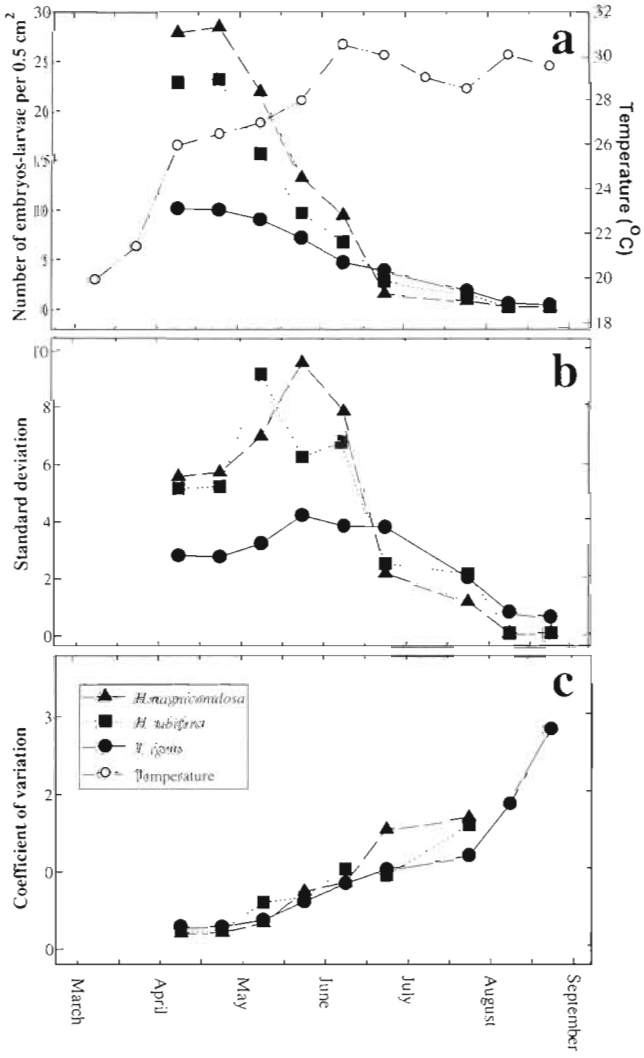

Fig. 4. Reproduction as a function of season: (a) water temperature and mean number of embryos or larvae 0.5 cm^{-2} over time, (b) standard deviation of the mean number of embryos or larvae 0.5 cm^{-2} over time, (c) coefficient of variation of the number of embryos or larvae over time

length:width ratios between 1.5 and 2, whereas 10 to 12 h old larvae become somewhat flatter and longer (length:width ratio = 2.5). The mean length in this species was 318 ± 45.3 µm for larvae younger than 6 h and 468 ± 67.3 µm for larvae older than 12 h. *Haliclona tubifera*, *Sigmadocia caerulea*, and *Tedania ignis* larvae measured 425 ± 79.68, 629.2 ± 95.8 and 758.7 ± 110.64 µm, respectively, with constant length:width ratios over time.

Larvae of all 4 species swim with a clockwise corkscrew motion and with the flagellar end directed backwards, but there are interspecific differences in swimming behavior. Larvae of *Haliclona tubifera*, *Sigmadocia caerulea*, and *Halichondria magniconulosa* swim actively near the water surface for 6 to 12 h after release. Then, swimming speed gradually decreases and larvae exhibit an 'exploratory' behavior, moving closely to the bottom of containers where they remain for between a few minutes and 90 h. By contrast, *Tedania ignis* larvae never swim vigorously; they remain near the bottom unless disturbed by water turbulence.

Settlement occurs in different ways, depending upon the species. Larvae of *Haliclona tubifera* and *Sigmadocia caerulea* position themselves perpendicular to the bottom, with the anterior pole oriented toward the substratum, and they spin with a clockwise rotation for between a few minutes and up to 3 h. While rotating, threads of a transparent solid substance (basal spongin?) are released around the future settlement spot. Spinning stops prior to settlement, and larvae always attach by the anterior end. The flagellar tuft beats for between 15 and 40 min after settlement, and then is shed. In *Halichondria magniconulosa*, there is no spinning phase. The corkscrew swimming movement ceases and the flattened larvae crawl on the bottom by one of their lateral sides for between a few minutes and 4 h. Larvae may attach either by the anterior end or one lateral side. About 40% of the time, attachment is by a lateral side. The whole flagellar tuft is instantaneously expelled just before settlement. Larvae of *Tedania ignis* stop corkscrew swimming after 12 to 72 h, crawl for a variable period of time and finally attach by the anterior pole. Fusion of larvae and early juveniles was common in *T. ignis*, *S. caerulea* and *H. tubifera*, especially when larvae were high in density in the containers. A single osculum appeared in juveniles of all 4 species about 2 d after settlement.

There was approximately 50% mortality of *Haliclona tubifera* and *Halichondria magniconulosa* after 8 d in the laboratory dishes. More *in vitro* mortality occurred in juveniles than in swimming larvae. Juveniles were especially vulnerable to mortality 24 to 48 h after settlement, and prior to the formation of any oscular finger. Survival of *Tedania ignis* and *Sigmadocia caerulea* was clearly higher than those of the other 2 species (Fig. 5). It is noteworthy that *T. ignis* larvae that did not settle within about 96 h lost body ciliation and became lethargic. Nevertheless, most of these lethargic larvae were able to attach about 2 d later, and they ultimately became healthy juveniles. We also found that *H. tubifera* larvae that swam for at least 72 h were able to settle successfully. These observations are contrary to the observations reported by Woollacott (1993) indicating that larvae of this species that do not settle within 24 h die before an additional 8 h passed. Juveniles from larvae that had delayed settlement showed a short-term mortality (after 4 d) similar to that of juveniles from larvae swimming for less than 72 h.

Effects of light and water flow on settlement and recruitment

Larvae of all 4 species were photonegative during the whole swimming period in laboratory conditions. Response to light, as measured by larval swimming speed in response to lateral illumination, was different in each species. Mean swimming speed (±SD) was 0.36 ± 0.05 cm s^{-1} in *Sigmadocia caerulea*, 0.27 ± 0.04 cm s^{-1} in *Haliclona tubifera*, 0.17 ± 0.07 cm s^{-1} in *Halichondria magniconulosa* and 0.098 ± 0.06 cm s^{-1} in *Tedania ignis*. Our results, along with larval measurements reported for *Halichondria melanodocia* (Woollacott 1990), indicate that the capacity to swim in response to light is related to the ratio of flagellar tuft length:body length (r^2 = 0.859, n = 5, p = 0.024; Fig. 6).

Larvae placed in the flume initially demonstrated a positive rheotaxis. They initially faced into the current, but were unable to swim against it because the mean

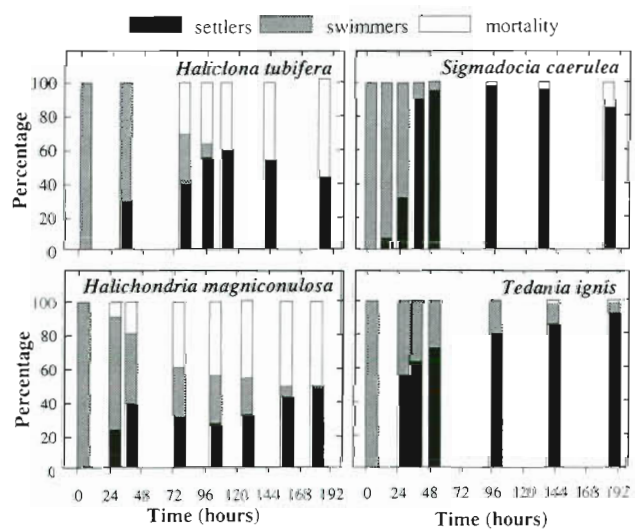

Fig. 5. Cumulative percentages of free-swimming larvae, settlers and mortality in laboratory dishes over time

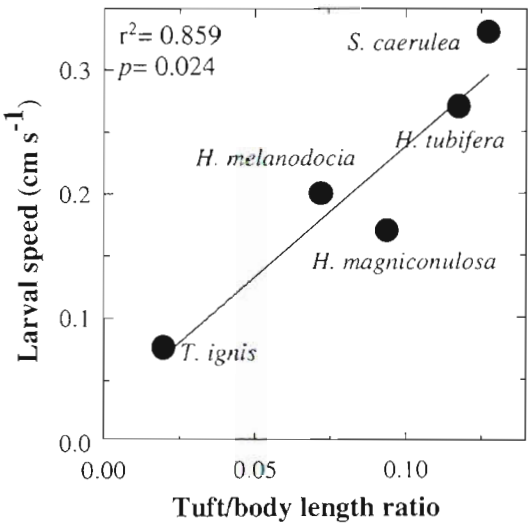

Fig. 6. Linear regression showing the relationship between tuft:body length ratio and larval swimming speed while exhibiting a photonegative response

Table 2. Row by column tests of independence between success/failure at settlement and presence/absence of current in circular aquaria. χ^2_Y: Yates-corrected chi-squared; +: presence of current; –: absence of current

	Tedania ignis		*Sigmadocia caerulea*		*Haliclona tubifera*		*Halichondria magniconulosa*	
	Current		Current		Current		Current	
	+	–	+	–	+	–	+	–
Alive	40	39	26	28	38	37	26	28
Dead	0	1	14	12	2	3	14	12
	$\chi^2_Y = 0.000$		$\chi^2 = 0.228$		$\chi^2_Y < 0.001$		$\chi^2 = 0.228$	
	$p = 1$		$p = 0.633$		$p = 1$		$p = 0.633$	

flow speed (3 cm s^{-1}) was much higher than the larval swimming speed. The presence of the flow did not affect settlement success in any of the 4 species (Table 2). Larvae of *Sigmadocia caerulea*, *Haliclona tubifera* and *Halichondria magniconulosa* significantly preferred shaded sites ($\chi^2_{adj} = 9.292$, $p < 0.005$; $\chi^2_{adj} = 21.33$, $p < 0.001$; and $\chi^2_{adj} = 5.35$, $p < 0.025$, respectively), but this choice was made independently of the presence or absence of current (Table 3). Flow increased the chance that *Tedania ignis* larvae would settle in shaded sites.

The recruitment of sponges on ceramic tiles placed in the field varied substantially among species. After 35 d, we recorded 258 recruits of *Tedania ignis* and 30 of *Sigmadocia caerulea* on the tiles, but only 1 recruit for each of *Haliclona tubifera* and *Halichondria magniconulosa*. The recruitment of sponges and most other invertebrates occurred mainly on the undersurfaces of the tiles, regardless of which location in the major community microhabitats the tiles were placed in. The exposed surface was always fully covered by fine sediment and usually by green filamentous algae as well.

The distribution of the recruits of *Tedania ignis* after 35 d was significantly higher in the shaded parts of the community (Fig. 7, Table 4), irrespective of flow conditions. The abundance of small recruits and the scarcity of large recruits suggests that there is high mortality within a few days of settlement. After 96 d, the number of surviving recruits of *T. ignis* had decreased in all microhabitats, except the one characterized by high water movement and high irradiance (Fig. 7). Recruitment after 96 d, unlike that after 35 d, was not affected by light differences among microhabitats, but it was influenced by water movement (Table 4). At this time, juvenile survivorship for *T. ignis* was significantly higher in all sites with high water movement, irrespective of light conditions. Recruitment of *Sigmadocia caerulea* after 35 and 96 d was not affected by either light or hydrodynamic conditions of microhabitats (Table 4). The number of survivors did not substantially vary between 35 and 96 d. *Haliclona tubifera* and *Halichondria magniconulosa* had low numbers of recruits after 35 and 96 d.

Temperature effects on larval behavior and settlement

The larval release season of *Haliclona tubifera* and *Halichondria magniconulosa* begins in late April, during a period when water temperature is rising (Fig. 4a). Larval release under natural conditions occurs at temperatures between 25 and 31°C. Experimental results

Table 3. Row by column tests of association between experimental conditions of light and current as cues used by sponge larvae at settlement. χ^2_Y: Yates-corrected chi-squared; +: presence of factor; –: absence of factor; φ: phi coefficient of contingency

		Tedania ignis		*Sigmadoria caerulea*		*Halichlona tubifera*		*Halichondria magniconulosa*	
		Current		Current		Current		Current	
		+	–	+	–	+	–	+	–
Light	+	11	20	5	10	11	6	7	11
	–	29	19	21	18	27	31	19	17
Statistics		$\chi^2_Y = 4.684$		$\chi^2 = 1.826$		$\chi^2_Y = 1.733$		$\chi^2 = 0.927$	
		$p = 0.030$		$p = 0.177$		$p = 0.188$		$p = 0.336$	
		$\varphi = -0.244$							

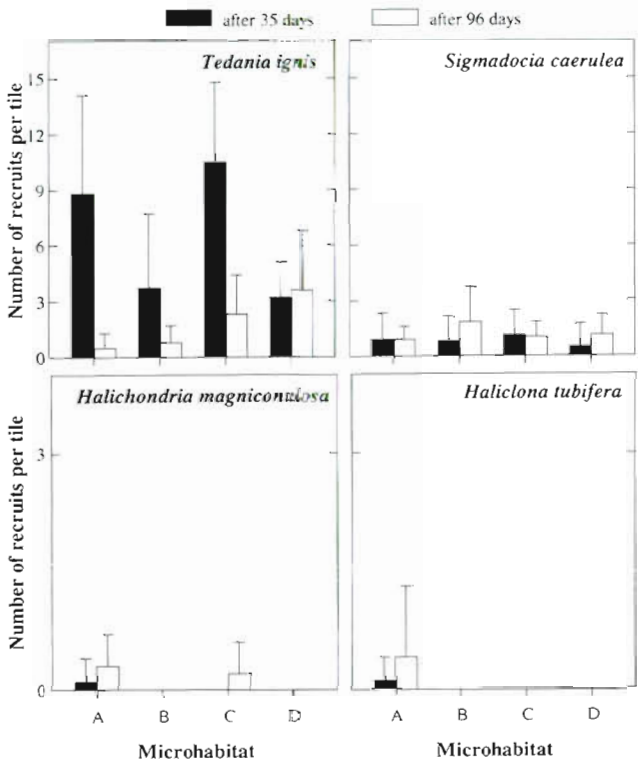

Fig. 7 Mean number of recruits per tile in the major community microhabitats after 35 and 96 d. Error bars are standard deviation. A: sites with low irradiance and reduced water movement; B: sites with high irradiance and reduced water movement; C: sites with low irradiance and high water movement; D: sites with high irradiance and high water movement

Table 4. *Tedania ignis* and *Sigmadocia caerulea*. Two-way analyses of variance on the effect of flow and light on recruitment (after 35 and 96 d) to ceramic tiles placed in the 4 major microhabitats

Source	df	MS	*F*	p
T. ignis after 35 d				
Flow	1	3.600	0.210	0.649
Light	1	384.400	22.472	<0.001
Flow × Light	1	12.100	0.707	0.406
Error	36	17.106		
T. ignis after 96 d				
Flow	1	52.900	12.645	0.001
Light	1	6.400	1.530	0.224
Flow × Light	1	2.500	0.598	0.406
Error	36	4.183		
S. caerulea after 35 d				
Flow	1	0.025	0.014	0.908
Light	1	1.225	0.669	0.419
Flow × Light	1	0.625	0.341	0.563
Error	36	1.831		
S. caerulea after 96 d				
Flow	1	0.900	0.585	0.449
Light	1	2.500	1.625	0.221
Flow × Light	1	1.600	1.040	0.315
Error	36	1.539		

indicated that water temperature is very important in controlling the length of the free-swimming period of *H. tubifera* and *H. magniconulosa* larvae (Figs. 8 & 9). At 10 and 15°C, the swimming phase was reduced and larvae settled in less than 24 h. However, juvenile mortality was high (close or equal to 100%) after 2 d, since attached larvae apparently could not complete metamorphosis. Those larvae that were unattached after 24 h at 15°C became lethargic, demonstrating some ciliary activity but no swimming capability. Approximately 20% of these lethargic larvae resumed swimming and were able to complete metamorphosis when they were transferred into warmer water after less than 12 h exposure to low temperatures. It is noteworthy that low temperatures induced a major shape change in larvae of *H. magniconulosa*. The body lengthened to between 50 and 100% of the original length when exposed to temperatures of 10 and 15°C.

At 20 and 25°C, the swimming phase lasted longer in both species and settlement was enhanced. Survival after 170 h was much higher in *Haliclona tubifera* than in *Halichondria magniconulosa* (Figs. 8 & 9). The mortality patterns in *H. tubifera* were similar at both temperatures, whereas they were notably different in *H. magniconulosa*. Nevertheless, the final percentage of mortality in this latter species was similarly high at both temperatures after 170 h.

DISCUSSION

Reproductive timing

In all 4 species of sponges, larval release extends over a long period of time, and it is not a synchronous event at either the individual or population level. Asynchrony in larval release has also been reported in other viviparous species inhabiting both tropical and subtropical areas (Reiswig 1973, Hoppe 1988, Ilan & Loya 1988, 1990) and also in regions with marked seasons (Fell 1974, Wapstra & van Soest 1987). This asynchrony in larval release, both at the population and the individual level, probably reflects an asynchrony of gametogenesis in viviparous sponges (Ilan & Loya 1988, Witte et al. 1994). Oviparous species, on the other hand, have been reported to have a very precise synchrony in gametogenesis and spawning both at the individual and population level (Sará 1961, Siribelli 1962, Reiswig 1970, 1976, Hoppe & Reichert 1987, Fromont 1988, Fromont & Bergquist 1994). Asynchrony in gametogenesis in viviparous species probably leads

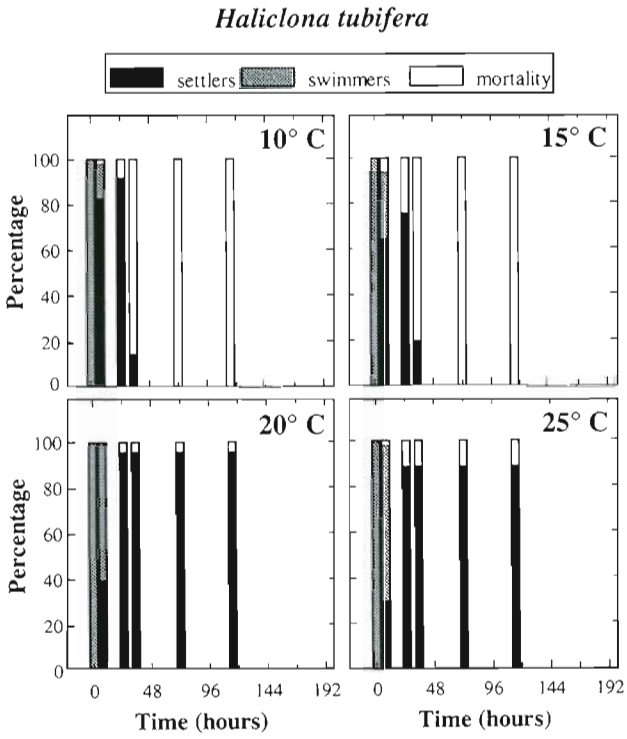

Fig. 8. *Haliclona tubifera*. Cumulative percentages of free-swimming larvae, settlers and mortality over time at 4 different water temperatures

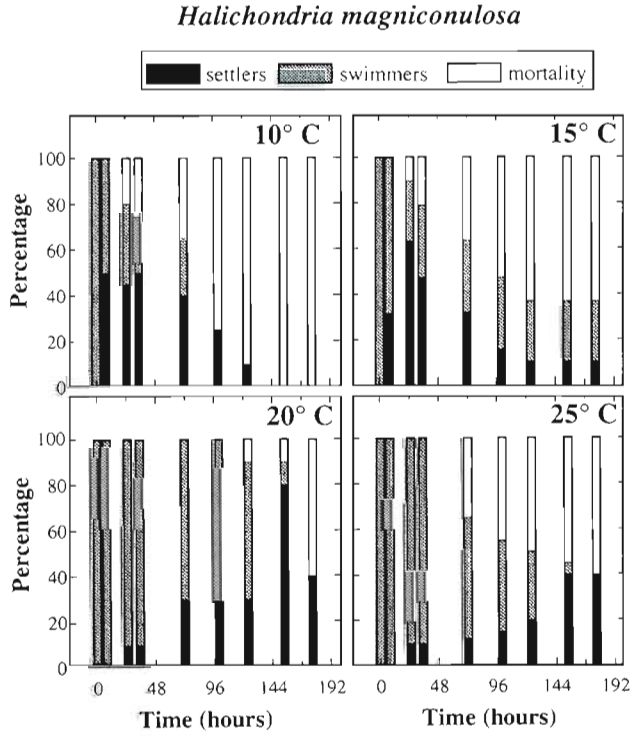

Fig. 9. *Halichondria magniconulosa*. Cumulative percentages of free-swimming larvae, settlers and mortality over time at 4 different water temperatures

to a lengthening of larval release over time at the population level. An extended larval release period spreads the risk of mortality across time; so the sponge does not risk losing the entire reproductive effort because of isolated, stochastic events (Ilan & Loya 1990).

Larval release is triggered in many sponges by a photoperiod-based mechanism. For instance, Amano (1986) demonstrated that the onset of darkness of the preceding day is the stimulus triggering larval release in *Halichondria panicea*, whereas the stimulus for *Callyspongia ramosa* is sunrise the day before (Amano 1988). We induced larval release in *Haliclona tubifera* and *Halichondria magniconulosa* by strong illumination, after keeping ripe adults for variable times in darkness. The mechanisms by which the light cue is translated into larval release remain completely unknown. So far, no photoreceptor has been identified in either adult sponges or larvae. It is possible that light cues are not perceived by adults, but by larvae only. Larvae of *Mycale americana* have been observed swimming actively in the subectosomal spaces of adults for several hours before being released through oscula (authors unpubl. data). This has also been observed in other species (Lévi 1956, Fell 1989).

We failed to find a stimulus triggering larval release in *Tedania ignis*; larval release was not induced by manipulating light or temperature conditions. However, larvae were sometimes released spontaneously during the early morning hours of sunny days. Larval release was also apparently unrelated to lunar phases, which are known to control gametogenesis in some oviparous sponges (Hoppe & Reichert 1987, Fromont & Bergquist 1994).

In most demosponges, larvae are expelled through the oscula by the outgoing water effluent. Larvae of *Tedania ignis* leave the body of the parent by creeping through the ectosome. This is quite an exceptional pattern in demosponges, although a similar release pattern has been described in an unrelated species, *Callyspongia diffusa* (Sivaramakrishnan 1951).

The total number of larvae released per individual cannot be easily calculated. We have estimated that the mean density of embryos and larvae brooded 0.5 cm^{-2} is about 10.6 in *Tedania ignis*, 22.8 in *Haliclona tubifera* and 27.8 in *Halichondria magniconulosa*. Fell et al. (1987) reported an average of 205 embryos cm^{-2} in *Haliclona loosanooffi* and up to 343 cm^{-2} in an unidentified species of *Halichondria*. These values are about 5 times higher than the values for co-generic species in our study. Uriz (1982) reported an average of 6 larvae cm^{-2} in a species of a related genus, *Hymeniacidon sanguinea*. According to data from the literature, oviparous species show somewhat higher densities. For instance, Fromont &

Bergquist (1994) found values of about 93, 156, and 510 oocytes 0.5 cm^{-2} in 3 different species of the genus *Xetospongia*. It is also known that not all brooded larvae are actually released; some may be resorbed (Bergquist & Sinclair 1968).

Larval behavior

Most sponge larvae, including those in this study, display a crawling behavior for a few minutes or hours just before settlement. This behavior is accompanied in some cases by extrusion of the anterior end of the larva. Although it appears that larvae are searching and testing surfaces, this point remains mere speculation because no tactile or chemical receptors are known in sponge larvae.

Photoreceptors, which would explain the larval phototaxis, do not exist in parenchymella larvae either, or at least they have not yet been found. The posterior flagellar tuft of uniformly ciliated larvae, such as parenchymella larvae of demosponges, has traditionally been thought to provide maneuverability (Konstantinova 1966, Chia et al. 1983), but the flagellar tuft may also control swimming speed (Woollacott 1993). It has also been suggested that cells forming the posterior tuft are involved in photoreception (Woollacott 1993). The strong association between the larval swimming speed when moving away from a source of light and the ratio of larval body:flagellar tuft length suggests that the posterior tuft is highly involved in phototaxis either as receptor, an effector, or both. Posterior flagellar cells contain large granules apparently filled with pigment. A similar structure consisting of pigment granules and a paraflagellar swelling associated with a flagellum is responsible for the photoresponses of the protozoan *Euglena* (Wolken 1971). The pigment and associated organelles function as the photoreceptor and the flagellum functions as the effector. However, in *Euglena*, microfibrils connect the pigment granules and the flagellum, and such microfibrils have not been found in sponges. The bundless of microtubules forming the flagellar roots, which are present in all invertebrates, have not been found in parenchymella larvae either (Nielsen 1987), although they are described in the larva of the calcareous sponge *Ascandra falcata* (Borojevic 1969). Further ultrastructural studies are needed to understand the cytological basis of the larval photoresponse.

Settlement and recruitment

Parenchymella larvae usually attach by the anterior end. Nevertheless, we have observed attachment of *Halichondria magniconulosa* larvae by a lateral surface, corroborating previous reports of this particular feature in other species of this genus (Wilson 1935, Wapstra & van Soest 1987) and in the related genus *Hymeniacidon* (Uriz 1982).

In experimental conditions, the presence of 3 cm s^{-1} flow had no effect on the success of larval settlement. The boundary shear stress at this flow rate did not prevent larvae from attaching to the smooth glass surfaces, despite the fact that larvae could not swim upstream. Furthermore, larvae of all 4 species significantly preferred shaded sites at settlement. This result suggests that, despite the possible existence of a passive transport by near-bottom flows, the active larval response to light is still important in determining the small-scale spatial distribution at settlement. However, larval responses in nature may differ from the laboratory responses as, apart from flow regimes, many other environmental factors can affect the larval choice at settlement (substratum characteristics, chemical cues, pycnoclines occurrence, turbidity, etc).

Light conditions in the natural microhabitats were not significantly related to the adult abundance of 3 species; the only exception was *Sigmadocia caerulea*. After 96 d, recruitment and adult abundances of *Tedania ignis* and *Haliclona tubifera* respectively were associated with the current regimen. This suggests that although the presence of water current does not affect larval choice for settlement, it can influence the post-larval survivorship of some species. High light intensities have been demonstrated to kill some sponges (Jokiel 1980). However, it is also true that many shallow-water Caribbean demosponges grow in sites with strong light (de Laubenfels 1950). Some of our observations suggest that post-settlement mortality is not affected by the presence of light itself, but by associated factors. For example, juvenile sponges growing on horizontal surfaces are highly exposed to light, but they are also highly exposed to siltation and algal overgrowth, both factors known to have negative effects on sponge survival (Sará & Vacelet 1973, Wilkinson & Vacelet 1979, Zea 1992, 1993). Although many adult sponges in our study sites were clearly exposed to light, it is possible that their original settlement sites may actually have been located in small crevices or holes where early juveniles were protected from siltation and algae. It seems, therefore, that although light is used by larvae as a major cue for orientation and selection of settlement sites, light provides only an indirect cue for sites where the survivorship may be enhanced.

Survival of *Tedania ignis* and *Sigmadocia caerulea* was much higher than that of *Haliclona tubifera* and *Halichondria magniconulosa* after 8 d in standard laboratory conditions. Mortality in laboratory conditions was virtually zero in the first 2 species, while it was

about 40 to 50% in the latter two (Fig. 5). The major sources of mortality in the laboratory were bacterial infections and predation by ciliates. Although laboratory mortality and its sources are not necessarily related to those operating in natural conditions, it is clear that larvae of *S. caerulea* and *T. ignis* larvae are generally less susceptible to mortality than the other species. Recruitment on experimental surfaces placed in the natural community indicates that the 2 species that survived well in the laboratory also recruit better in the field. This could explain the higher adult abundances of these 2 species in many shallow-water Caribbean assemblages (e.g. Hetchel 1965, Zea 1987).

Water temperature controls the duration of the different behavioral phases of larval life. At 10 and 15°C, swimming speed decreased, the free-swimming phase was dramatically shortened, and metamorphosis was inhibited. Low temperatures, therefore, potentially prevent larval dispersal and recruitment into cold waters and might restrict the distribution of *Haliclona tubifera* and *Halichondria magniconulosa* to tropical areas and warm shallow waters. Sharp temperature changes also resulted in lengthening and shortening of the larval body in *H. magniconulosa*. This body reshaping could result from flattening and 're-shaping' processes of the high, cuboidal epithelial cells.

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