

Sources of variation in the response of embryonic *Membranipora sp.* to prolonged UVA radiation exposure

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Abstract

Phenotypic plasticity in larval organisms plays a large role in individual survival and population-wide recruitment. Intrapopulation variation that occur at the adult stage in response to different habitats and adaptive mechanisms are often passed on to progeny. In this study, we looked at the response of embryos of *Membranipora sp.* to prolonged UVA radiation exposure. We compared embryos of eggs from colonies collected in different light environments as a means of assessing embryonic variability in response to environmental stress. Colonies of *Membranipora sp.* were collected from two depths (0-0.5m and 1.8-2m) off of the dock at Friday Harbor Laboratories on San Juan Islands, Washington. Stimulation of zygote release was achieved using Ethylenediaminetetraacetic acid (EDTA) at 0.1 μ M concentrations, and embryos were irradiated with UVA light from a lamp continuously for 61 hours. We measured rate of development by counting hatched blastulae at semi-regular intervals over the course of this time period. Our results show that embryos from colonies at greater depth in the ocean are more susceptible to developmental delays caused by UV radiation than those from shallower depths. We believe this variation in response reflects the impact that local conditions have on parental colonies of *Membranipora*. These results suggest that there may be an acclimation response in parent colonies that can be conferred to their offspring.

Introduction

Early life-history (ELH) stages in marine invertebrates are vulnerable periods when individuals face many potential dangers, including risks of predation and various environmental stressors (Allen 2008, Lenz et al. 2019, Byrne 2011, Przeslawski et al. 2015). Many studies have shown that phenotypic plasticity has a significant effect on larval fitness and recruitment success across multiple species (Bergenius et al. 2002, Green & Chambers 2011, Pan et al. 2016). Members of the order Cheilostomata, a clade of bryozoans, are well-known for exhibiting a wide range of phenotypic variation, and zooid-level plasticity has been observed within colonies across several different studies (Cheetham et al. 1995, Harvell 1998). However, the presence of phenotypic variation in the pre-colonial life stages of cheilostome bryozoans has yet to be subject to intensive study. One way to address the question of phenotypic plasticity in pre-colonial cheilostomes is to investigate the variability of responses exhibited by embryos to environmental stressors. Among the most straightforward of environmental stressors to measure is developmental response to UV radiation (UVR). Indeed, the differential response of invertebrate organisms within a given population to high-level UV-exposure has been studied in the past as a feature of phenotypic plasticity (Aranda et al. 2011, Gleason & Wellington 1995).

The effects of ultraviolet radiation on larval survival can be influenced by an adult's environment (Gleason & Wellington 1995, Loayza-Muro et al. 2013). Several aquatic species show (apparently) adaptive responses to intense ultraviolet radiation in the form of photoprotective pigments (Loayza-Muro et al. 2013) and mycosporine-like amino acids (Gleason & Wellington 1995) which absorb UVB radiation. There appears to be a range of phenotypic plasticity in these adaptive responses depending on the environment in which the larvae develop (Cahenzli & Erhardt

2013). We chose UVR as a stressor because of extensive previous research and its well-noted effects on embryonic and larval development.

The larval form of the bryozoan genus *Membranipora*, commonly referred to as a cyphonautes, is a planktotrophic larvae with a characteristic bivalved triangular shell (Stricker et al. 1988). *Membranipora* larvae have been subject to various investigative studies concerning motility and feeding due to their unusual morphology and feeding behaviors (Atkins 1955, McEdward & Strathmann 1987, Nielsen & Worsaae 2010). Despite this fact, there has been little research conducted on their early development through various environmental stressors, like ultra-violet radiation (UVR) exposure. We investigated how UVA exposure affects the survivorship of embryos from the zygote until the earliest larval stage from colonies collected from two different depths in the water column - from 0 to 0.5 meters and from 1.8 to 2.0 meters of depth. We hypothesize that embryos from colonies collected at greater depth will have more developmental delays upon continuous irradiation compared to embryos from colonies closer to the ocean's surface. In order to address this question, we studied the variation in developmental timing to UVA radiation of *Membranipora* bryozoan embryos from different members of a given population. The potential differences in response between embryos from colonies growing in different microhabitats could explain differential survival of offspring in the early stage of the bryozoan life cycle.

Methods

Shallow colonies of *Membranipora* were collected by cutting the fronds of the macroalga *Iridaea cordata* at depths of 0-0.5m depth off the side of the floating dock at Friday Harbor Labs,

University of Washington, San Juan Islands, Washington. Deep colonies on kelp (1.8-2.0 m depth) were collected from the same location. Colonies were soaked for one hour in 0.1mM Ethylenediaminetetraacetic acid (EDTA) in 15 μ m filtered seawater at sea surface temperature (Temkin 2014). This procedure activates and releases eggs held in the coelom or intertentacular organ (ITO) of constituent zooids (Temkin 2014). Eggs ($n=50\pm 10$ per container) were counted out and placed into separate containers according to treatment groups (2 replicates per treatment): 1) Deep UV-exposed, 2) Deep Non-UV-exposed, 3) Shallow UV-exposed, 4) Shallow Non-UV-exposed (Figure 1). Only eggs that clearly had not begun the cleavage process were collected and put into our treatments to standardize the starting age of each embryo. Rearing containers were filled with 15 μ m filtered sea water and set in a sea table to maintain ambient sea surface temperature with a UVA lamp (340nm) illuminating all dishes (Figure 1). Cylindrical, open-topped opaque plastic containers covered with Plexiglass lids (14 cm x 14 cm) blocked the UVR from reaching embryos in our non-UV treated group (Figure 1).

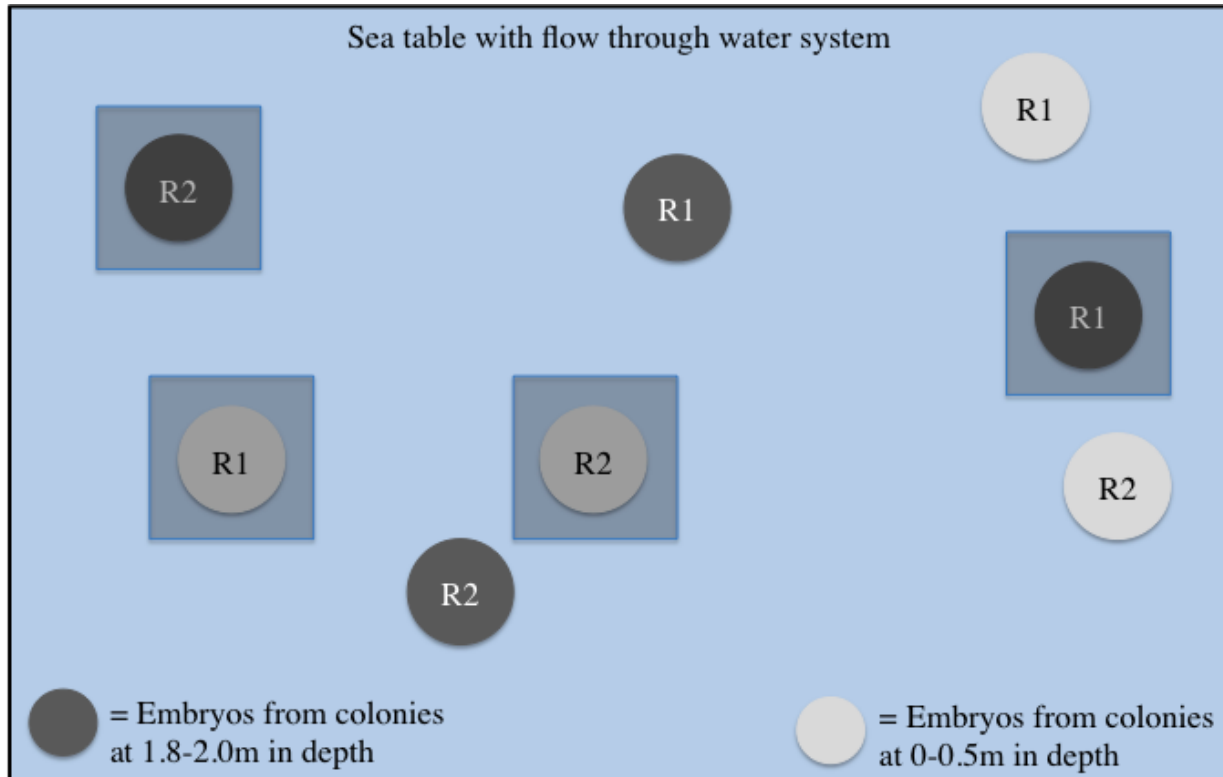


Figure 1. Diagram of experimental set-up. Embryos from colonies collected at two depths were placed randomly throughout table with two replicates per treatment (either UVA-treated or non-UVA-treated). PlexiGlass lids (depicted as transparent squares) and opaque outer containers were used to filter out UVA radiation from our non-UVA treatments. $n=50\pm 10$ per replicate.

Our UVA source was a 340nm UVA light, 1.2m x 0.25m in size. The UVA lamp was manufactured by Q-Panel company, or Q-LAB. The emission spectrum ranges from wavelengths of 300 nm to 400 nm, with a mean wavelength of 340 nm. The UVA lamp was mounted on a polyvinyl chloride (PVC) pipe stand approximately 0.5m above the sea table. To assess the effectiveness of plexiglass as a shield, we measured its absorption at different wavelengths of light across the UV spectrum with a spectrophotometer (Figure 2). These data show that over 90% of the incoming UVR from our lamp was attenuated by the plexiglass for the non-UV-treated embryos. Plexiglass lids were placed over the open ends of the opaque cylinder containers housing the non-UV-treated embryos in order to ensure minimal UVR exposure.

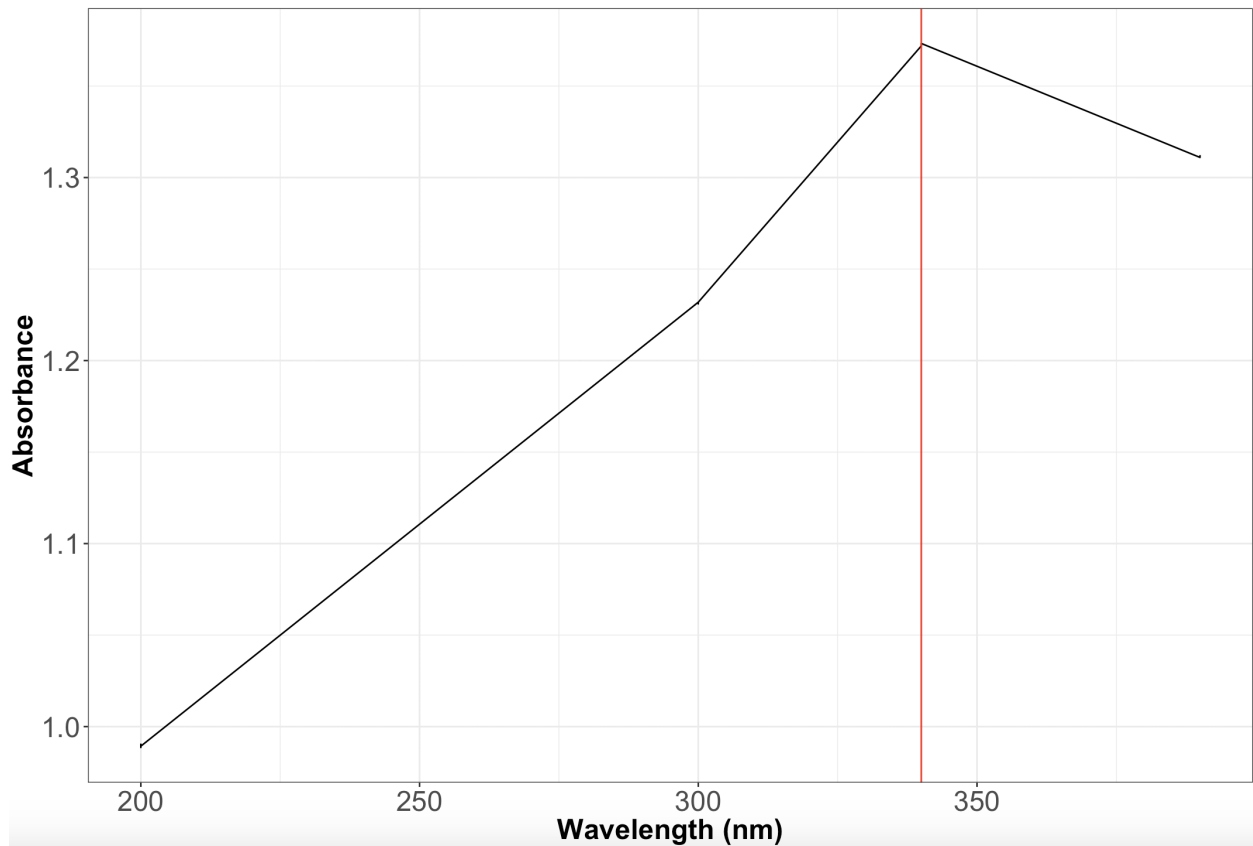


Figure 2. Absorption of UV light with varied wavelength. The red line (wavelength = 340 nm) is the UV emission average of the lamp we used in the experiment.

To assess the impacts that UVR has on developing *Membranipora* embryos, we looked at rate of development (parametrized by time from zygote activation to hatching). After 20 hours of uninterrupted UVR exposure, embryos were assessed every two hours until the majority of embryos hatched in each of the treatments. We counted hatched blastulae at 2 hour intervals until embryo hatching stopped. We used standard error as a means of assessing the statistical significance of our results. Computations were completed in RStudio (RStudio version 1.1.463).

Results

The embryos from colonies living at 1.8-2m below the ocean surface appeared to suffer more developmental delays when exposed to continuous UVA radiation. The rate of development for UV-exposed deep colony embryos was lower than those for embryos from shallow colonies exposed to UVA (Figure 3). Shallow UV Replicate 1 (R1) was compromised midway through the study, so the data from that replicate have been omitted beyond the point at which the sample was disturbed.

The three samples of shallow-water embryos (2 without UV and 1 with UV) that we followed through the entire embryo stage are not significantly different from one another, which suggests that UVR exposure has a minimal effect on their embryonic development. However, deep-water embryos were statistically distinct between UV treatments in how quickly they developed.

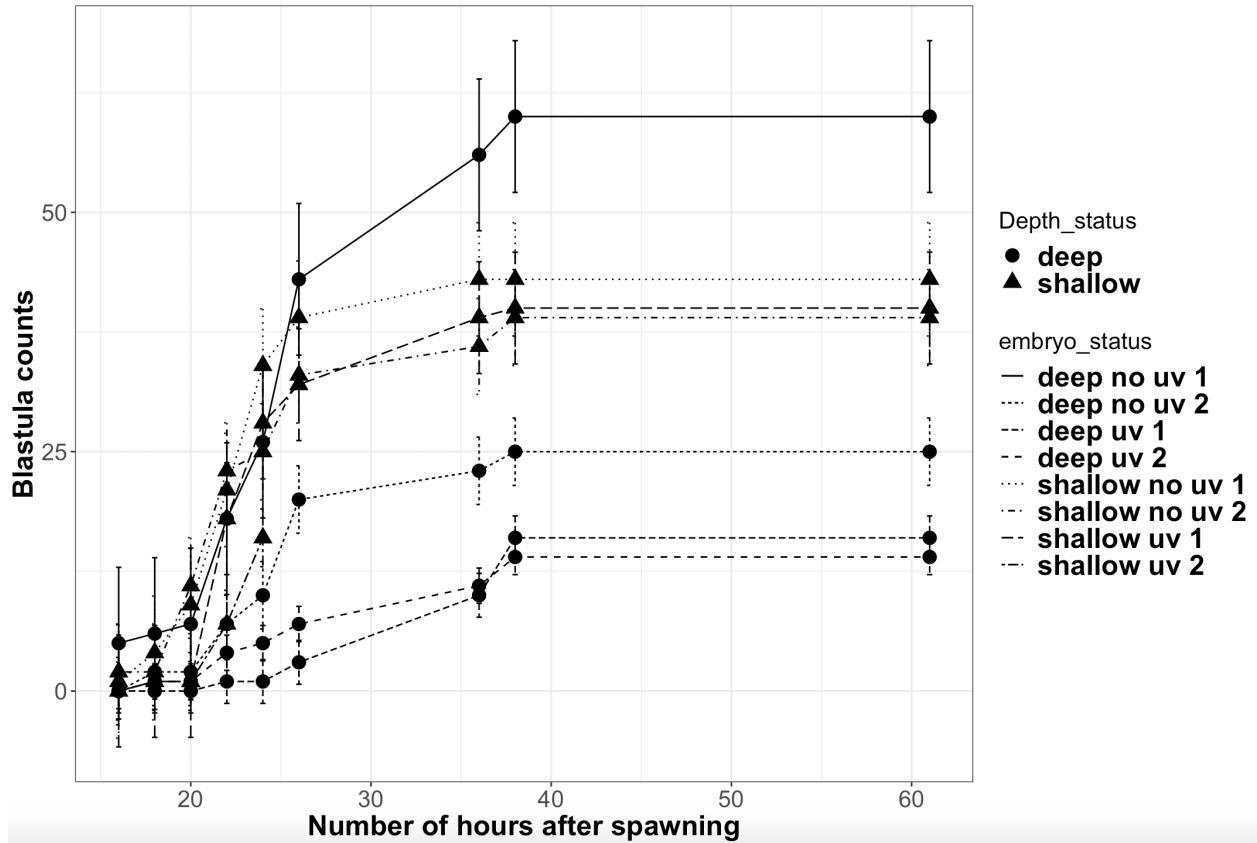


Figure 3. cumulative hatched embryos (blastula) across time intervals. Vertical bars denote standard error.

We used standard error as a means of assessing significance, with non-overlap indicating statistically significant results (Greenland et al 2016) (Table 3). We chose this method of statistical analysis because we lost a replicate of our shallow water UV-exposed embryos, and there was not enough replication to run reliable mean-comparison statistics.

Colony ID	Standard error	Final blastula count
Shallow UV R1	5.84	40

Shallow no UV R1	5.92	43
Shallow no UV R2	5.01	39
Deep UV R1	2.29	16
Deep UV R2	1.84	14
Deep no-UV R1	7.92	60
Deep no UV R2	3.52	25

Table 1. Final blastula counts and standard error for all treatments and replicates.

Discussion

Shallow-water colonies face less developmental delay (lag in hatch time) in the face of long-term UVA exposure than their counterparts from deeper-water colonies. This result was anticipated by our hypothesis. Differential acclimation to habitat between populations of *Membranipora* bryozoans could explain this finding. Shallow-water colonies face exposure to higher levels of UVA than those from deeper waters, due to the attenuation of UV light intensity with depth in the water column. Shallow adults colonies may consequently endow their eggs with the ability to endure greater UV stress as a result of habitat acclimation. There is evidence that habitat acclimation influences parental effects; acclimated parents can pass on acquired stress resistance to offspring in some animals (Cahenzli and Erhardt 2013, Gleason & Wellington 1995).

Our findings may also be explained by various other factors that are not related to plastic traits. Previous studies have suggested that cyphonautes larvae often preferentially settle on the younger parts of kelp blades (Denley et al. 2014). Considering the geometry of the kelp blades we used for our experiment (the younger parts of the kelp are at a shallower depth than the older parts), there is a chance that colonies present further down on the kelp are just colonies that are older than those from further up on the kelp blade (i.e., the part of the kelp blade closer to the meristem). Perhaps our result is merely a function of colony age instead of adaptability to UV light exposure. Older colonies may produce less robust offspring, and in the face of an environmental stressor like UVR, the offspring of older colonies may fare worse than those from younger colonies based on age. Further investigation is necessary to decouple age and depth in the water column.

Due to the loss of one of our replicate samples, we have limited data to assess the significance of our results. However, even if we used our second replicate, we only had two replicates per treatment, which makes statistical analysis difficult. A repeat trial of our experiment using more replicates would further substantiate our results. Additionally, it would be helpful to pick parent colonies from a wider range of depths - from surface to at least 10 meters below the surface. The strong attenuation of UV light in the first few meters of water (Fleischmann 1989) makes our spatial scale (from 0-2m below the surface) meaningful, but a larger range of water depths would provide more exhaustive coverage of habitats where this bryozoan occurs.

Additionally, due to the time constraints of collecting *Membranipora* zygotes before they had begun the cleaving process - which, by our observations, can start as early as 1 hour from release from the intertentacular organ - there was a large error in counting and collection accuracy, with one treatment (deep, non-UV-exposed replicate 1) where we had 10 more blastulae than we started with. The colonies collected from the deeper habitat were covered in detrital matter, algae,

and larval crustaceans making sorting through EDTA baths for embryos extremely challenging. A filtration process post-release was considered but was not conducted due to fear of damaging the delicate embryos. Given the possible limit to accuracy of our initial counts of embryo, survival to hatch metrics were determined not to be robust and were not reported in our results.

Overall, our study suggests a differential response of *Membranipora* embryos to UVA exposure. There may be parental effects in the form of habitat acclimation occurring within populations of *Membranipora* bryozoans that contribute to the observed variability in response of embryos to environmental stressors. These results coincide with findings from similar studies investigating the response to UVR in larval from different rearing habitats (Gleason and Wellington 1995, Loayza-Muro et. al. 2013). Larvae from source populations that settle in shallower water may be more resistant to the deleterious effects of UV exposure, and confer to their offspring a similar trait, and vice-versa for colonies that settle in deeper-water locations.

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