Effects of thermal stress on growth and mortality of juvenile *Crepidula fornicata* in New England

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ABSTRACT: As a result of Climate Change, the oceans have become warmer and more acidic, causing additional stress on organisms as well as changing the diversity and health of the ecosystem. Previous studies have examined the effects of climate change on pelagic, subtidal, and intertidal zones and have found that while ocean acidification has an effect on calcification, the change in temperature has negative effects on health and survival in the case of calcifying organisms (Castillo et al. 2014). Based on this information, this study focuses on the effect of thermal stress on a species of molluscs, *Crepidula fornicata*, a native intertidal limpet found along the eastern coastline of the United States. While this species appears relatively benign, it has managed to invade areas such as the UK and the Mediterranean affecting aquaculture and environmental conditions (Montaudouin, et al 1999). Understanding the effect of global warming on these snails can help create more accurate predictions regarding the health of the ecosystem heavily impacted by this species. In this study, the mortality and growth of juvenile *C. fornicata* subjected to heated conditions (30°C) did not significantly differ from those held in control conditions (19°C). However, subjects in the heated treatment used significantly more dissolved oxygen than those in the control, possibly indicating a change in metabolism.

Introduction

The Earth's atmospheric temperature has increased by approximately 0.6 °C over the past 100 years, affecting a broad range of organisms across diverse geographical regions (Walther *et. al.* 2002). These drastic changes in temperature have been found to be caused by human actions. Much of the increased warming in the past 50 years has been linked to an increase in greenhouse gas concentrations being released into the atmosphere, which leads to an increase in the concentration of gases in the atmosphere that absorb or scatter radiant energy (Oreskes 2004). Global temperatures are expected to rise by at least 2°C by 2050, exceeding the previous rate over the past 420,000 years (Hoegh-Guldberg *et. al.* 2007). The adverse effects of climate change are expected to have a greater impact on these organisms as Earth's physical conditions continue to change. Though some organisms now struggle to survive in their changing environment, others have become resilient to these new conditions through adaptations. These adaptations are perceived to reduce the effects of climate change on organisms. (Hulme 2005).

The intertidal zone is an extremely stressful environment, forcing organisms to cope with rapid changes in conditions such as temperature and salinity (Diederich & Pechenik 2013). One of the most successful species in the intertidal zone is *Crepidula fornicata,* a species of gastropods native to the eastern coastline of North America (Blanchard 1997). Due to globalization, this organism has expanded its spatial distribution and invaded areas such as England and the Mediterranean

(Bohn *et. al.* 2012). Since these snails heavily impact ecosystems in the form of spatial competition, bottom raising, and trophic competition, it is crucial to understand how increasing sea temperatures will affect their population (Blanchard, 1997). Like any species, a sudden change in population density, either increase or decrease, can have cascading effects on the ecosystem around them.

Previous studies have investigated thermal stress on *C. fornicata*. However, these studies focused primarily on larval and adult life stages. Pechenik (1984) suggested that larvae grew faster when in warmer water. In addition, Diederich and Pechenik (2013) found that *C. fornicata* larvae and embryos were more tolerant to temperature stress than adults. Lastly, it has been suggested that as the temperature rises, dissolved oxygen decreases (Newell *Et al* 1978; Blumberg & Di Toro, 2011) and metabolism increases (Lesser & Kruse, 2004).

We hypothesize that with increased temperatures, growth should increase along with mass. However, due to decreased levels of dissolved oxygen (DO) in heated tanks, we also believe there will be an increase in mortality. Due to the lack of studies on juvenile snails, this study aims to focus exclusively on juvenile snails and their reaction to thermal stress. A comparison between the juvenile snails' reaction to thermal stress and previously observed reactions by larvae and adult snails can therefore be made. Therefore, this study focuses on the effect of thermal stress on the physiology of juvenile *C. fornicata* to determine their resilience when placed in an environment affected by climate change.

Methods

Field Site: For the purpose of this study, we collected intertidal juvenile *Crepidula fornicata* (N = 60) from Nahant, MA (42.43572 °N 70.93848 °W) (Fig. 1) on November 2, 2017. This field site is also the location of the Lynn Shore and Nahant Beach Reservation, which consists of 67 acres of beach and recreational areas, spanning approximately 2,100 meters.

Figure 1: Map of Boston area, with inset map of eastern Massachusetts. The sampling site (red dot) indicates that snails were collected from Nahant, MA

Experimental Setup: After collection, snails were maintained for four weeks at control conditions in one tank measuring 25.4 cm in length, 13 cm in width, and 16.2 cm in height. The snails were randomly assigned across six plastic bins (N=10 per bin), each of which was placed in a separate experimental tank (N=3 per treatment). These plastic bins were used for ease of measurements as repetitive scraping could damage individuals and cause mortality and stress. Bins had mesh sides and lids, allowing water flow. Prior to being transferred to bins, each snail was marked with a unique nail polish label in order to keep track of individual growth rates and mortality. Three control tanks were maintained at 19°C, the recorded average warm temperature during the summer months in Nahant, MA (Nixon *et al.* 2004) and three experimental tanks were heated at a constant rate overnight until they reached a temperature of 30°C – the predicted highest temperature in the year 2100 for this area (Rogelj *et al*. 2012) The experiment was conducted over a 12-day period. Water quality (temperature, salinity, and pH) was assessed every morning in order to verify that all conditions except temperature were kept constant across control and experimental treatments. Salinity was measured using a digital refractometer and pH was measured using a Pinpoint® pH meter.

Feeding and Dissolved Oxygen: Subjects were fed 0.5 mL of Shellfish Diet 1800® twice daily. Water flow was turned off and phytoplankton was subsequently added to each tank. Water flow remained off to allow for filter feeding. During the morning feeding, snails were allowed to filter feed for 15 minutes prior to water flow being turned back on. After 10 minutes, water flow was turned off again and an initial dissolved oxygen (DO) level was measured for each tank. Flow then remained off for four hours before a final DO level was measured. Flow was kept off to stop the introduction of new oxygen from entering the tanks and thereby allowing for assessment of the effect of temperature on snail respiration. After the final DO measurement, phytoplankton was added to the tanks a second time and snails were allowed to filter feed for one hour, after which flow was resumed and remained on until the morning feeding period.

Mortality and Growth: Snail mortality was assessed in the morning and afternoon. Mortality was determined when the snail was no longer attached to its shell. Initial shell length and width of each snail, and collective weight of the 10 snails in each bin was also recorded at the beginning of the experiment. Snail shell length and width, and bin mass were measured every 5 days. Shell length and width were measured using digital calipers. Bins were initially weighed prior to the addition of snails; collective snail mass was determined by subtracting the measured weight from the pre-weighed value. During weighing, each bin was removed from the tank, drained of water, and lightly dried with a paper towel.

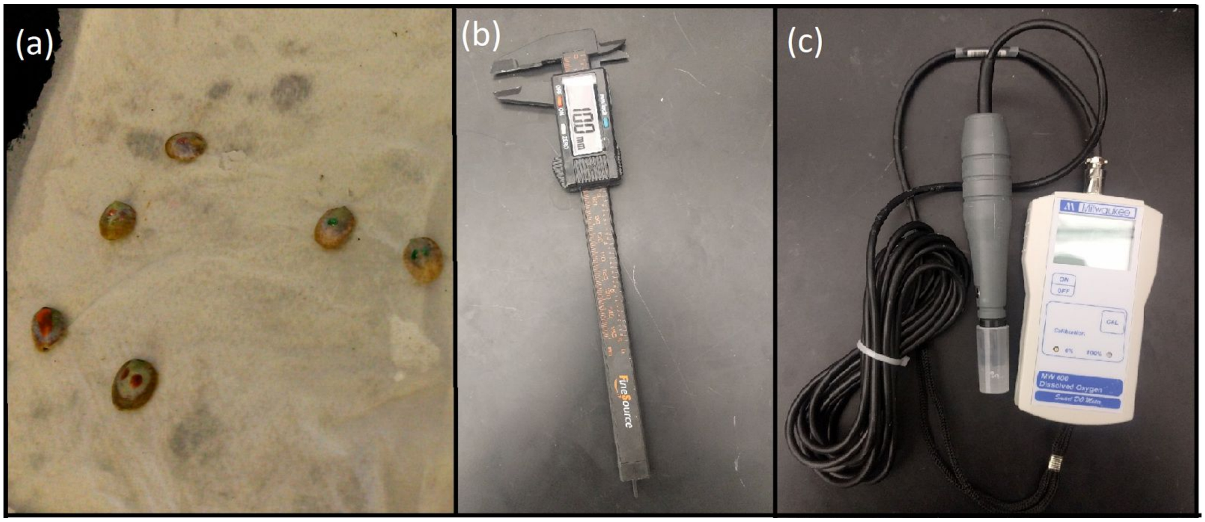

Figure 2: Images of snails and measuring tools used during experiment. (a) Nail polish dots were painted on the snail shells to keep track of growth for individual snails (b) A digital caliper was used to measure the length and width of each snail shell (c) Dissolved oxygen meter was used to measure.

Statistics: Data was analyzed using R to determine any correlations between experimental treatment and mortality, growth, and DO. R was used to run linear models which assessed significant differences between control and heat treatments. Changes in growth (shell size and snail weight), mortality, and DO were examined over the course of two weeks. These measurements were analyzed using linear regressions and cox-proportional hazard models, respectively. The correlation between shell length and width was analyzed, and the measurements over time were compared between each treatment. Snail mass between treatments was compared over time. DO was standardized by subtracting initial (morning) DO from final (afternoon) DO and dividing it by number of snails in each bin to determine the change in DO for each snail. Since the treatments were done in triplicates, the values for each bin was averaged based on treatment.

Results

Dissolved oxygen: A linear model of DO measurements showed a significant change in dissolved oxygen levels ($p=0.0003201$; Fig. 4). The DO values included in this model represent the difference between the DO measurements taken in the afternoon and those taken in the morning. A statistically significant change in DO measurements suggests that the subjects in the heated experimental tanks displayed higher oxygen intake during the 4-hour period between measurements than those in the control tanks.

Mortality: Mortality difference between the heated tanks and the control tanks was found to be insignificant by a linear model ($p=0.474$; Fig. 3). This implies that there was no significant difference between the amount of subjects that died in the heated experimental group when compared to the control experimental group.

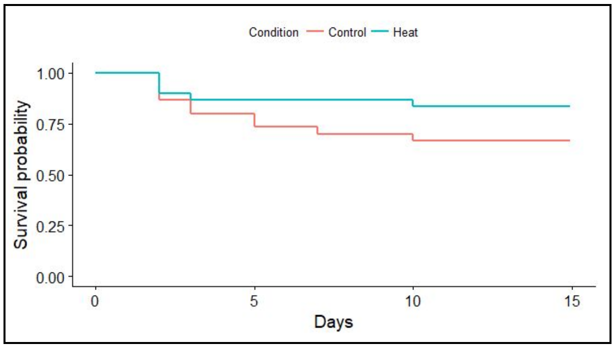

Figure 3: Cox proportional hazard regression illustrating the survival of snails subjected to both heated and control environments. While not significant (p = 0.474), there were more deaths at the control temperature.

Growth: Linear models showed that there was no statistically significant change in snail shell length (p=0.6135; Fig. 5) and width (p=0.9313; Fig. 5). However, there is a significant correlation between shell length and width (Adjusted R-squared= 0.8579, p=0.005041). Snail mass was found to be statistically insignificant (p=0.07201; Fig. 5).

Discussion

Daily DO measurements were used to calculate the average oxygen intake per snail, per tank. These calculations were then averaged between treatments to allow for comparison between heated and control tanks. The snails in the heated tanks were found to use significantly more dissolved oxygen than the snails in the control tanks (Fig. 4). This supports our hypothesis. This increased DO usage may be due to increased metabolism in the warmer environment.

It should be noted that there is also a certain amount of air-water exchange which naturally causes an increase in dissolved oxygen in the system (Schladow *et al.* 2002). There was nothing in this experiment to account for this. Though this means that natural air-water exchange affected both experimental tanks, therefore both treatments were affected in the same way. Oxygen intake from snails in the heated treatment negates this exchange for the most part and tends to further deplete DO. If we were to repeat the experiment, a respirometer would be used to calculate the exact oxygen intake of each subject. If a respirometer were used, the snails would be kept in the dark to prevent any photosynthetic activity impacting the dissolved oxygen. This study focused on comparing the change in DO between the heated tanks and the control tanks each day, therefore the average DO per snail was taken for each tank.

Average mass per snail decreased for the heated group from day 5 to 15, and for the control group from day 10 to 15 (Fig. 5d). This decrease was unexpected, as snails should not lose mass while still growing in both length and width. This does not support our hypothesis, as we expected to see a continuous increase in snail mass over time. This decrease may have been due to an error when initially weighing the bins, or possibly due to the mortality of some large snails which, when removed, would cause a decrease in the average snail mass. Since they are an intertidal species, the subjects may also have retained different amounts of water when removed from the tank, which would affect the mass of the bins.

Ideally we would have measured each snail separately, to get individual snail mass. However, this would have caused more stress for each snail as they would have to be removed from the plastic bins. Another way we could have measured mass is through buoyant weights, keeping them in the plastic bins and weighing the whole bin. Similarly, this would would have resulted in an average snail mass per bin. Another issue to be accounted for if using buoyant weights would be the buoyancy of the plastic bin.

We observed an increase in both shell length and width of all snails apart from one - Sparkly Green 1 (SG1) in Control Tank 1. SG1 had a shell length of 10.1cm on day 0, which increased to 13.1cm on day 5. The shell length then decreased to 10.6 cm on day 10 and increased slightly to 11 cm on day 15. Days 10 and 15 support our hypothesis, as we predicted snails would continue to grow with time. The 13.1 cm reading may have been due to a measuring error where the caliper was not calibrated. It is unlikely that the snail grew 3 cm in five days, then shrunk within the following five days and subsequently followed the expected growth rate. For this reason, we decided to eliminate SG1 from our analysis. We found that snails in both conditions were growing at similar rates (Fig. 5c), therefore the difference in growth rates is not significant. However, there is a significant correlation between an increase in length and an increase in width (R-squared=0.8579). Overall, though there was an increase in growth for all snails, there was no significant difference in length or width over time, which supports the results of a previous study done by Lima and Pechenik (1985).

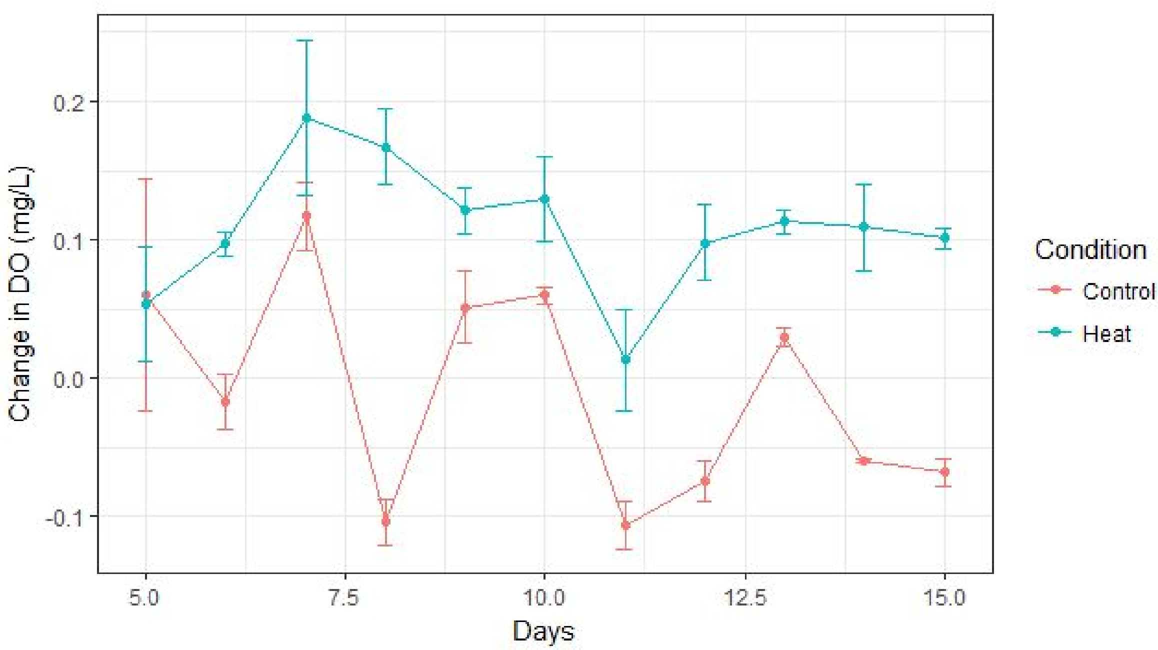

Figure 4: Illustrates the change in dissolved oxygen (DO) between control and heat conditions over a 4 hour period while water flow is off ($p<0.005$). Change in DO was calculated by subtracting the afternoon measurement from the morning measurement. Higher points represent more DO usage by the snails. When the DO value is negative, there is more oxygen being added to the system than is being taken up by the snails

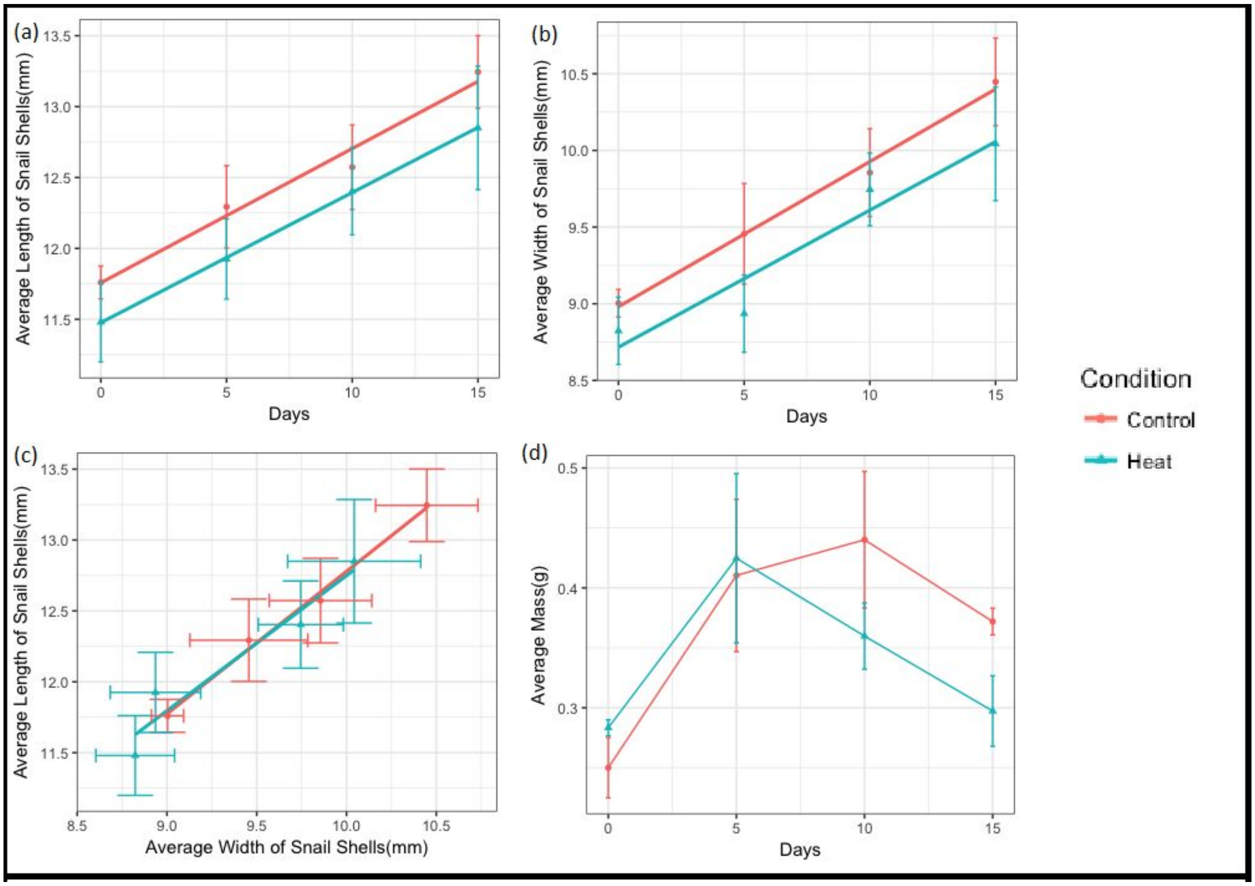

Figure 5: Mass measurements, as well as shell length and width, were averaged for all snails in each bin and averaged across treatment for each measurement day during the experiment for both control and heated tanks. (a) Shell length (mm) was analyzed over time, showing no significant difference between treatments (p=0.6135) (b) Shell width (mm) was analyzed over time, also showing no significant difference between treatments (p=0.9313) (c) Shell length (mm) was compared to width (mm). The two factors are shown to be heavily correlated (Adjusted R-squared= 0.8579, p=0.005041) (d) Change in average snail mass (g) was analyzed over time, showing no significance (p = 0.07201).

We also predicted higher mortalities in the heated tanks, as was shown in previous studies (Diederich & Pechenik 2013). There were more mortalities in the control tanks (Fig. 3), however these results are not statistically significant. This therefore does not support our hypothesis. This may be because *C. fornicata,* being an intertidal species, is used to being out of the water for a certain amount of time, however they were only removed from the tanks briefly every morning and afternoon to check mortality, and every five days when measurements were taken. This lack of constant change may have contributed to the amount of stress they experienced, and therefore the number of mortalities. There have also been previous hypotheses that intertidal *C. fornicata* live in sub-optimal conditions, and have not fully acclimated or adapted to the environment in which they are found (Diederich & Pechenik 2013), which could explain the lack of tolerance to the colder temperatures in the control tanks.

Conclusion

Although our results were not significant, we observed that the snails in the heated tanks had lower mortality. Further research should be done to investigate this. It is possible that our results would show significance if the study was replicated over a longer period of time; exposing the snails to these experimental temperatures over months would provide a better understanding of how they cope with thermal stress over time. Future studies could also look at increasing the temperature further to see how juvenile *C. fornicata* cope with higher temperatures. As well as this, since we observed more mortalities in the colder tank, it may be interesting to observe their reactions to colder temperatures as well. In order to replicate their natural intertidal environment, snails could be removed from the water for a certain amount of time every day. We found the increase in snail growth to be insignificant, however the upwards trend suggests that further study on growth rates in thermal stress could be beneficial. Further study could also be done on the metabolism of *C. fornicata*, as the DO results in this study were shown to be significant.

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