Masters Thesis: Coded in R

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Facing Physiological Plasticity: Investigating the Interactive Effects of pH and Temperature Variation on the Metabolic Demand of an Intertidal Gastropod *Tegula funebralis* Amidst a Fluctuating Tidal Environment

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By

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"Against this cosmic background the lifetime of a particular
plant or animal appears, not as a drama complete in itself,
but only as a brief interlude in a panorama of endless change."

— Rachel Carson, *Undersea* (1937)

"For nothing is fixed, forever, forever, forever,
it is not fixed;
the earth is always shifting,
the light is always changing,
the sea does not cease to grind down rock.
Generations do not cease to be born,
and we are responsible to them
because we are the only witnesses they have.

The sea rises, the light fails,
lovers cling to each other,
and children cling to us.
The moment we cease to hold each other,
the moment we break faith with one another,
the sea engulfs us and the light goes out."

— James Baldwin, *Nothing Personal* (1964)

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Being a part of the CSUN and the Los Angeles community the past three years has been some of the most formative and fulfilling experiences of my life and for my career moving forward. Studying climate change in an era marked by rapid ecological change and numerous interconnected crises, encompassing racial capitalism and the climate emergency, particularly during a time in which society stands at a pivotal crossroads for humanity's future can feel rather dismal. Yet the support and knowledge I've received from my community and mentors as well as the collective imagination of the communities I am a part of has instilled within me an unbridled sense of optimism. That amid these challenges, we possess all of the solutions

to the crises we face, and that within our collectivities, there are reservoirs of hope for the future - a future where our individual lives matter not less, but more, as they form the pixels shaping a panorama of endless change.

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Abstract

Ocean acidification (OA) is occurring across a backdrop of concurrent environmental changes that may in turn influence species' responses to OA. Temperature affects many fundamental biological processes and governs key reactions in the seawater carbonate system. It therefore has the potential to offset or exacerbate the effects of OA. While initial studies have examined the combined impacts of warming and OA for a narrow range of climate change scenarios, our mechanistic understanding of the interactive effects of temperature and OA remains limited. Here, we use the black turban snail, Tegula funebralis, as a model species to test how OA affects the respiration rate of a herbivorous invertebrate across a wide range of temperatures encompassing their thermal optimum. Snails were exposed in the laboratory to a factorial combination of low and high pCO2 (400 and 1200 µatm CO2) and temperatures (12, 14, 16, 18, 20, 22, 24, 26°C) for two weeks. Results indicate that the effects of OA on respiration rate are highly dependent on temperature. Although high CO2 significantly increased respiration rate at 20°C, this effect gradually lessened with successive warming to 20°C, illustrating how moderate warming can mediate the effects of OA through temperature's effects on both physiology and seawater geochemistry. Together, these results highlight the importance of considering the physiological and geochemical interactions between temperature and carbonate chemistry when interpreting species' vulnerability to OA.

1 Introduction

Organisms and environments are entwined, as the relationship between organisms and the environments they are embedded within, is in constant flux through inextricable links and flows of energy. The relationship between organisms and environments varies through both space and time and is influenced by a mosaic of dynamic biotic and abiotic drivers **??**. Organisms are active participants in constructing their environment, from altering seawater biogeochemistry through physiological processes to organisms constructing biogenic structures; plenty of studies demonstrate that organisms influence their environment **??**. Organisms and ecosystems, while playing an essential role in structuring the physical and biological environment, are simultaneously governed by the ability to perform and function under a myriad of complex interactions, as each act to influence one another non-contemporaneously and contemporaneously through direct and indirect feedback loops **??**. Organisms must physiologically cope with the conditions of the environment they are situated within, which inevitably influences community structure and populations **?**. Further, the biogenic structures created by organisms are highly dependent upon the environmental regime the organism develops in, living beyond the life of the organism itself; organisms are thus simultaneously creators and products of the environment. In a geological epoch of rapid ecological change, it is increasingly imperative to understand how and the extent to which organisms can respond and perform to abiotic drivers and how the legacy of the structures (e.g., shells, reefs) that organisms create may influence other species indirectly.
Understanding physiological responses, interactions, and constraints of marine organisms to anthropogenic climate change is perhaps the sine qua non for understanding the changes between marine organisms and the ecosystems they construct. This research intends to inform how the changing oceanic environment may affect organismal physiology by teasing apart the relationship between environmental drivers and physiological performance. This research also intends to elucidate how changes within physiological processes in one organism may have indirect effects on other species long after the organism persists. In this regard,

the fate of organisms is intertwined as the abundance and growth of one species codetermines the other, illustrating that organisms are directly or indirectly the subjects and objects of ecological change **?**.

2 Organisms as the Subjects of Ecological Change

Environments are governed by natural spatiotemporal variation of abiotic and biotic drivers that, in turn, influence the structure and processes of communities, drive ecological change, and create a mosaic of microhabitats **???**. For example, within marine ecosystems, the combination of oceanographic processes and local coastal geography may create an array of patterns and variability in abiotic drivers such as temperature, flow, pH, dissolved oxygen, etc., that may impact the structure and processes of ecological communities on distances ranging from microscale to macroscale **??**. Such heterogeneous patterns are naturally occurring and create complex gradients that shape ecological communities and influence physiological processes within individual organisms **?**. However, due to the connotation of stress as a negative response and the ability for organisms to adapt and evolve to changing conditions over time, the term driver has been utilized to describe an environmental parameter that influences organisms and environments across a spectrum ranging from enhancing, optimal, or stressful conditions **??**. Many organisms have evolved to withstand complex and variable environmental gradients through physiological mechanisms such as phenotypic plasticity and acclimatization **??**. According to the metabolic theory of ecology, environmental gradients and changes in abiotic factors may result in physiological trade-offs due to the alterations within the energetic partitioning of an organism's metabolism **??**. The physiological processes of metabolism are the total sum of biological and chemical processes in converting energetic resources and materials into biomass and activity **?**. Comparing physiological responses to gradients of abiotic drivers may allow us to quantify and compare the tolerance limits of organisms **??**. The role of biotic and abiotic drivers in influencing

metabolic processes has been of primary interest to the field of ecology as changes in metabolism directly affect the survival, behavior, and energy requirements of organisms, thereby impacting fitness and ecosystem function **?**.

Temperature and pH are important for determining physiological processes and metabolic rates for marine organisms and thereby play a large role in affecting the functioning and physiology of ecosystems **?**. Temperature is the key driver in determining physiological rates of organisms as the kinetic energy of biochemical reactions is temperature dependent **???**. Biological processes such as organismal and ecological interactions are also strongly influenced by temperature **?**. The relationship between temperature and body-size exemplifies this as organisms develop faster yet decrease in size under elevated temperatures **?**. Metabolic rates are strongly influenced by an organism's body size and temperature and are subject to change due to changes in abiotic drivers and the natural variability of drivers **??**. Further, organisms adapt to local temperatures to match optimal conditions for physiological processes and acclimatize to a range around these values **?**. Any range too far beyond the ability of an organism to acclimatize influences survival, fitness, and population densities **?**. Studies have shown the influence of sea surface temperature on metabolic processes such as growth, feeding, reproduction, and influencing the range of species distributions **???**. However, it is essential to note that temperature is not the only driver of biological processes and temperature has interactive effects with other abiotic drivers **?**. pH is also an important abiotic driver that impacts the physiological performance of marine organisms and influences the biogenic structures that organisms create **?**. pH plays a vital role in metabolic processes due to its effect on biochemical pathways and internal acid-base balance **?**. For example, low pH is often associated with elevated metabolic rates due to the increase in energetic costs in creating calcified structures such as the formation of shells in mollusks or the skeletons of corals and echinoderms **??**. Due to differences in the energetic costs associated with calcification, there are significant differences in the ability to control acid-base regulation between species **?**. Consequently, changes in physicochemical parameters

of the environment affect species differently, impact the interaction between species and, in turn, affect the structures of ecological communities; therefore, studying how differences between abiotic drivers affect organismal physiology will have ecosystem-level implications **??**.

3 ORGANISMAL PHYSIOLOGY IN A CHANGING ENVIRONMENT

As the atmospheric carbon dioxide (CO2) concentration continues to surpass the limits of the earth system, marine organisms will be forced to endure profound transformations of the envrionment, from shifts in temperature to altered geochemistry (richardson2023earth, portner2008physiology}. Ocean warming (OW) and ocean acidification (OA) represent two of the most significant changes occurring in marine ecosystems across the globe, both driven by the unremitted rise of anthropogenic-induced carbon dioxide emissions. OW and OA are not isolated phenomena; they share a common origin, and in a rapidly changing world, their combined impacts on organismal physiology necessitate special attention as multiple drivers of change may act interactively **?**. Since the beginning of the 20th century, the global mean sea surface temperature (SST) has increased by 0.88 [0.68–1.01] °C, and is further projected to warm by 2.89°C [2.01–4.07°C] at the end of the century, which surpasses the thermal tolerance limits of many marine species (following the representative concentration pathway 8.5 emission scenario) **????**. Concurrently, the ocean has absorbed ~30% of anthropogenic CO2 **?**, altering the carbonate chemistry of seawater through a decrease in the concentration of carbonate ions CO_3^{2-} and a decline in seawater pH **?**. Mean surface ocean pH values have declined by 0.1 units since the pre-industrial era, with a further projected diminution of 0.1 - 0.4 units by the end of the century **??**, posing a unique threat to calcifying marine organisms. Consequently, the impacts of OW and OA will not be consistent across geographic regions, leading to differential effects that will modify already variable spatial and temporal environments. Building a mechanistic understanding of how the combined impacts of ocean

warming and acidification affect marine organisms is integral for reliable projections of how climate change may continue to affect marine organisms.

Coastal marine organisms frequently encounter a wide range of temperatures and experience fluctuations in biogeochemistry, resulting from temporal variations, such as tidal and seasonal cycles. The rocky intertidal system is one such system that is known for its variable conditions on both temporal and spatial scales, making them a model ecosystem for understanding how organisms interact and respond to change ????. Organisms within the rocky intertidal zone must contend with alternating periods of immersion and emersion of tidal fluctuations, which commonly lead to large variations in temperature, oxygen availability, and pH, ?. Of these naturally occurring changes, thermal variability within the intertidal zone is believed to be a dominant driver in structuring the vertical and latitudinal distribution patterns by limiting upper zonation through abiotic stress and lower zonation through biotic influence ???. Daily temperature fluctuations are drastic enough to elevate the body temperatures of marine organisms by more than 20°C during a tidal emersion event ??. Furthermore, changes in pH within tidepools may exceed 1 unit when nighttime respiration rates exceed photosynthetic rates ??. Such highly variable abiotic changes are naturally occurring and create complex gradients that shape ecological communities and influence physiological processes within individual organisms ?. Given that organisms within the intertidal zone simultaneously face drastic fluctuations from abiotic drivers, and experience conditions far beyond what is expected in the future, understanding organismal performance in these ecosystems may provide a window for looking toward the future.

The role of biotic and abiotic drivers in influencing metabolic processes has been of primary interest to the field of ecology as changes in metabolism directly affect the survival, behavior, and energy requirements of organisms, thereby impacting organism and ecosystem function ????. Physiological processes are heavily influenced by environmental factors, and many marine organisms undergo biological responses to natural diel variability present

within environments \cite{hofmann2010living. Temperature is the primary environmental driver regulating physiological rates of ectothermic organisms, as kinetic energy of biochemical reactions are temperature dependent **???**. Further, temperature is the key determinant in the regulating rates of biological processes, ranging from metabolic rates **?** to species-interactions **?**, such as growth, feeding, reproduction, and determining the range of species distributions \cite{kordas2011community, sanford2002feeding, pinsky2013marine. The physiological processes of metabolism are the total sum of biological and chemical processes in converting energetic resources and materials into biomass and activity **?**. Changes in pH also play a vital role in metabolic processes due to its effect on biochemical pathways and internal acid-base balance **?**. Specifically, declines in seawater carbonate ions and pH attributed to OA are strongly correlated to decreases in calcification and growth rates of many marine organisms **?**. Due to species-specific differences in the energetic costs associated with calcification, there are significant differences in the ability to control internal acid-base regulation between species **?**. Ultimately, changes in the environment that lead to alterations in organismal energetic requirements will scale up to affect the processes of ecological communities; therefore, studying how multiple abiotic factors affect organismal physiology has ecosystem-level implications **???**.

The use of performance curves can help to quantify the relationship between abiotic drivers and physiological rates to forecast future effects **?** and can allow for comparative assessments across different biological rates and environmental conditions (Figure 1.) **????**. Further, thermal performance curves have been suggested to fill in the gap of uncertainty between multiple stressors as they empirically characterize the relationship between biological performance rates across a wide range of temperatures **???**. Thermal performance curves are a univariate function that describes how some measure of performance (e.g., metabolic rate) varies with temperature. As temperature increases so do the biochemical and physiological rates until they reach a species-specific optimal temperature. Beyond the optimum rate, further increases in temperature denature proteins, stunt growth, and cause

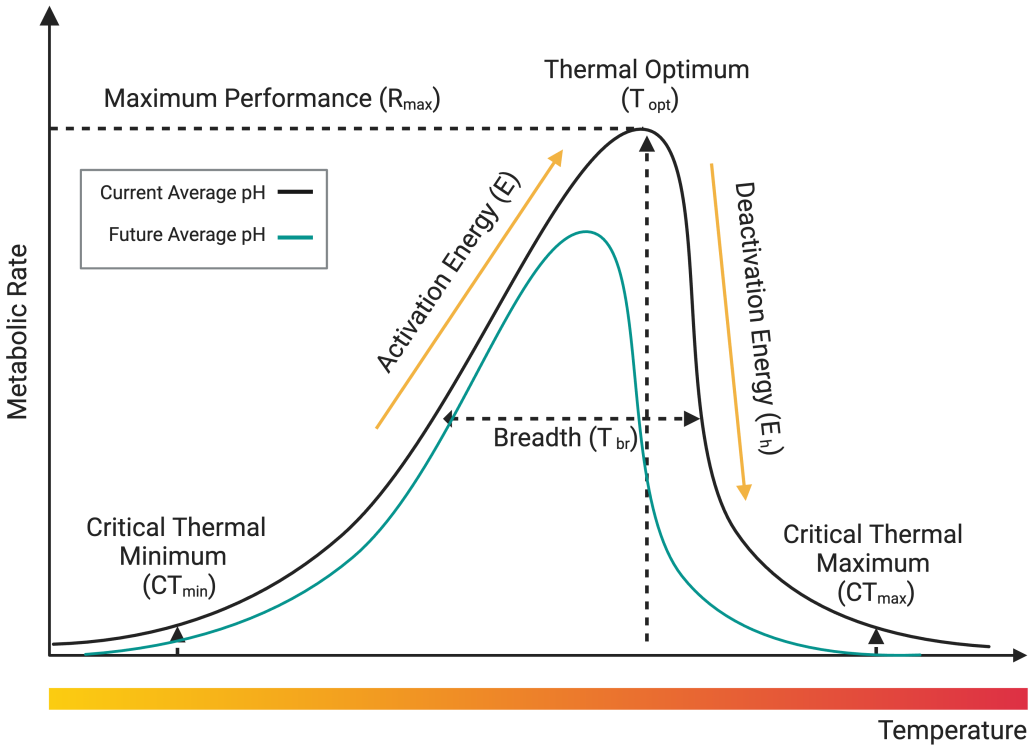

Figure 1: Thermal performance curve schematic illustrating the relationship between biological rates and temperature, including critical thermal maximum (CTMax), critical thermal minimum (CTMin), thermal optimum (Topt), activation energy (E), deactivation energy (Eh), and the thermal breadth of the curve (TBr). Hypothesized characteristics of a thermal performance curve exposed to ocean acidification, including reduced thermal optimum and reduced performance ay maximum physiological rate and breathe of the curve.

reductions in performance and survival **??**. Thermal performance curves are typically left-skewed and hump-shaped and include several metrics, including but not limited to a thermal optimum (TOpt)—the temperature at the highest rate of performance—and a critical thermal minimum (CTMin) and a thermal maximum (CTMax)— the upper and lower thermal limit that an organism can tolerate **???**. These tolerance thresholds and the range they encompass are governed by an organism's ability to respond to sub-lethal and lethal conditions through an organismal-level response and molecular-level responses such as anaerobic metabolism and heat shock response **??**. Further, exposure to concurrent drivers of ecological change, like OA, are expected to constrict an organism's performance curve and thermal limits, such as decreasing the breadth of thermal performance **??**. Comparing physiological responses to gradients of abiotic drivers may allow us to quantify and compare the tolerance limits of organisms **?**.

Specifically, we ask the question: how does exposure to decreased pH influence thermal performance curves of respiration of an intertidal gastropod, Tegula funebralis? We anticipate that the thermal optimum (TOpt) for respiration rates will shift towards lower temperatures, indicating a reduced ability to sustain optimal metabolic activity in the face of ocean acidification. Additionally, we expect a decrease in the thermal breadth of the curve (TBr), indicating a narrower range of temperatures at which the gastropod can effectively maintain its respiratory rates. In this study we... (give an overview of your experimental design to set up expectations on how you plan to answer these questions)

4 Methods

4.1 (1) Mesocosm Design

The Silbiger Lab mesocosm system at California State University, Northridge was used to emulate experimental conditions of a semi-diurnal tidal fluctuation across a gradient of temperatures and blocked exposure to either low or high pH. The facility operated as a closed-loop system, wherein water from individual tanks was continuously recirculated back into a central holding reservoir (sump). Unbuffered natural seawater was collected from the Southern California Marine Institute (SCMI) in San Pedro, CA and filtered through three mesh filters (20 μm, 5 μm, 1 μm) prior to being introduced into the sump of the mesocosm system. Within the system, recirculating seawater underwent further filtration through three 50 μm carbon bag filters, eight mesh filters, a UV sterilizer (Comet Series 95 Watt Lamp), and a chiller (Aqua Logic Delta Star, DS-4) which maintained water quality and chilled seawater to ambient conditions. Weekly water replacements, accounting for approximately 50% of the total volume, were conducted to prevent the accumulation of metabolic waste and to maintain stable carbonate parameters within the system. The mesocosm system was equipped with 16 experimental tanks (53.9 cm (L) $\times$ 31.75 cm (W) $\times$ 34.29cm (H)) with individual controls for temperature, light intensity, and water flow. Each tank was outfitted with a submersible powerhead pump (Hydor Nano Koralina 240 powerhead, 240 GPH), 200 W Heater (Hydor aquarium heater), temperature probe (Neptune Systems, $\pm$0.1 degree C), pH probe (Neptune Systems, Lab Grade Double Junction, measures pH from 4.0 to 12.0 $\pm$0.1), three flow sensors (Apex, FS25 ¼" fitting, flow rates from 3-12 GPH (12-24 LP)), and a temperature logger (HOBO TidBit MX2203, $\pm$0.2 degree C). LED lights (Halo Basic M-110) in each tank followed a 12:12 day/night cycle, which mimicked the local light conditions using a sunset and sunrise table.

Each individual tank was programmed to experience tidal fluctuations as well as

temperature/pH controlled seawater conditions for their respective treatments. Programmable solenoid valves (Apex Neptune) were utilized to adjust the seawater flow rates to each tank, ensuring that either inflow rates exceeded outflow drain rates simulating a high tide condition or outflow drain rates exceeded inflow rates to simulate a low tide condition. This emulation aimed to replicate the semi-diurnal tidal characteristic of the Pacific Coast. Within a 24-hour period, two high tide and two low tide fluctuations, each lasting six hours, were generated by either opening or closing the solenoids. Flow rates were meticulously maintained on a daily basis using a graduated cylinder and timepiece to ensure a programmable inflow of 10 L/h, constant total inflow of 10 L/hour, and a constant outflow drain rate of 15 L/hour, thereby creating the desired tidal effect. Precise control over temperature in each tank was achieved by employing a programmable thermostat (Neptune Apex), which automatically activated or deactivated heaters in response to temperature deviations from the set range. Individual tank pH levels were regulated using a pH-stat set-up through the direct bubbling and mixing of CO2 facilitated by a pH logger and solenoid valves (Neptune system) attached to a CO2 tank (PhosBan Reactor 150). Additionally, in each tank, a venturi connected to an aquarium pump facilitated the mixing of ambient air to stabilize the pH levels in the treatment tanks. After recirculation into the sump system, the sweater was chilled to ambient condition and scrubbed of CO2 using a phosban reactor (Phosban 150 Reactor).

Throughout the experiment, various water quality parameters were regularly measured to monitor environmental conditions within the tanks. pH, dissolved oxygen (DO), and temperature were assessed daily at consistent times to ensure accurate readings and facilitate the calibration of in-tank temperature probes for precise measurements. pH and dissolved oxygen levels were measured daily, within each tank using a Termo Specific ORION ISE instrument with a resolution of 0.1 mV and an accuracy of ± 0.2 mV or $\pm 0.05\%$. Simultaneously, temperature readings were obtained using a Thermo Fisher Trace digital thermometer. The temperature data also aided in calibrating the thermostat sensors within

each tank, which were adjusted once a day to maintain accurate temperature control. pH on the total scale was calculated from mV and temperature by using a multipoint calibration to a tris standard solution from the Dickson Lab at Scripps Institution of Oceanography following Dickson SOP 6a **?**. Accuracy of the pH was tested against a Tris buffer of known pH from the Dickson Lab at Scripps Institution of Oceanography **?**. The pH values for the individual aquaria were calculated using the seacarb package in R, accounting for temperature corrections specific to each tank **?**. I also measured total alkalinity (TA) from water samples collected once every few days (3-4 days) from each experimental tank and sump. All total alkalinity (TA) water samples were collected and stored in 125 ml Nalgene containers. Prior to use, these containers underwent thorough cleaning in a 10% HCl bath for 24 hours, followed by rinsing with deionized (DI) water. Additionally, during sample collection, the containers were rinsed three times with sample water to ensure a representative water quality sample. Collected samples were analyzed within 24 hours of collection using a T-5 automatic titrator (Mettler Toledo) following the best practices for ocean CO2 measurements **?**. To verify accuracy, a certified reference material (Reference Material for Oceanic CO2 Measurements, A. Dickson, Scripps Institution of Oceanography) was run prior to each total alkalinity measurement with an error no greater than 1.0% off from the certified value **?**.

4.1.1 INSERT TABLE. Summary statistics (means ± SE) for tank parameters at each of the three timepoints.

4.2 (b) Species Collection and Maintenance

4.2.1 INSERT FIGURE 2 MAP

For this experiment, black turban snails (*Tegula funebralis*) (N=80 individuals) were collected haphazardly from tidepools in Point Fermin, San Pedro, CA (Figure 2.) on August 16, 2022 (SCP ID: S-220520002-22054-001). All collections were made and transported

during low tide to minimize any physiological variation that might be related to endogenous tidal rhythms. Individuals of *T. funebralis* were measured for shell width (dorsal to ventral) between 18-22 mm using Vernier calipers, since shell height is a reliable predictor for body mass. Organisms were then transported back to California State University, Northridge in a wet insulated container where they were measured for blotted wet mass (g), volume displacement (mL), shell height (mm), and shell width (mm) and tagged using a previously weighed FloyTag placed at the apex of the dorsal side of the shell with coraffix glue. The snails were then randomized and assigned to an experimental treatment as detailed below. Each snail was randomly assigned to one of 16 experimental aquaria across a range of 8 temperatures from 12 to 26 °C and two pH treatments, and placed into their respective experimental tanks (n=4 per treatment). To adjust the snails to their treatment temperatures, all snails started in ambient temperature conditions (16 degree C), and temperatures were then increased or decreased at a rate of up to 2 ^{o} degree C per day until reaching the set treatment temperature. The changes in pH for the acidification treatments were simultaneously reduced with temperature changes at a rate of up to ~0.5 units per day during this period as this is the fluctuation of pH that organisms in the intertidal experience in a single day **?**. Organisms were adjusted to experimental conditions for a week before the experiment began. Throughout the experiment, snails were fed giant kelp wrack *Macrosystis pyrifera*, a highly preferred food, was collected from Point Fermin, CA to feed organisms and placed on 3 inch PVC disks every three days throughout the experiment. *M. pyrifera* was rinsed with fresh water to remove epiphytes prior to feeding.

4.3 (c) Temperature and pH Treatment

4.3.1 INSERT FIGURE 2 SST AND PH FROM NEARBY SHORE STATION SCOSS AND CITE

Sea snails were subjected to one of eight temperatures ranging from 12-26 degree C (12, 14, 16, 18, 20, 22, 24, 26; n=8) and either low or high pH conditions (7.7 or 8.0; n=2),

resulting in 16 experimental treatments. based on average facility tank temperatures, or a realistic marine heatwave occurring on top of ambient conditions. Nine tanks (three tanks per size class) underwent a marine heatwave manipulation, while the remaining nine tanks were maintained at ambient controls. Temperature conditions were chosen based on sea surface temperature ranges and variability at a nearshore shore station. pH was chosen due to the expected decreases of pH expected under future conditions.

4.4 (d) Survivorship

Snail survivorship was monitored daily during the experiment. Snails that exhibited signs of distress, such as being unable to adhere to tank surfaces, being found at the bottom of the tank, or showing no movement for a period of 24 hours, underwent sensory tests to assess potential mortality. Specifically, snails were gently held w and touched along their foot with forceps. If there was no response within thirty seconds, they were considered deceased and subsequently removed from the tank. Additionally, olfactory cues were also considered as indicators of potential mortality.

4.5 (e) Metabolic Experiment

4.6 Figure 2.4. Wet weight (g) to organic biomass (ash free dry weight; AFDW (g)) curve from preliminary trial used to calculate dry weight of the 90 experimental abalone ($y = 0.093x + 0.049$, $R^2 = 0.98$).

Respiration rates were measured after a 7-day adjustment period and a 10-day exposure to the treatment conditions. Prior to conducting respirometry, each individual snail shell was thoroughly scrubbed using an acrylic brush to remove any epibiont communities that could potentially obscure respiration rates. Snail respiration rates were assessed by measuring

oxygen evolution within sealed, water-tight respirometry chambers (650 mL) for each individual. A mesh wire separated the top and bottom sections of the chamber, with a magnetic stir bar (200 rpms) placed in the bottom section to ensure proper mixing of water and prevent oxygen stratification. During the respirometry trials, temperature was carefully controlled and stabilized using an insulated container and a programmable thermostat system (Apex Controller, Neptune Systems ± 0.1 degree C). Temperature adjustments were made using a submersible water heater (Finnex 300W Titanium Heater) and a water chiller (Aqua Logic Delta Star, DS-4). Oxygen measurements were taken at a frequency of 1 Hz using an oxygen probe (Presens fiber optic oxygen dipping probe, DP-PSt8 ± 0.1 degree C) and continuously monitored throughout the 45-minute respirometry trial using Presens Software. To ensure experimental consistency, four organisms from each pH treatment (n= 8 snails) and one blank control from each pH treatment (n = 2 blanks) were run together at the same treatment temperature. This resulted in a total of 10 individual chambers placed in the respirometry stand and measured simultaneously. Each chamber was fully submerged within the water bath to maintain a controlled temperature inside. Since each respiration chamber functioned as a sealed system, the oxygen consumption rate of each individual organism ($(\mu mol\, O_2\, g^{-1} h^{-1})$) was calculated and normalized to ash free dry weight. To obtain final wet mass, snails were blotted with a paper towel to remove excess water, scrubbed with a toothbrush to remove epibiont communities, and then weighed using an electronic balance to the nearest 0.0001 g. Organic biomass and ash free dry weight was obtained after the experiment, during which snails were placed in a drying oven (Fisher Scientific Isotemp Drying Oven) at 60°C for 72 hours and then in a muffle furnace (Fisher Scientific Isotemp Muffle Furnace) set to 450°C for 5 hours.

4.7 (f) Statistical analyses

To analyze the thermal performance curves of respiration rates and determine the shape, we used the Sharpe Schoolfield high activation energy model. statistical analysis was

conducted in R software. The Schoolfield model is widely used to describe the thermal performance curves of biological rates of ectotherms. the Schoolfield model was implemented in R to fit the data and estimate the parameters of the model. The fitting process involved using the nmls and rTPC packages in R to optimize the model parameters and estimate their uncertainty **?**. The data were fitted to the Sharpe Schoolfield model (high) using AIC values between relevant performance models for ectotherm species to evaluate the model's performance and the quality of the fit. Furthermore, model selection techniques, such as comparing different models based on their statistical criteria (e.g., AICc), were employed to identify the most suitable model (e.g., gaussian, sharpe-schoolfield low, sharpe-schoolfield full, sharpe-schoolfield high, weibull) that accurately described the observed thermal performance curve. Furthermore, I conducted bootstrap resampling to estimate confidence intervals for the model predictions.

5 Results

Some more guidlines from the School of Geosciences.

This section should summarise the findings of the research referring to all figures, tables and statistical results (some of which may be placed in appendices). - include the primary results, ordered logically - it is often useful to follow the same order as presented in the methods. - alternatively, you may find that ordering the results from the most important to the least important works better for your project. - data should only be presented in the main text once, either in tables or figures; if presented in figures, data can be tabulated in appendices and referred to at the appropriate point in the main text.

Often, it is recommended that you write the results section first, so that you can write the methods that are appropriate to describe the results presented. Then you can write the discussion next, then the introduction which includes the relevant literature for the scientific story that you are telling and finally the conclusions and abstract – this approach is called writing backwards.

6 Discussion

the purpose of the discussion is to summarise your major findings and place them in the context of the current state of knowledge in the literature. When you discuss your own work and that of others, back up your statements with evidence and citations. - The first part of the discussion should contain a summary of your major findings (usually 2 – 4 points) and a brief summary of the implications of your findings. Ideally, it should make reference to whether you found support for your hypotheses or answered your questions that were placed at the end of the introduction. - The following paragraphs will then usually describe each of these findings in greater detail, making reference to previous studies. - Often the discussion will include one or a few paragraphs describing the limitations of your study and the potential for future research. - Subheadings within the discussion can be useful for orienting the reader to the major themes that are addressed.

7 Conclusion

The conclusion section should specify the key findings of your study, explain their wider significance in the context of the research field and explain how you have filled the knowledge gap that you have identified in the introduction. This is your chance to present to your reader the major take-home messages of your dissertation research. It should be similar in content to the last sentence of your summary abstract. It should not be a repetition of the first paragraph of the discussion. They can be distinguished in their connection to broader issues. The first paragraph of the discussion will tend to focus on the direct scientific implications of your work (i.e. basic science, fundamental knowledge) while the conclusion will tend to focus more on the implications of the results for society, conservation, etc.

8 Appendix(ces)

A last section may contain supporting data for the text in the form of one or more appendices. Appendices should be placed after the bibliography. The appendices must fall within the margin requirements and may be single-spaced if necessary. The ETD website gives students the option to upload "Supporting Files" in addition to the thesis/dissertation. Supplemental files can include large appendix type material, videos, images, audio files, PowerPoint presentations, and any other file type, which will not be embedded into the main thesis document.

8.1 Appendix A: additional tables

Insert content for additional tables here.

8.2 Appendix B: additional figures

Insert content for additional figures here.

8.3 Appendix C: code

Insert code (if any) used during your dissertation work here.