

Come on baby light my larva: exploring sponge larval fluorescence



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Background

Fluorescence techniques can provide a quick and effective means of viewing specimens of interest in real-time. Fluorescence is the emission of light by a substance that has absorbed/ been excited by incidental light of certain wavelengths. We used fluorescence microscopy both in the field and in the laboratory to explore its applications for answering questions pertaining to larval ecology of sponges. This includes using a fluorescence stain for detecting larval lipid reserves, observing symbiotic cyanobacterial transmissions over embryogenesis and detecting potential differences in light sensitivity in larval photoreceptive pigmented rings from multiple sponge species.

Larval energetics

A fluorescent lipophilic stain, Nile Red, was used to highlight lipids in larvae of *Rhopaloeides odorabile* from inshore and offshore reefs to investigate whether maternal investments to larval energetics are depressed under sub-optimal conditions at inshore habitats. Nile Red staining (**Figure 1a**) together with confocal microscopy confirmed the identity and location of lipid droplets in larval sponge when compared to staining by Herxheimer lipid stain in light microscopy (**Figure 1b**). We found up to 60% reduction in the level of lipids in inshore larvae compared to offshore larvae.

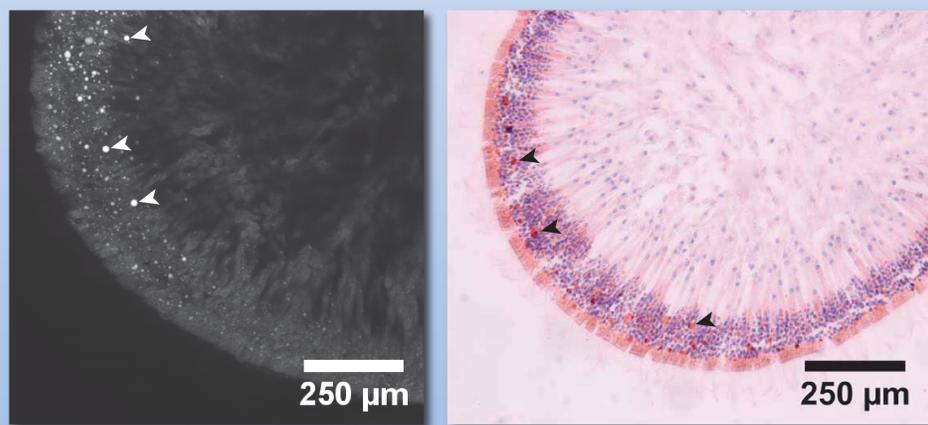


Figure 1: *Rhopaloeides odorabile*. Cryosection of larva stained with a) Nile Red (left) and b) Herxheimer stain (right) for lipids. White and black arrowheads point to lipid droplets in section.

Sponge-cyanobacteria associations

Carteriospongia foliascens is a phototrophic sponge possessing symbiotic cyanobacteria (Wilkinson 1983; Webster et al. 2012). *C. foliascens* larvae possess a dark interior at release, suspected to be aggregations of cyanobacteria vertically transmitted from adult sponges (see **Figure 3**). We used confocal microscopy to detect the location of cyanobacterial cells over embryogenesis in histological sections (**Figure 2**). Oocytes did not possess any internal cyanobacteria with aggregations of cyanobacterial cells detected in surrounding adult mesohyll. Cyanobacteria are subsequently detected in embryos and larvae suggesting that vertical transmission may have occurred at later stages of larval development in this species.

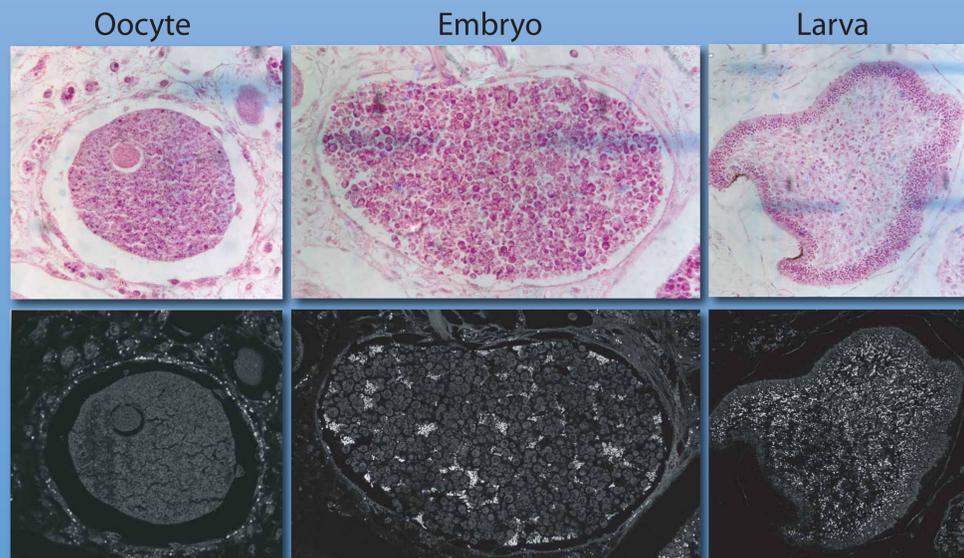


Figure 2: *Carteriospongia foliascens*. Light micro-photographs (top) and fluorescent micro-photographs (bottom) of the same histological sections. White dots represent cyanobacterial cells. Auto-fluorescence revealed the location of cyanobacterial cells over embryogenesis undetected by light microscopy.

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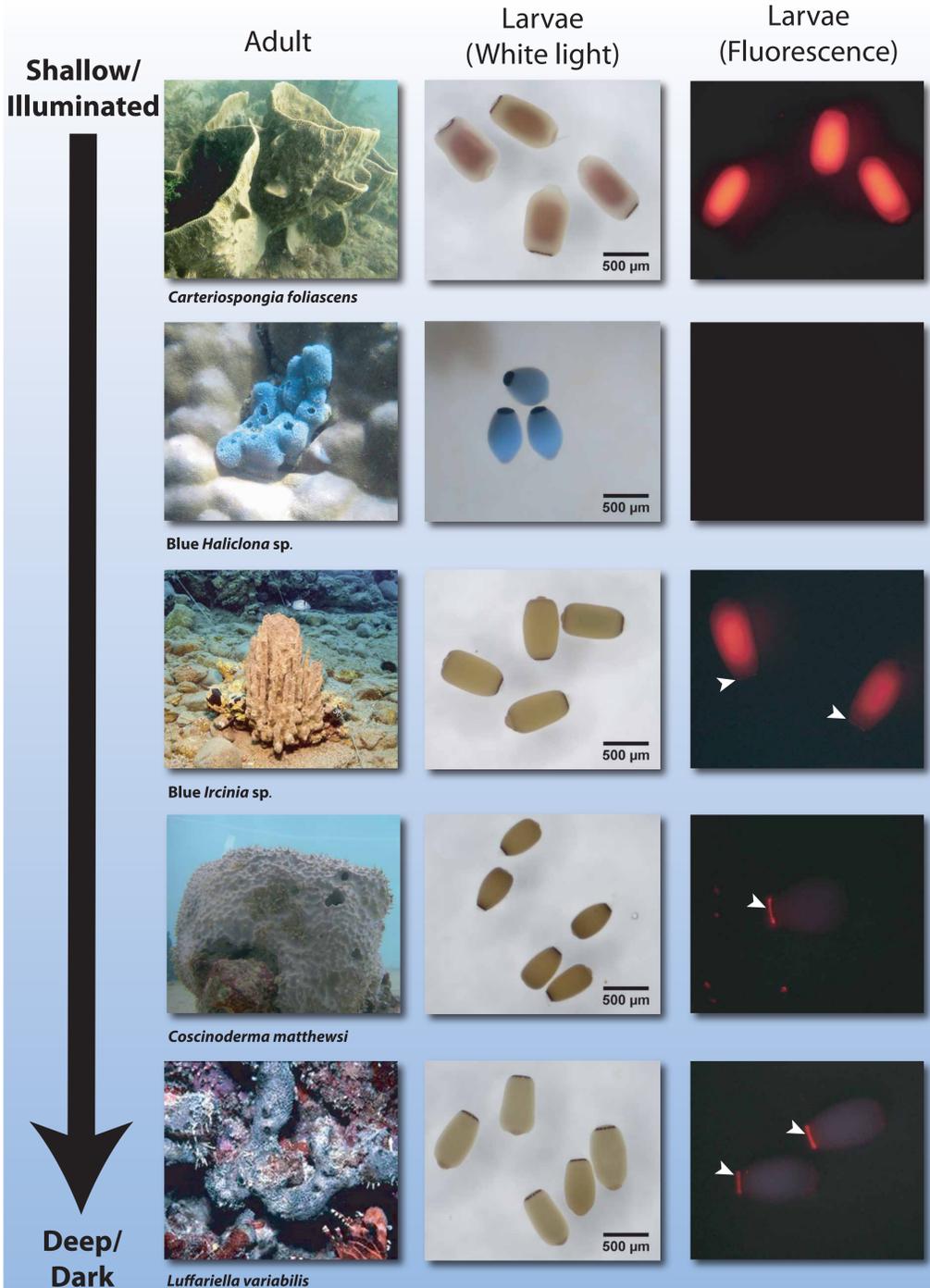


Figure 3: Adult sponge, larval light micro-photographs and larval fluorescent micro-photographs of five co-occurring Great Barrier Reef sponge species. White arrowheads point to auto-fluorescence in pigmented posterior rings.

Variations in larval posterior ring auto-fluorescence

Depth and habitat distribution of sponges can be apparent at some locations, with species occupying specific environmental niches (i.e. shallow/deep and illuminated/dark). With directional swimming to light (phototaxis) in tufted parenchymellae influenced by the pigmented posterior ring (PPR) (Leys et al. 2002; Rivera et al. 2012), can species depth (shallow vs deep) and habitat (illuminated vs dark) distribution be explained by larval innate light sensitivity?

We used epi-fluorescence microscopy in the field to view auto-fluorescence in PPR of newly released larvae from five co-occurring Great Barrier Reef sponges (**Figure 3**). We found that heterotrophic species occupying deeper and darker habitats (i.e. in crevices), have higher intensity of auto-fluorescence in their PPR (see *C. matthewsi* and *L. variabilis*). Species occupying shallow and illuminated habitats had no/ minimal auto-fluorescence in their PPR (see *C. foliascens* and *Haliclona* sp.). *Ircinia* sp. which is suspected to be mixotrophic appeared to have intermediate levels of PPR auto-fluorescence. We hypothesize that if these variations in PPR auto-fluorescence correspond to light sensitivity in larvae, then species depth and habitat distribution may, in part, be determined by innate larval light detection capability.

References

Leys et al. (2002) J. Comp. Physiol. A 188: 199-202
 Rivera et al. (2012) J. Exp. Biol. 215: 1278-1286
 Webster et al. (2012) Front. Microbiol. 3: 444
 Wilkinson (1983) Science 219 (4583): 410-412