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The Effect of Environmental Conditions and Predator Presence on the Metabolic Rate of Red
Abalone

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Abstract

Climate change threatens to disrupt marine ecosystems, prompting concern for their long-term health. Understanding how environmental conditions and predator presence affect the energetic costs of organisms can provide insight into the fitness of individual species. Until now, the metabolic response of red abalone (*Haliotis rufescens*) to these variables have not been quantified. Furthermore, this study quantified the effect of increased temperature and decreased pH on the metabolic rate of juvenile red abalone under the presence or absence of predators. Abalone were exposed to a fully factorial design crossing two temperatures, two levels of pH, and predator presence or absence (12 abalone per treatment conditions) for 5-7 days. The effects of these variables were tested against three size classes: small (17-21 mm), medium (22-30 mm), and large (35-41 mm). Post exposure, the metabolic rate of abalone from each treatment combination was estimated using closed chamber respirometry and subject to ANOVA analyses to determine the individual and interactive effects of temperature, pH, and the presence of predators on abalone metabolism. The effect of predators and pH were insignificant on the red abalone's metabolic rate across size classes. Similarly, the effect of temperature was also insignificant in the medium and large groups and might be possibly significant within the small

abalone. In addition to prior studies, our results suggest *H. rufescens* might be particularly vulnerable in their early life-history stages (up to ~20 mm) and potentially experience a bottleneck but have greater resiliency when greater than 20 mm.

Introduction

The concentration of atmospheric carbon dioxide has spiked in recent decades affecting interactions amongst marine species. Once introduced to the atmosphere, carbon dioxide can absorb long-wave radiation emanating from Earth's surface and re-emit this energy as heat resulting, in part, in the warming of oceanic ice fixtures. In addition, a warmer atmosphere interacts with the oceans surface layer leading to a general increase in ocean temperatures. These rising ocean temperatures have been shown to lead to local extinctions (Nicholas et al., 2006, Hughes et al., 2017), influence spawning (Lawrence & Soame, 2004; Travers et al., 2009), and the growth of marine organisms (Lord et al., 2017). These organismal effects can negatively affect the species population as a whole.

Similarly, increasing concentration of atmospheric CO₂ reacts with seawater, resulting in a decrease of free carbonate ions and an increase in hydrogen ions. The addition of free hydrogen ions increases the acidity of a region while the formation of bicarbonate ions reduce the amount of carbonate ions present. This reduction of carbonate ions negatively impacts calciferous organisms that use these ions to build their shells, especially gastropod mollusks like abalone, and can decrease their ability to defend themselves from predators. In particular, elevated acidity has been shown to negatively affect growth of invertebrates (Kim et al. 2013) as well as overall physiology (Gibson et al., 2011).

Changing ocean conditions have been shown to increase the metabolism of cold-blooded invertebrates (Gibson et al., 2011). Marine organisms allocate energy to various biological processes within a specific span of temperature, dissolved oxygen concentration, and pH. These organisms evolved to optimally operate within a range of environmental conditions known as their thermal window (Pörtner & Farrell, 2008). Climate change will push an organism to or past the boundaries of their thermal window decreasing the efficiency of their energy expenditure and increasing the likelihood of mortality. These effects can scale up from an organismal to population level and can cumulatively pose serious threats to species survival. Similarly, climate change will alter the behaviour of individual organisms that can affect the community they inhabit (Nagelkerken & Munday, 2016).

Most studies have looked at the physiological effect of climate change on a single species. However, any given species can belong to at least one food web. Although predators can affect plant abundance through direct consumption of herbivorous marine animals, predators can also non-consumptively interact with prey species resulting in changes to prey physiology. When predators eat prey, chemical cues are released by predators that prey can interpret as a threat to their survival. In response, nearby prey can move into hiding or outside the range of direct harm. Thus, predators can still affect the population abundance of plants through indirect interactions mediated by herbivores in a process known as Trait Mediated Indirect Interactions (TMII). Through this, predators can affect lower trophic levels to a greater extent than through direct consumption.

Although understanding the effect of increased temperature and decreased pH on specific aspects of a species physiology is crucial, it does not represent the cumulative effect of environmental and community interactions on a given species. In fact, relatively few studies seek

to understand the combined concomitant effect of climate change and predator-prey interactions on a given species. One exception is a study conducted by Lord et al. (2017) that looked into the effect of climate change on direct and indirect species interactions.

To understand the effect of climate change on a community, Lord et al. (2017) constructed a fully factorial experiment. To test the effects of temperature, incoming seawater was offset by 2°C to reach a low and high of 14.2°C and 16.2°C, respectively. Similarly, the pH was offset by -0.3 to average a low of 7.52 and a high of 7.82. The indirect effect of predators were tested through placement of *Haliotis rufescens* in cages while *Pachygrapsus crassipes* were allowed to roam free. Using these conditions, the study constructed a fully factorial design crossing temperature, pH, and predator presence. A combination of these three variables represented one treatment that abalone were exposed to for ten weeks. The feeding behavior was examined and quantified through the abalones daily consumption of kelp. At the end of the experiment, the growth of abalone shell was measured in addition to the weighing of somatic tissue. Their results showed a significant decrease in the amount of kelp that red abalone consumed in the presence of predators. When predator presence and environmental conditions were coupled, temperature did not seem to have an effect on the growth or feeding of abalone. However, both pH and predator presence had a significant negative effect on the growth of abalone shell and somatic tissue as well as the amount of food consumed.

The experiment conducted by Lord et al. (2017) indicated that environmental conditions and predators have an affect on abalone growth and feeding; however, it was unclear whether this change was due to behavior or differences in metabolism. To understand the underlying energetics, this study incorporates similar temperature and pH variation as well as indirect predator effects to test the role of metabolism on the metabolic rate of abalone under predator

presence. It is hypothesized that long-term exposure of abalone to high temperature, low pH, and predator presence will increase metabolism.

Methods

This study focused on benthic organisms found along the west coast of North America including red abalone (*Haliotis rufescens*), crabs (*Pachygrapsus crassipes*), and kelp (*Macrocystis pyrifera*). Red abalone is a commercially important species whose population is especially threatened by overfishing and climate change. This experiment is the first to assess the individual and cumulative effects of temperature, pH, and predator presence on red abalone as well as to determine the metabolic rate of abalone at these environmental settings. One of our goals is to assess how variations of these factors might affect the environment abalone inhabits. It is hypothesized that, individually, increased temperature and predator presence will increase the metabolic rate of abalone. Cumulatively, it is believed that these factors will act synergistically to significantly increase metabolic rate. In the long-term, elevated temperature, decreased acidity, and predator presence could lead to a decrease in red abalone survival rates. Therefore, understanding the individual and cumulative effects of varied environmental conditions and predator presence on red abalone will be useful in the management of kelp forests.

Juvenile red abalone and kelp were purchased from the Monterey Abalone Company (17-41 mm carapace width [CW]) while crabs were collected from Moss Landing Harbor (35 mm CW). *P. crassipes* was chosen because they consume juvenile red abalone. Similarly, *M. pyrifera* was chosen because *H. rufescens* readily consume them. The red abalone used in this experiment were divided into three size classes: small (17-21 mm), medium (22-30 mm), and large (35-41 mm).

Red abalone were held in aquaria with flowing seawater for approximately six months prior to the experiment in a temperature of about 13°C. Crabs were collected from the intertidal shore in Moss Landing, California.

To understand the individual and cumulative effects of temperature, pH, and predator presence a fully factorial design was constructed. To reach this, we exposed eight groups of twelve abalone to either a high or low temperature, high or low acidity, or both. Four of these tanks were exposed to a predator. A cross of temperature, pH, and predator presence is referred to as a treatment. In each of the eight rectangular experimental tanks, the abalone roamed free while those with a crab were caged. Eight rectangular containers were used to test the average low and high temperatures of 14.2°C and 16.2°C from the work of Lord et al. (2017). The average low of 7.52 and high of 7.82 pH was also used. Flowing seawater from Monterey Bay was pumped into an overhead tank that provided water to 4 small overhead conditioning tanks (approximately 2 gallons) with a water flow rate of $1230.00 \pm 33.54 \text{ mL min}^{-1}$. The four conditioning tanks were used to control the temperature and CO₂ settings. To do so, a pump was attached to each conditioning tank where it would provide a specific air and carbon dioxide measure as a means of controlling the acidity. Another pump would provide either ambient or warmed seawater. For both temperature and pH, an offset of +2°C and -0.3 pH units (total pH scale) was used and allowed provision of naturally fluctuating seawater. The temperature and pH offset was maintained using LabVIEW™. Each conditioning tank had a thermistor, pH sensor, and dissolved oxygen sensor to maintain the chosen conditions. All pH sensors were calibrated with a pH 6.8 and pH 7.7 buffer.

Within the experimental tanks, crabs were caged within a spherical plastic cage with a diameter of 10 cm. Of the crabs kept within cages, they were fed a piece of squid each day

approximately 2 cm by 3 cm in size. Previous studies have demonstrated that exposure of a prey to a predator actively feeding releases chemical cues that elicits a flight or fight response from prey (Manzur et al., 2014). Each tank consisted of 12 abalone representing the small, medium, and large size classes and were fed kelp daily *ad libitum*. Each day, any leftover kelp was removed to reduce microbial presence.

The abalone were exposed to their treatments for 5-7 days. Following the exposure of abalone to their treatments, closed chamber respirometry was conducted. Each respirometry chamber was attached, by tube, to their corresponding experimental tank to maintain treatment conditions (both environmental and predator presence or absence). One large, two medium, and four small abalone were placed into three respirometry chambers, respectively. Once placed into the chambers, they were held in baths overnight to maintain temperature. The following day, the abalone were placed atop stirring plates. Drawdowns were run in triplicate for ten minutes. Once a run began, flow to the chamber was stopped using luer locks and the oxygen concentration and temperature were measured. At the end of each run, flow was restored allowing replenishment of oxygen to starting levels.

Once abalone underwent respirometry, the weight of the chamber (inclusive of water and abalone) were taken at the same time temperature was. The weights of the abalone with and without a shell were also taken. Calculations were done to determine the amount of oxygen present in the chamber from its final concentration. Analysis was conducted with a single and 2-way ANOVA.

Results

Abalone in the small size class had a metabolic rate between 479.43 to 867.45 mgO₂/hr per mg dry weight, the medium class had a rate between 330.37 to 628.21 mgO₂/hr per mg dry weight, and the large class had a metabolic rate between 316.84 to 672.60 mgO₂/hr per mg dry weight (Fig. 1a).

The effects of predator presence and size class were analyzed with a 2-way ANOVA (n = 8; Fig. 1b). The effects of predator presence on metabolic rate across size classes were insignificant (p = 0.27). Size class did have a significant effect on metabolic rate (p < 0.01). Both predator presence and size was insignificant on metabolism (p = 0.12). A Tukey Post Hoc test was conducted to determine the effect of size class on metabolic rate. The effect of the small group on metabolism was significantly higher (p < 0.01) than the medium and large (p > 0.15).

An ANOVA test on temperature and pH was conducted with size class as a covariate. The effect of these environmental variables on metabolic rate were insignificant across sizes (p = 0.16; Fig. 2a). To test the effect of temperature on metabolism, a t-test was conducted pooling all treatments into either high or low temperature bins. Within the small group, the effect of temperature yielded a p = 0.08 whereas the same temperature effects were insignificant for the medium and large classes (p > 0.1). For each size group, the sample size was 8 (Fig. 2b).

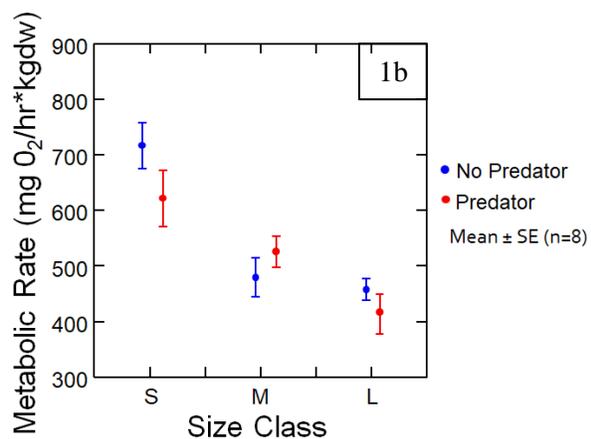
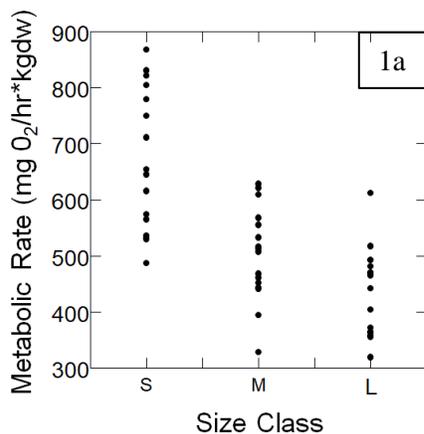


Fig. 1a. The range of metabolic rates ($\text{mgO}_2/\text{hr} \cdot \text{kgDW}$; dry weight (DW)) per size class. All treatments were pooled ($n = 16$ per size class). Fig. 1b. The mean and standard error (SE) of metabolic rate was determined for each size class. The effects of predator presence were insignificant whereas the effect of size was significant for the small group.

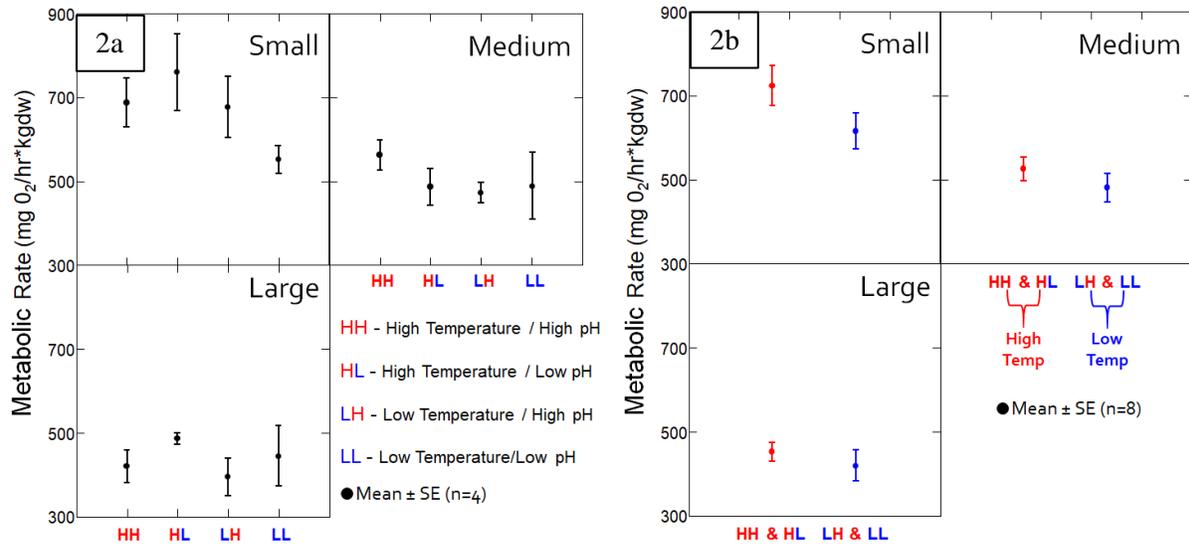


Fig. 2a. The effect of temperature and pH on metabolism were tested. Each bar represents the mean and standard error ($n = 4$). There was a significant effect of environmental conditions on the small class unlike the medium and large groups. Fig. 2b. To test the effect of temperature, pH and predator presence were pooled ($n = 8$). Each bar represents the mean and standard error.

Discussion

These results build upon previous studies conducted on the potential bottleneck juvenile abalone may experience in its early life-history stages. Our work suggests that small abalone (>21 mm) are affected by changing ocean conditions to a greater extent than medium (22-30 mm) and large (35-41 mm) juvenile abalone. By testing the effect of climate change on different size classes of abalone in the presence or absence of a predator, we were able to quantify how these variables interact. In the context of a study conducted by Lord et al. (2017), our results suggest that reductions in abalone tissue and shell growth were due to behavioural changes and not metabolism in medium and large juvenile abalone. Notably, the small group did experience

elevated metabolic rates suggesting certain treatment conditions can potentially interact with behavioural changes to negatively compound their ability to survive under multiple stressors.

After a long 5-7 day exposure of abalone to crabs, the effect of predators on abalone metabolism were insignificant (Fig. 1a). This could be due to the *H. rufescens* acclimation to the presence of *P. crassipes* (Steiner & Van Buskirk, 2009) and subsequent depression of metabolic rate from their initial flight or fight response. This experiment relied on chemical cues produced by caged crabs to elicit a physiological response in free-roaming abalone. However, this may have been more effective if the abalone were caged and the crabs were free. This would prevent abalone from moving to the opposite side of their experimental tank, increasing the distance between them and their predator, and decreasing the effect of predator presence. Nonetheless, the length of predator exposure is applicable to actual conditions abalone might face in the wild and thus, the effects of predator presence on abalone metabolism.

The slight increase in temperature used in this experiment (+ 2°C) was not outside the normal range of fluctuation abalone might experience in the summertime. Therefore, the effect of temperature on metabolic rate may have been subdued and could be increased in future experiments to elicit a greater metabolic response from abalone. The smallest group of abalone were most significantly impacted by a temperature increase. Due to the sample size used in this study (n =4), it is suggested that future work be done incorporating a greater sample size to definitively determine whether these results are meaningful or spurious. Previous research on the effects of climate change on juvenile abalone has indicated they are more susceptible to changes in ocean conditions up to 20mm (Kim et al., 2013, Boch et al., 2017, Lord et al. 2017). Our small size class (>21 mm) overlaps with the sizes used in these experiments. Therefore, the effects seen in our experiment suggest this size group is more susceptible to climate change and might

experience a bottleneck up to ~21 mm (Fig. 2b). The diminished effect of temperature on abalone greater than 21 mm hints to a greater resiliency as well as an exclusion from any potential bottlenecks (Fig 1a-2b).

The effect of pH on abalone metabolism was insignificant across all sizes. Acidity has been shown to hinder the growth of abalone shell and somatic tissue, however, it lacked any significant effect on feeding rates (Lord et al. 2017). Furthermore, results from Lord et al. (2017) do not specify whether reductions in shell and tissue growth were related to changes in feeding behaviour or to differences in metabolic energy allocation. Our results suggest that the reduction seen in growth was due to behaviour instead of changes in metabolism (Fig. 2a).

Conclusion

The effect of *P. crassipes* on the metabolic rate of *H. rufescens* after 5-7 days of exposure were insignificant across size classes. Similarly, the effects of temperature and pH were also insignificant except for the small juvenile red abalone that might undergo increased metabolism under elevated temperature. Therefore, this study contributes to a growing body of research that has indicated red abalone, approximately less than 20 mm, experience a bottleneck in their early life stages. Our research also suggests red abalone greater than 20 mm seem to have greater resiliency and are excluded from a potential bottleneck. Furthermore, this study elucidates previous work and suggests that reductions in shell and somatic tissue under climate change and predator presence is due to changes in behaviour instead of differences in metabolic energy allocation.

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