Investigating Sensory Integration and Settlement Response to Sensory Stimuli in the Hydrozoan *Ectopleura crocea*

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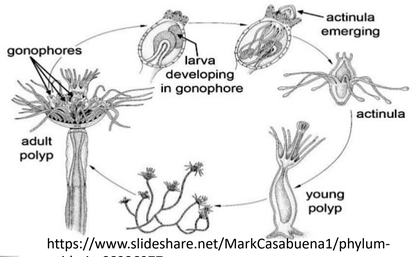
Introduction

The hydroid *Ectopleura crocea* has an indirect lifecycle that produces a dispersive larval stage called actinula. Motile larvae are a common feature of benthic marine invertebrates.

Actinulae larvae select the substrate upon which they settle by integrating sensory cues from the environment.



https://www.slideshare.net/MarkCasabuena1/phylum-cnidaria-66086977

Ectopleura have become a nuisance species that biofouls structures such as aquaculture nets but little is known about larval settlement biology

Here we examine the molecular genetics underlying the behavioral response to environmental cues during settlement of the actinula larva.

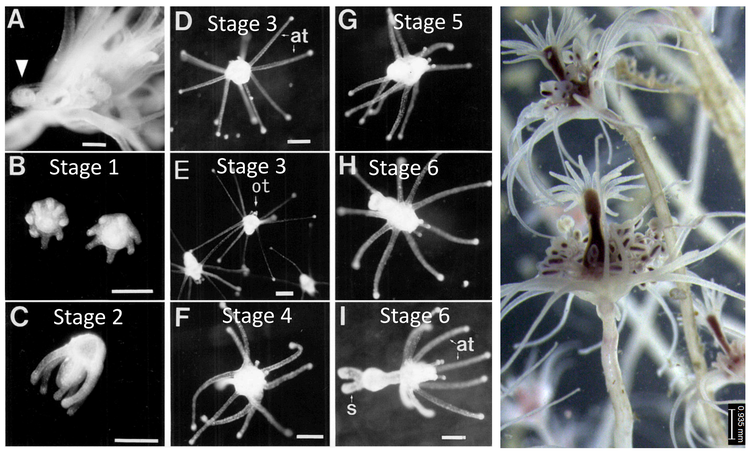


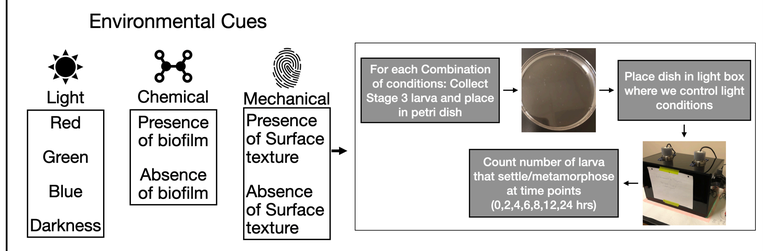
Figure 1. Left: Stages of actinula larva through settlement (Yamashita et al., 2003) **Right:** Adult polyps of *E. crocea*

Major Questions

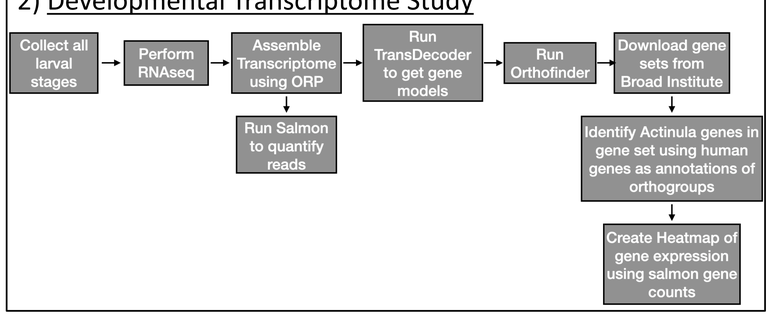
- **Q1:** What are the sensory modalities being utilized in the settlement decision? (Environmental cues)
- **Q2:** How do larvae integrate sensory information during settlement at the genetic level and when does this develop?

2 Major Approaches

1) Factorial Larval Settlement Study



2) Developmental Transcriptome Study



Preliminary Results: Larval Settlement Study

Light Enhances Larval Settlement compared to darkness

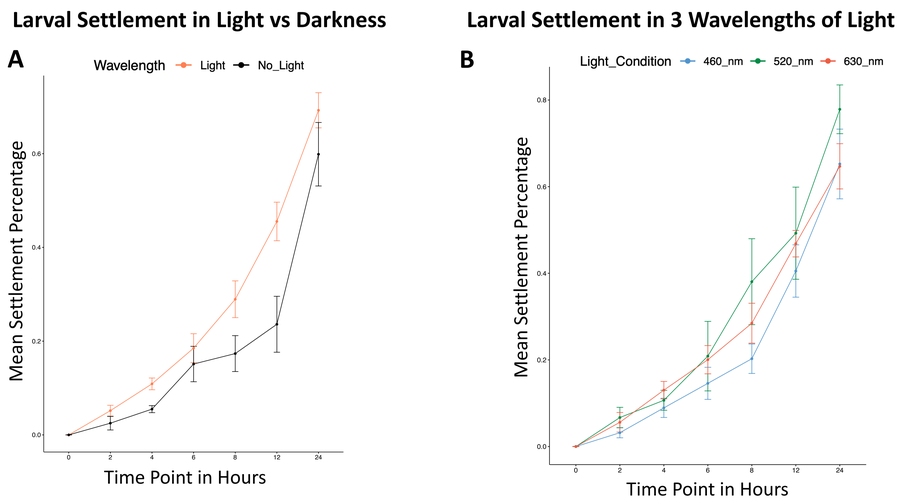


Figure 2. Results of the light experiments for the larval settlement study. **A:** Larval Settlement in light conditions compared to darkness. We found that the presence of light significantly enhances larval settlement compared to darkness (p = 0.012). **B:** Larval Settlement in the 3 wavelengths of light we tested. We found no significant effect of wavelength on settlement (p = 0.357).

Chemical Cues from Biofilms Enhance Larval Settlement

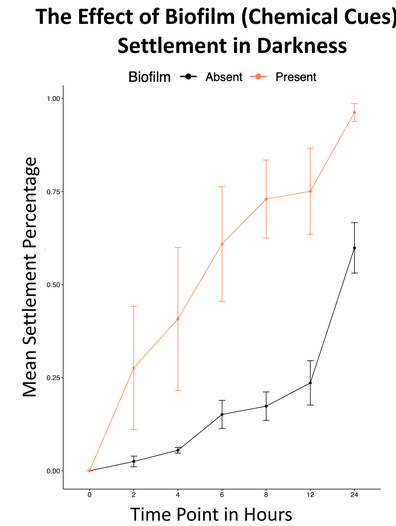


Figure 3. Results of the chemosensory control experiment for the larval settlement study. We found that the presence of chemical cues from biofilms significantly enhance larval settlement (p = 0.0157). This finding is consistent with the literature and is what we expected to find.

Preliminary Results: Developmental Transcriptome Study

Upregulation of TF and Photosensory Pathway Genes in Stages 3 and 4

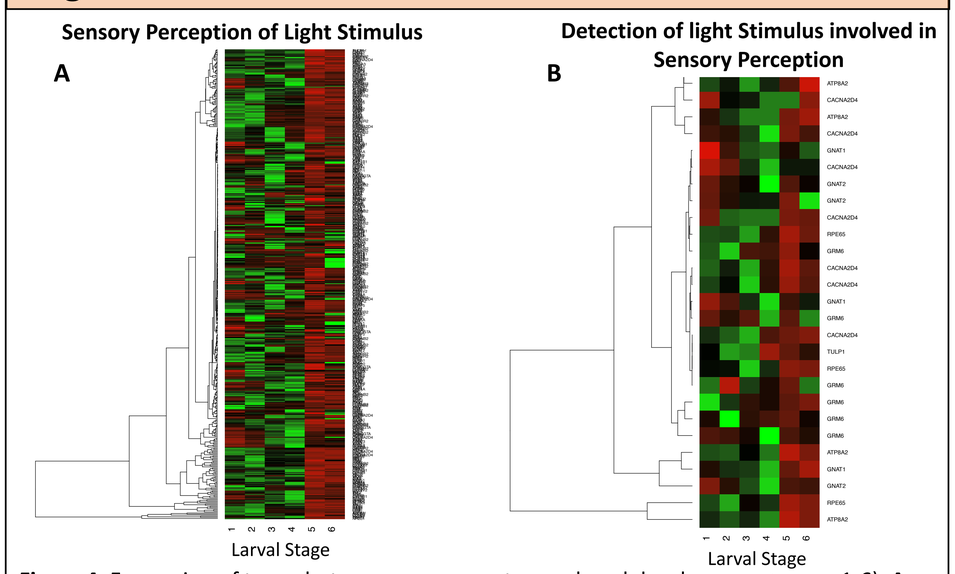


Figure 4. Expression of two photosensory gene sets over larval development (stages 1-6). **A:** There is an upregulation in genes involved sensory perception of light stimulus up until larval settlement (stage 5) where many sensory genes are then downregulated. Stage 6 shows the expression of juvenile polyp sensory genes (after metamorphosis). **B:** We see the same pattern as in part a with this sensory gene set.

Summary

- We report that the presence of light enhances larval settlement, however the wavelength does not appear to be an influencing factor.
- The presence of chemical cues through biofilms greatly enhances settlement in our control experiment.
- Sensory genes are upregulated during stages 3 and 4 which are the hypothesized competent stages. These genes are then downregulated during metamorphosis (stage 5) and stage 6, the metamorphosed polyps, exhibit less sensory genes than the larva.

Future Work

- Confocal imaging of actinulae
- Finish Factorial behavioral experiments testing the effects of:
  - Different wavelengths of light and intensities on settlement
  - The influence of biofilms on settlement
  - The influence of mechanical stimuli on settlement
- Finish analyzing larval and adult Transcriptome
- Perform microbial analysis on biofilm

Citations

Yamashita, K., Kawaii, S., Nakai, M., & Fusetani, N. (2003). Larval behavioral, morphological changes, and nematocyte dynamics during settlement of actinulae of Tubularia mesembryanthemum, Allman 1871 (Hydrozoa: Tubulariidae). *The Biological Bulletin*, *204*(3), 256-269.

Zardus, J. D., Nedved, B. T., Huang, Y., Tran, C., & Hadfield, M. G. (2008). Microbial Biofilms Facilitate Adhesion in Biofouling Invertebrates. *Biol. Bull.*, *214*(February), 91–98. https://doi.org/10.2307/25066663

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Upregulation of Taste Transduction Pathway Genes in Stages 3 and 4

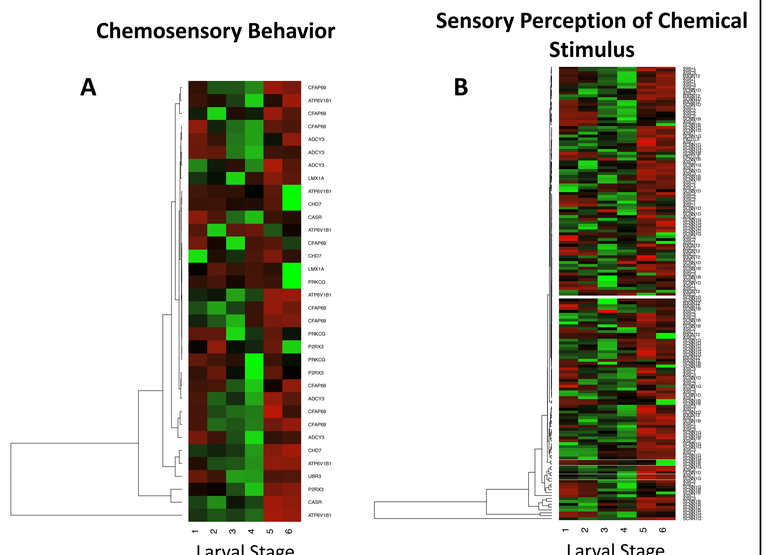

Figure 5. Expression of two chemosensory gene sets over larval development (stages 1-6). **A:** There is an upregulation in sensory genes involved in chemosensory behavior in stage 3 and 4 and then a downregulation in genes during metamorphosis, stage 5, with few sensory genes being expressed in the adult, stage 6. **B:** The same pattern emerges in this gene set as in A.