

## SPONGE LARVAL PHOTOTAXIS: A COMPARATIVE STUDY

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### ABSTRACT

Recent work has shown that larvae of the tropical demosponge *Reniera* sp. are capable of instantaneous responses to abrupt changes in light intensity, a behaviour that allows them to settle in dark areas under coral rubble on the reef flat at Heron Is. GBR. To determine how widespread this kind of phototactic behaviour is among sponge larvae, ontogenetic changes in the photoresponse of larvae from two temperate demosponges and a calcareous sponge were studied. Most larvae from *Scypha* sp. swam away from a white light source for 3 days until settlement and metamorphosis; *Haliclona* cf. *permollis* larvae swam away from light for 48 hours; and larvae from *Halichondria panicea* were benthic until settlement and metamorphosis, and showed no responsiveness to gradients of light intensity. These results demonstrate that sponge larvae are capable of responding to environmental stimuli like other metazoan larvae and show that a coordinated behavioural response to stimuli is possible even in the absence of neurons or junctions that would allow electrical signalling between cells.

### KEY WORDS

Larvae, behaviour, phototaxis, photoreceptor.

### INTRODUCTION

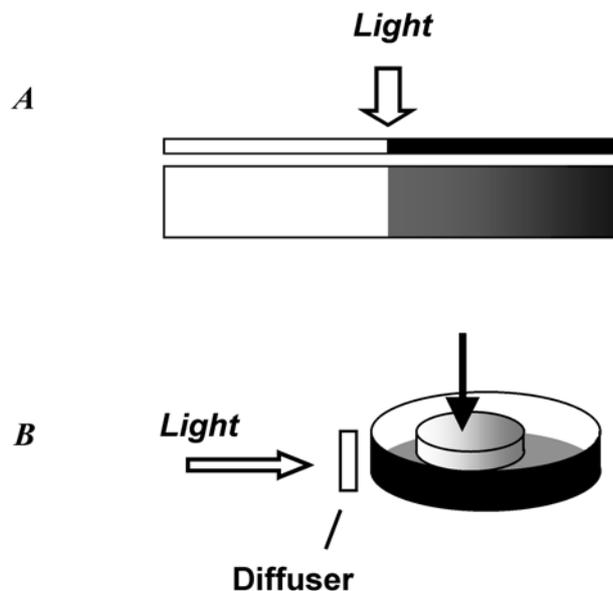
In the last century many attempts have been made to demonstrate the presence of neurons or sensory cells in the Porifera (*e.g.*, MCNAIR, 1923; PAVANS DE CECCATTY, 1955, 1959, 1962, 1971, 1974a; JONES, 1962). Despite histochemical evidence of the presence of neurotransmitters in some cell types (LENTZ, 1966; WEYRER *et al.*, 1999), and histological evidence of elongate cells in some sponges (PAVANS DE CECCATTY, 1959; PAVANS DE CECCATTY *et al.*, 1970), specialists now agree that the Porifera is the only metazoan phylum that lacks a nervous system and that there is no evidence of gap junctions or other communicating junctions (PAVANS DE CECCATTY, 1974a, b, 1976; MACKIE, 1979, 1990; GARRONE *et al.*, 1980; LEYS & MACKIE, 1997). Nevertheless, sensory systems must be present in sponges, because phototaxis (WARBURTON, 1966; BERGQUIST & SINCLAIR, 1968; BERGQUIST *et al.*, 1970; WAPSTRA & VAN SOEST, 1987; WOOLLACOTT, 1990, 1993; MALDONADO & YOUNG, 1996, 1999), geotaxis (WARBURTON, 1966) and rheotaxis (MALDONADO & YOUNG, 1999) have all been documented in sponge larvae.

It has recently been demonstrated that parenchymella larvae of the haplosclerid demosponge *Reniera* sp. can respond to abrupt changes in the intensity of light by instantly straightening and bending long posterior cilia (LEYS & DEGNAN, 2001; LEYS *et al.*, 2002). In the absence of a nervous system, directional swimming of these larvae away from light (negative phototaxis) is caused by the summation of the individual ciliary responses to light. Such acute photoresponsiveness allows *Reniera*

larvae to seek the exact shading of the preferred habitat of the adult sponge (LEYS & DEGNAN, 2001). To better understand how universal this photosensory mechanism is across different sponge taxa, we have compared swimming ability and responsiveness to light in larvae from two temperate demosponges and a temperate calcareous sponge with respect to the patterns of locomotory cilia.

## MATERIALS AND METHODS

Adult sponges of *Haliclona* cf. *permollis* Bowerbank, 1866 and *Halichondria panicea* Pallas, 1766 were collected from rocks and mussel shells on Dixon, Seppings, and Wizard islands in Barkley Sound, British Columbia. Adults of *Scypha* sp. were collected from the inside of plastic plant pots that were suspended at 5 m depth from ropes off the docks at the Bamfield Marine Sciences Centre, Bamfield, British Columbia. Pieces of sponge were placed in bowls with approximately 200 ml of seawater and left at room temperature for up to three hours. During this time the water warmed from 9° C to approximately 15° C. Larvae were released from all three sponges after 1 - 3 hours and were collected by pipette and transferred to 12 well multi-well dishes for experiments, or directly into vials of fixative.



**Fig. 1. A,** A diagram of the test chamber for experiment (1) in which white light was shone from above onto a lid that was half opaque and half transparent. **B,** Diagram of the setup for experiment (2) in which larvae were pipetted into a test chamber (at the position indicated by the arrow) which was placed within a larger chamber filled with sea water. The outer chamber was blackened on all sides except that facing the light source. Light was shone through a diffuser of acrylic plastic to create a gradient of light across the chamber.

To examine the ontogenetic change in phototactic response two experiments were carried out. In the first experiment (1) 50 (*Scypha*) or 100 (*Haliclona* and *Halichondria*) newly released, 2hr, 6hr, 12hr, 24hr, 48hr, and 72hr-old larvae were pipetted into a rectangular aquarium 5 x 5

x 15 cm containing 0.45  $\mu\text{m}$  filtered seawater that was opaque on one half and transparent on the other (Fig. 1A). The transparent side of the chamber was illuminated with two arms of a cold-light source (Volpi Intralux 5000) held approximately 5 cm from the surface of the lid, so as to provide an even distribution of light over the chamber. The chamber was maintained at approximately 10° C throughout the experiment in a dish of ice. The larvae were placed at the transparent end, at the opaque end, or in the middle of the chamber, and the chamber was covered with a lid. After one hour the lid was removed, a small plastic divider was placed in the middle of the chamber separating the illuminated from the dark side. The number of larvae on each side were counted and the number of larvae at the surface and at the bottom of the water in the chamber was noted. In a second experiment (2), larvae from *Haliclona* and from *Scypha* were pipetted into a test chamber containing filtered (0.45  $\mu\text{m}$ ) sea water, which was immersed in sea water in a second chamber that was blackened on three sides to reduce reflected light (Fig. 1B). Light from a cold light source was passed through a diffuser made of acrylic plastic into the inner test chamber to create a gradient of light from the front to the back of the chamber (after LEYS & DEGNAN, 2001). The location of individual larvae after 1 minute was recorded and plotted as a circular histogram. The mean angle swum by the larvae was calculated and the measure of randomness was tested using the non-parametric Rayleigh test (an  $r$  value approaching 1 indicates the data are highly grouped).

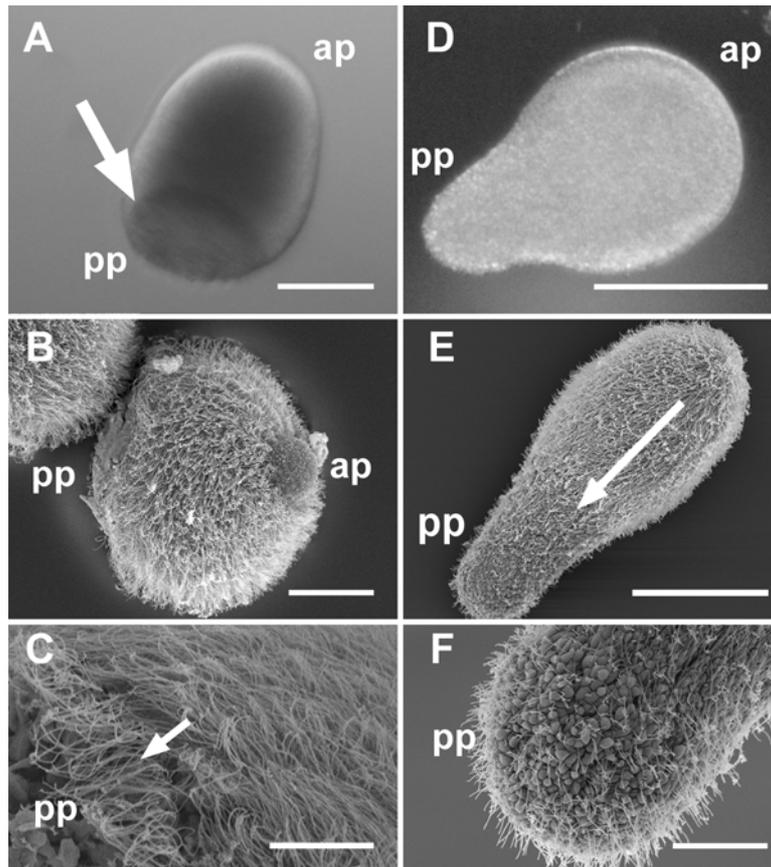
Images of live larvae were taken using an Olympus SZX-12 dissecting microscope equipped with a DP12 digital camera. Larvae were fixed and prepared for ultrastructural observations according to the methods of LEYS & REISWIG (1998).

## RESULTS

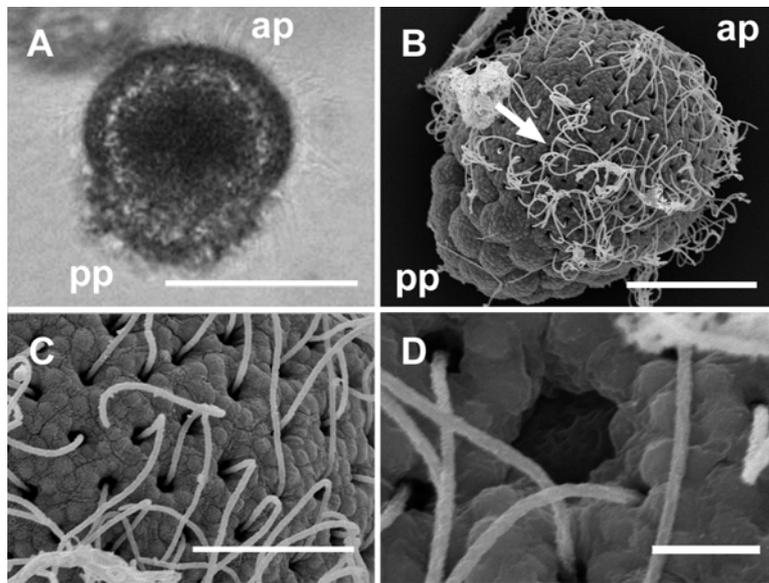
Larvae of *Haliclona* are cream-coloured, round parenchymellae, 140 - 190  $\mu\text{m}$  long and 130 - 150  $\mu\text{m}$  wide. They are ciliated at all but the anterior and posterior poles (Figs 2A, B). The bare region (80 - 95  $\mu\text{m}$  in diameter) at the posterior pole of the larva is circumscribed by a broad band of pigmented cells (Fig. 2A). A ring of 25  $\mu\text{m}$ -long cilia arises from the pigmented region (Figs 2A, C), while the cilia around the rest of the larvae are approximately 10  $\mu\text{m}$  long. *Halichondria* larvae are yellow, elongate parenchymellae, 240 - 280  $\mu\text{m}$  long and 90 - 100  $\mu\text{m}$  wide (Fig. 2D). They are narrowest at the posterior pole. These larvae are ciliated throughout, but the posterior third (a region approximately 80  $\mu\text{m}$  long) is very sparsely ciliated (Figs 2E-F). *Scypha* larvae are cream-coloured, round amphiblastulae, 55 - 72  $\mu\text{m}$  long and 45 - 55  $\mu\text{m}$  wide, with a small lightly pigmented region at the anterior end (Fig. 3). The larva has two distinct hemispheres: an anterior hemisphere composed of columnar ciliated cells, and a posterior hemisphere that is composed of large non-ciliated globular cells (Fig. 3B). The cilia on the anterior hemisphere are 15  $\mu\text{m}$  long and arise from a depression deep in the cell's apical surface (Fig. 3C). Just anterior to the unciliated posterior hemisphere the larvae possess four pits, one in each quadrant, which lie among, but do not appear to be associated with, the ciliated cells (Figs 3B, D).

*Haliclona* larvae are released singly, and intermittently, through the dermal surface of the adult, not through the oscula. Upon release from the adult sponge, the larvae swim upward rotating in a clockwise direction as seen from the posterior pole (a 'right-handed' rotation). The rate of swimming is 0.04 cm/s  $\pm$  0.004 cm/s. *Halichondria* larvae are also released singly and intermittently, but through the oscula of the adult sponge. Once clear of the osculum, these larvae rarely swim up into the water column; most creep along on the surface of the adult sponge or swim weakly

along the bottom in still water, rotating always in a right-handed direction. *Scypha* larvae are released in great numbers through the osculum of the sponge and swim upward at 0.009 cm/s, rotating in a right-handed direction; many become trapped in the surface tension of still water. Most larvae of all three species settle within several hours to 2 days after release, but some larvae can continue to swim for up to a week.

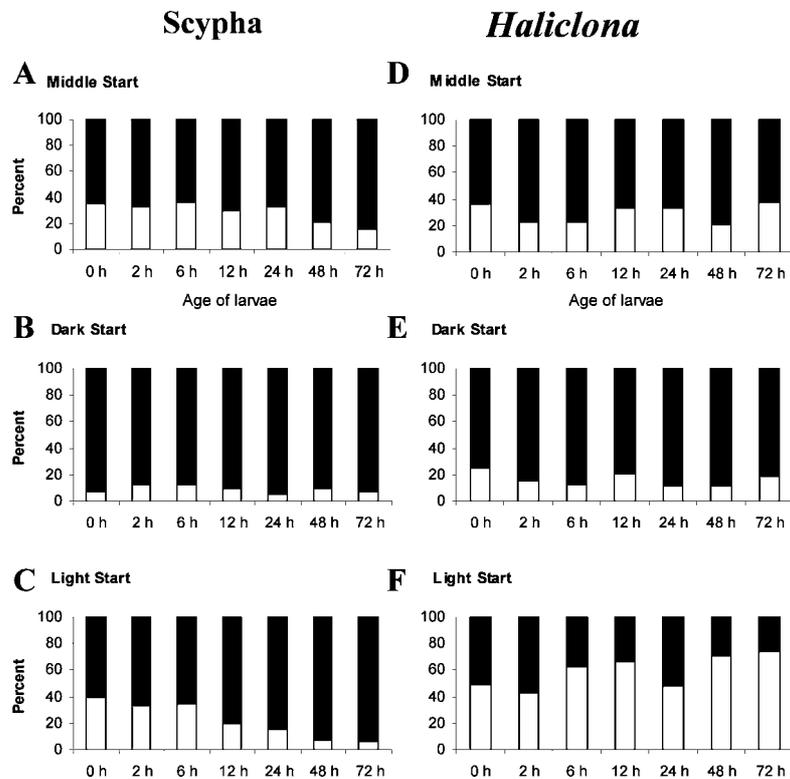


**Fig. 2.** Morphology of *Haliclona* cf. *permollis* (A-C) and *Halichondria panicea* (D-F) larvae. Light and scanning electron microscopy. The posterior pole (A, arrow) of *Haliclona* larvae has a ring of pigmented cells and a ring of cells with longer cilia (C, arrow). The anterior pole (B, ap) is bare. The posterior half of *Halichondria* larvae (E, arrow; F) is sparsely ciliated. Scale bars: a, b: 50  $\mu$ m; c: 20  $\mu$ m; d, e: 100  $\mu$ m; f: 25  $\mu$ m. Anterior pole (ap); posterior pole (pp).



**Fig. 3.** Morphology of larvae of *Scypha* sp. **A**, Light micrograph and **B** scanning electron micrograph of the whole larva. The arrow in **B** shows the location of one of four pits on the anterior hemisphere of the larva, shown in higher magnification in **D**. **C**, Ciliated cells of the anterior hemisphere of the larva. Scale bars: a: 50  $\mu$ m; b: 25  $\mu$ m; c: 10  $\mu$ m; d: 2.5  $\mu$ m. Anterior pole (ap); posterior pole (pp).

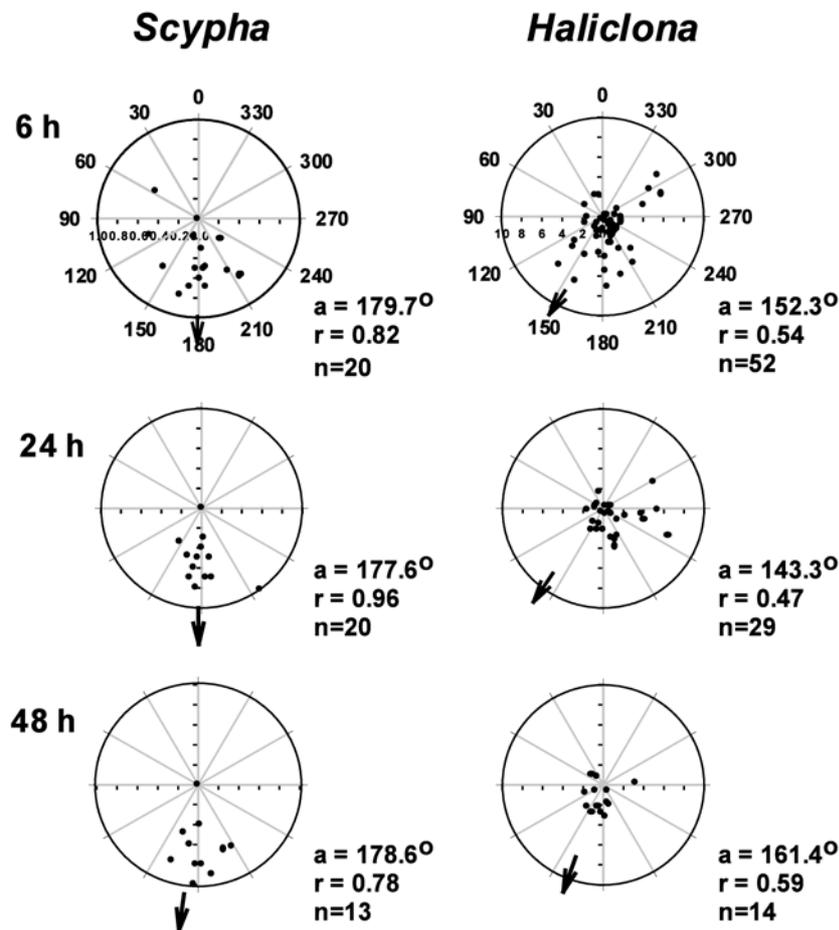
*Haliclona* and *Scypha* larvae are negatively phototactic for at least 3 days after release. Most larvae of all ages of both species placed in the middle, dark or light side of the test chamber were found in the dark side of the chamber after 1 hour (Fig. 4). *Haliclona* larvae swam in the upper portion of the test chamber for at least 24 hours after release from the adult, after which time most larvae sank or swam to the bottom of the dish. *Scypha* larvae, however, continued to swim in the upper portion of the test chamber for at least 72 hours after release. *Halichondria* larvae are indifferent to gradients of light intensity. Larvae placed in either side or in the middle of the test chamber remained where they were placed. Fig. 5 shows the response of *Haliclona* and *Scypha* larvae to directional white light (experiment 2). *Scypha* larvae swam away from directional white light for a certain distance and then appeared to stop swimming. Even though the mean angle of the location of all *Haliclona* larvae after one minute was in the direction away from the light, these larvae appeared to be relatively indifferent to directional white light.



**Fig. 4.** The light response of *Scypha* (A-C) and *Haliclona* (D-F). Newly released larvae, and 2, 6, 12, 24, 48, and 72 hour-old larvae were placed in the dark side, light side, or middle of a test chamber (see text for details). Shaded bars indicate the percent of larvae found in the dark side; open bars indicate the percent of larvae found in the light side after one hour.

## DISCUSSION AND CONCLUSIONS

The results presented here confirm that many sponge larvae are capable of coordinated behaviour in response to environmental stimuli. However, the different responses to light shown by the three larvae in the present study, and the radically different patterns of locomotory cilia each has, suggest that while ciliary sensory systems are widespread across the Porifera the sensitivities of these cells are probably very different.



**Fig. 5.** Circular histograms showing the location of individual larvae of *Scypha* and *Haliclona* after exposure to diffuse white light for 1 hour. The mean angle of all the larvae from one age cohort is given by 'a'; the regression 'r' of the Rayleigh's test indicates whether the larvae are grouped (a value approaching 1). The distance swum by the larvae is given in centimeters and is displayed as distance from the centre of the circle after one minute.

Most sponge larvae are thought to locomote by the beat of cilia in metachronal waves (waves coordinated by the viscous coupling of the cilia in the fluid medium). Considering sponges lack nerves or communicating (*e.g.* gap) junctions between cells that would allow the rapid coordination of cilia, sponge larvae must use non-nervous mechanisms to respond to stimuli. In *Reniera* sp., it was demonstrated that long cilia which form a band around the posterior pole of the larva respond to increases and decreases in light intensity by straightening and bending (LEYS & DEGNAN, 2001). As the larva rotates through the water, the cumulative effect of all the ciliary

responses allows the larva to swim away from light. Given that this is the only known mechanism by which sponge larvae can steer to or away from light, the very different patterns of cilia on the three larvae studied here suggest that the mechanism of response to photic stimuli must be very different in each larva.

Like other haplosclerid larvae, *Haliclona* larvae have a ring of longer cilia surrounding the posterior pole (see review by WAPSTRA & VAN SOEST, 1987), and a broad band or ring of pigment adjacent to or underlying the ring of longer cilia. Unfortunately, the long cilia forming the ring are too small to directly observe any movement in response to changes in light intensity, but presumably the pigmented band shades the base of the longer cilia in these larvae similar to those in *Reniera* sp., and the cilia respond to increases and decreases in light intensity by straightening and bending, thus causing the larva to swim away from light. The lack of a ring of longer cilia and of a pigmented region at the posterior pole in *Halichondria* larvae may be the reason these larvae appear to be indifferent to changes in light intensity, but it is unclear what function the narrower, sparsely ciliated posterior pole may serve in larval behaviour.

*Scypha* amphiblastulae are quite different from the parenchymella in general morphology and in pattern of locomotory cilia. The anterior hemisphere is so sparsely ciliated that it is difficult to say whether the cilia are able to beat in metachronal waves or how the larva propels itself. All cilia are of equal size, approximately 15 µm long. In most preserved larvae the cilia appear disordered, but in larvae that have just been released from the parent sponge the cilia are all directed toward the posterior pole, which suggests that they may beat in metachronal waves. Although there appears to be a pigmented spot in the centre of the anterior hemisphere, it is unclear how this pigment would shade any of the cilia to generate a shadow response (such as that demonstrated in the parenchymella of *Reniera* sp.). Each cilium arises from a small indentation in the cells' apical surface which may shade the basal portion of the cilium. A more intriguing feature is the presence of four pits, one on each quadrant of the larva's anterior hemisphere. These pits may correspond to the four 'cross cells' ('cellules a croix') that TUZET (1973) indicated might be involved in photoreception. If the pits are photoreceptors, then the mechanism of photodetection in amphiblastula larvae is significantly different than that in parenchymellae.

The demonstration that sponge larvae differ greatly in their responsiveness to changes in light intensity is not new. But the interpretation of these results is new. Previous authors have interpreted a lack of responsiveness to changes in light intensity as a lack of sensitivity (MALDONADO & YOUNG, 1999). We suggest, however, that all the larvae studied here are very sensitive to different light intensities. Larvae that do not show a strong response to gradients in light intensity remain where they are, rather than swimming up and out into currents (*e.g.* *Halichondria*), and larvae that show an agile response to gradients in light intensity swim up and out into currents (*e.g.* *Scypha*). Continued work is focussed on examining how *Scypha* larvae respond to light and water cues in their microenvironment in order to determine how these animals coordinate their swimming behaviour so adeptly in the absence of a nervous system.

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