Developmental differences in Monterey Sea Lemon (*Doris montereyensis*) veliger larvae with temperature variation

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Abstract

As climate changes, many organisms are affected, and affect their biomes as a result. Nudibranchs, a ubiquitous group of marine organisms, are known for their beautiful colors, but not much for their developing forms. Recently metamorphized nudibranchs have fragile shells and survive in the benthos inhabiting a completely different niche than their grown forms. *Doris montereyensis* is a widespread dorid nudibranch species native in the Eastern Pacific from British Columbia to Baja California. Its pelagic form hatches in early summer and develops as the water warms. This study looked at how temperature variation affected the development of larval stages (shell length and cross-sectional area). Across three developmental temperatures (9, 15.5 and 20°C), shell length was not seen to increase singificantly, wherease average area was larger in colder conditions. The warmest water treatments saw death in all replicates. This may be due to experimental error or be a sign of a deeper scientific phenomenon. If temperature causes larvae to die, as sea temperature rises, this could be fatal to nudibranch populations, If, as other literature states, growth increases with slight increases in temperature, this may increase the natural range of many species, disrupting ecosystem function (Rose 1986). Regardless, further study is needed to quantify the effects of a warming ocean on nudibranch populations.

Introduction

Dorid nudibranchs are a variable group of marine invertebrates that populate every latitude of the Earth's oceans. As a group, they are spatially and temporally variable, and often ignored in large scale ecological surveys due to this variability (Domenench et al. 2002). Because of this, not much is known about why they are temporally variable. Their life cycle transitions from benthic egg masses to planktonic veliger larvae and ends with either a benthic or pelagic dorid adult (Fig. 2). This allows them –as a group– to occupy multiple niches and better utilize the ecosystems they live in throughout their lifetime. As larvae, veligers tend to be filter feeders, consuming phytoplankton within the water column during the season that they appear (Strathmann 1987). They then settle and consume sponges or bryzoans, with many species specializing in eating one kind of organism.

In the case of the Monterey Sea Lemon (*Doris montereyensis*), they consume only *Halichondria* sponges (Strathmann 1987). Their egg masses are laid in mid to late spring and hatch as veligers in late spring or early summer (Strathmann 1987). In summer waters, they then live as pelagic larvae, consuming primarily unicellular brown algae and dinoflagellates. *D. monetereyensis* has a very broad range in the Pacific, from Southern British Columbia to Baja, California and can tolerate a wide variety of temperatures (Strathmann 1987). However, because of a lack of extensive studies, it is unknown how these organisms will respond to rising sea temperatures, particularly as veligers, which are more variable with temperature (Scheltema 1967).

To this effect, the primary interest of this paper is to look at how veliger larval growth changes with temperature, to try and quantify the seasonal variation of *D. montereyensis*

populations, as well as look at how climate change may affect this species as sea temperature rises. Some studies have shown increases in developmental rate of larvae with temperature (Scheltema 1967; Strathmann 1987), which could dramatically affect ecosystem dynamics, changing timing of population increases and the livable range of organisms. Because of this, it was hypothesized that the larvae would develop more quickly as temperature increased., which could have population and ecosystem wide affects.

Methods

Larval Culturing

The egg mass used in this experiment was collected from a multi-species running seawater table at the Friday Harbor Laboratories on San Juan Island, Washington. The individual that laid the eggs was collected from Argyle Creek [48°31'18.2"N, 123°00'50.9"W] in the last week of March 2019, the mass was laid around April 22rd, 2019, and was collected with a razor blade for this experiment on April 26th. It was then put in a container with 0.5 mm mesh sides for three weeks within a running sea table with an average temperature of 10°C . Development was checked under a compound scope twice weekly by sampling an egg mass fragment. After 3 weeks, veligers were visible within the egg mass and the egg mass was moved to a two-liter air lifted droplet stirrer (Fig. 1) with a 28 micrometer (μm) mesh bottom within the same sea table. The egg mass was left intact for another three weeks with bi-weekly examination for developmental stage. Veligers reached the planktonic stage on May 26rd (three weeks after first veliger development) at which point the egg mass was divided into three replicates for each of three treatment (i.e., nine total fragments). The three treatments were a low temperature (9.13°C ± 0.47°C, a mid-range temperature (15.58°C ± 1.48°C), and a high range temperature (20.15°C ± 2.65°C), determined using Strathmann 1987 and in coordination with two other projects using

Pisaster ochraceus larvae and *Nereocystis luetkeana* spores respectively (all three of our projects occurred simultaneously using shared water baths). The low temperature was meant to represent average local sea temperatures in the Spring (US Department of Commerce, National Oceanic and Atmospheric Administration, National Weather Service 2012), the mid-range temperature was meant to mimic the upper range of sea temperatures (US Department of Commerce, National Oceanic and Atmospheric Administration, National Weather Service 2012), and the high temperature was meant to represent future sea temperatures with climate change (Pierce et al. 2009). The temperatures were maintained using heated water baths within a 10.5°C constant temperature room.

Using a compound scope, it was determined the egg mass had a concentration of approximately 200 veligers per centimeters squared (cm^2). As in accordance with Strathmann 1987, which says that *Doris montereyensis* larvae should be kept at concentrations around 100 to 400 individuals per milliliter (mL), one squared centimeter of egg mass was added to each replicate. The fragments were put in 100 mL finger bowls and were kept at between 18°C and 22°C with no air dropper while replicate photos were taken with an QImaging MicroPubliher 5.0 RTV camera attached to an Nikon eclipse e600 compound scope. Fragments were then placed in 100 mL filter containers with 15 μm mesh bottoms in 400 mL air lifted droplet stirrers (Fig. 1) within their respective water baths. Once in their respective water baths, each replicate was fed *Isochrysis galbana*. Concentration of *I. galbana* was determined with cell count using a hemocytometer (Hodin et al. 2019). Water baths were checked for proper water level and filter function twice daily and the larvae were fed again two days after being placed in the water baths. On the third day, larvae were removed from water baths and again held at 18°C to 22°C for two

hours for final data analysis (?) before being released off the Friday Harbor Laboratories floating dock.

Data Collection

Three photos were taken of one individual within each replicate using the aforementioned photomicroscopy setup under 200x magnification. Larvae were selected using a glass pipette to take a water sample halfway down into the culture water. If no larvae were present in the first batch, samples were taken with the glass pipette until live larvae were found. No photos were taken of dead larvae unless no live larvae were present. In the case of a majority dead replicate, the containers were examined under a dissecting scope to determine if any larvae survived.

Data Analysis

One of the three photos per individual was selected based on the visibility of the veliger for analysis. Photos were analyzed using imageJ 1.52a. To get area, photos were cropped, auto-thresholded, edited so larval shape was the only black in the image, filled in, then analyzed for area. Pixel area was then converted to squared millimeters (mm^2). Length was measured using a set scale (scale micrometer) to measure from the outer lip to the highest point of the shoulder of the shell. Both measurements were taken to eliminate the bias from differing photo angles and the presence of vela. Images were blindly analyzed to eliminate bias. Veliger areas and lengths were then analyzed using two-way ANOVA tests with replication in Excel 2016 and Post HOC Tukey tests for significance were conducted in R (Version 3.5.1). All statistical tests were done with an alpha value of 0.05.

Results

The date on which photos were taken (the first or fourth day) had a slight significant effect on the area of the veligers ($p = 0.047$) at 95% confidence. The temperature treatments the veligers were exposed to also had a significant effect on the area of the veligers ($p = 0.035$) at 95% confidence. However, the interaction between the temperature treatment and date of photo did not have a significant effect on area ($p = 0.14$). The post-HOC Tukey test showed that the average for area for day 0 was slightly but not significantly larger than day 4 (Fig. 3). However, it did show that the low temperature treatment average area was significantly larger than that of the mid and high temperature treatments, and that the mid-temperature treatment average area was significantly larger than that of the high temperature treatment (Fig.3). No significance was found in length between treatments or measurement days ($p\ = 0.143$ and 0.322, respectively) (Fig. 4). The low temperature treatment had more visible vela and swum more actively after the three-day period, while the higher temperature treatments became more retracted and less active. The high salinity treatment only had two living individuals within all three replicates and 600 individuals by the fourth day.

Discussion

Based on pervious papers, it was assumed the growth of veligers of *D. montereyensis* were maximized between 17° and 20°C (Scheltema 1967; Strathmann 1987). However, it was found in this study that the veligers in the lowest temperature treatment had the largest area. It was also found that the recently hatched larvae had a larger area than those four days after hatching. This is in direct contrast to previous papers, some of which even suggest that settlement can occur as soon as two days after hatching (McGowan and Pratt 1954). Vela were only out in some veligers at the timing of the photos, so this is thought to have significantly affected the data, as vela are proportionally large compared to total body size and can be

completely retracted into the veliger shell, they not present in other photos. This study also faced time and space constraints which greatly limited the scope of the experiment. Salinity and pH were not controlled for, which have been shown to affect veliger growth (Chia and Koss 1978; Rose 1986). There were also fluctuations in the water baths used, which may have brought the water temperature of the replicates above or below the controlled for temperatures. Further studies are needed to investigate why there was a larger portion of retracted vela in colder temperatures and earlier after hatching.

There may have been no change in growth in any treatment due to the time limitations of the experiment or the lack of other environmental cues in vitro. Other papers looking at how temperature effects dorid nudibranchs development have found that, in the case on *Rostanga* species, slightly warmer than average temperatures combined with slightly higher or lower than normal salinities can increase the rate of development (Chia and Koss 1978; Rose 1986). However, these same studies showed that if temperature increased too much or for a long enough duration, these conditions are fatal. While these studies used a different species and different temperatures, due to their similar physiology these organisms can be compared. A three day 22°C treatment was fatal to the *D. montereyensis* veligers, which may have been due to the duration, the intensity, or the fluctuations in the exposure. However, from this it can be learned that temperature does greatly affect veliger development. Taken together, data reported here and elsewhere suggests that increased temperature could lead to an increase in growth rate in developing dorids (see, e.g., Scheltema 1967; Rose 1986), which could change seasonal population dynamics, or a decrease in growth, leading increasing veliger fatality of *D. monteryensis* (this study). This may, in the future, lead to changes in ecosystem dynamics that could be dramatic but may be negated with further understanding of developing veligers.

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Figures

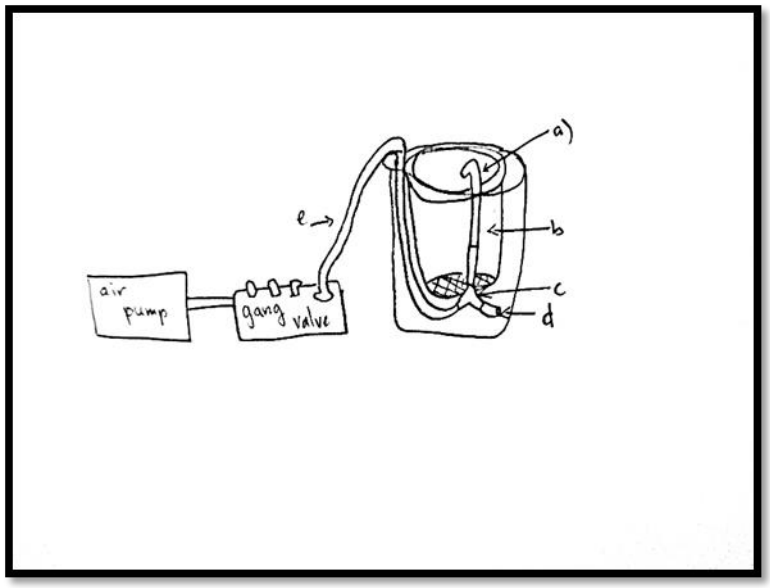


Figure 1: Airlifted droplet stirrer used to culture larvae. Disposable beaker with bottom removed and replaced with mesh placed inside a plastic E container filled with filtered sea water. Air pump pushes drops of water into disposable beaker, preventing trapping in surface tension. A is glass piping, b is disposable beaker, c is a t-joint, di is the input of water. Output of air, and e is the tubing from the gang valve to the t-joint. See also Strathmann (1987).

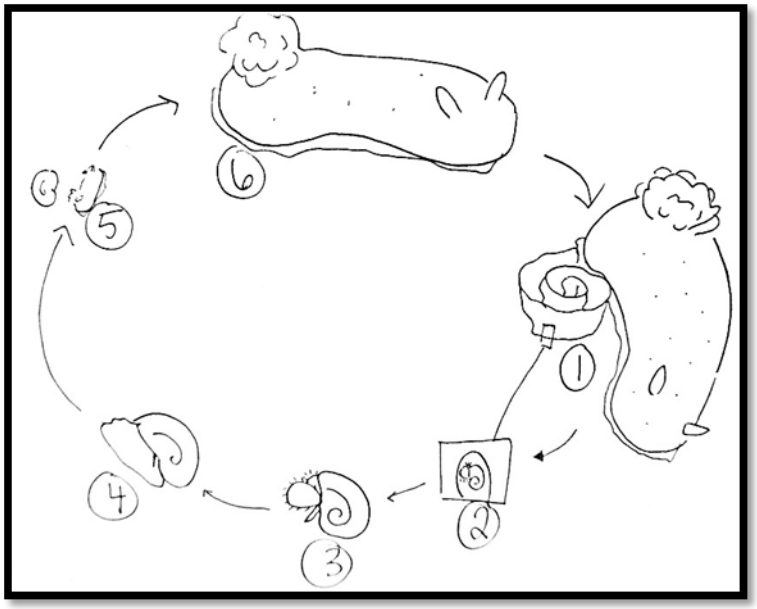


Figure 2: Life cycle of a dorid nudibranch. 1) nudibranch lays spiral egg mass 2) veliger develops in egg mass 3) veliger leaves egg mass, becomes pelagic 4) veliger settles, starts to lose shell 5) nudibranch fully loses shell, no longer a larva 6) Adult dorid.

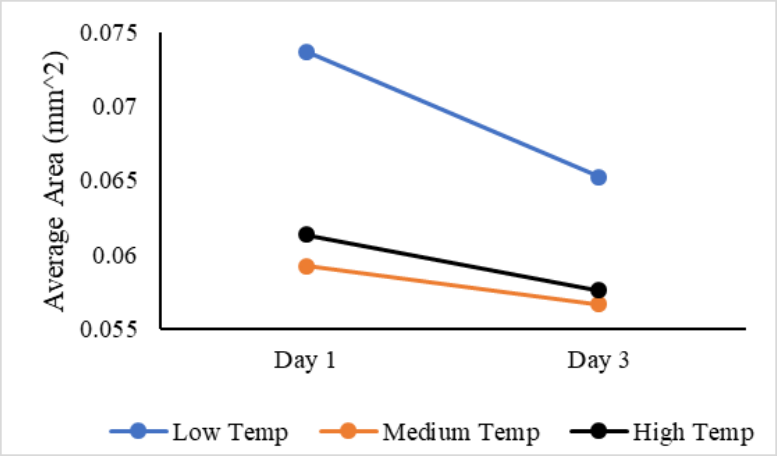
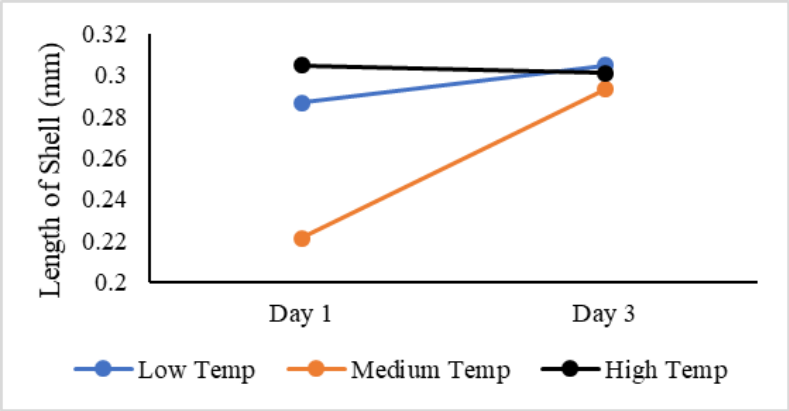


Figure 3: Graph of day versus total area in millimeters squared. Replicates averaged and treatments in key.



FFigure 4: Graph of day versus total length in millimeters. Replicates averaged and treatments in key. No significance found.