Masters Thesis: Coded in R

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      First and foremost, I would like to express my gratitude to our ocean for the generosity it bestows upon us daily and for the awe it instilled in me as a young child—a wonder that has grown into a lifelong dream and pursuit. I am deeply grateful to Dr. Nyssa J. Silbiger, my advisor, for her unwavering support, encouragement, inspirational attitude, and collaboration on this project. Thank you for believing in me, urging me to become the person I need to be, equipping me with the knowledge, tools, and skills needed to achieve this, and for ignoring the lies we often tell ourselves about ourselves. To my thesis committee, Dr. Peter J. Edmunds and Dr. Kerry J. Nickols, thank you for your invaluable guidance and advice throughout this process. Thank you to my mentors, Dr. Aradhna Tripati, Dr. Rachel Bay, Dr. Tessa Hill, and Dr. Melanie Okoro, who have acted as academic mothers, paving the way and uplifting me throughout my academic trajectory. I would also like to extend my gratitude to the Silbiger and Nichols Lab team, including D.M. Barnas, J.R. Kerlin, C. Fajardo, and H. Merges. In particular, I want to give a special thank you to J.B. Fields and T. Smith for their monumental support and dedication in ensuring that everything ran smoothly inside and outside of the lab; they were always there as friends to call on and played pivotal roles in ensuring the success of my degree. Without all of you, none of this would have been possible. Throughout endless sleepless nights and days of struggle, thank you for lifting as you climbed. A special thank you to Ana and the rest of the custodial staff, whose skills are the cornerstone of the entire institution, and whose presence makes the halls of academia feel more like home as they sweep through the halls, serenaded by the rhythm of bachata. This research couldn’t have been possible without the financial support from the National Science Foundation Graduate Research Fellowship Program, UCLA Center for Diverse Leadership in Science Early Career Fellowship, UC Davis Sustainable Oceans Scholars Program, and the National Science Foundation CAREER grant OCE-2044837 awarded to Dr. Nyssa Silbiger, the material in this study based upon work supported by the U.S. National Science Foundation, the CSUN Department of Biology, and research activities that were conducted under scientific collecting permits issued by the California Department of Fish and Wildlife (ID: S-220520002-22054-001).

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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.

Ocean acidification (OA) is occurring across a backdrop of concurrent environmental changes that will reverberate to affect energy flow throughout ecological communities. Ocean acidification and warming will profoundly affect marine organisms, the ecosystems that organisms are embedded within, and the services and functions ecosystems provide to humanity. However, a mechanistic understanding of the interactive effects of ocean acidification and warming on organismal physiology remains a critical gap of uncertainty in a field that has prioritized a reductionist approach to studying future oceanic scenarios as isolated phenomena. The highly variable coastal environment of the rocky intertidal, and the organisms within the ecosystem, provide a model system to understand the physiological mechanisms for how organisms respond to environmental change. To address these limitations, we used thermal performance curves to empirically characterize the relationship between biological rates of respiration and grazing of , an intertidal herbivore ubiquitous to the highly variable rocky intertidal ecosystems of California. This study measured thermal performance across a range of eight temperatures crossed with a blocked exposure to either current average () or future () average oceanic pH predictions. Measurements for respiration rates were taken after a 10-day exposure thereby enabling us to quantify energetic expenditure. Our results show no statistical difference between thermal performance metrics (, , , ), yet there were differences in energy expenditure between the high and low pH treatments as temperature increased. Our results empirically characterize how measurements of performance will respond to ocean warming and acidification and exemplify how organismal-level interactions may scale up to affect different ecosystem functions.

# 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 .

## Organisms as the Subjects of Environmental 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 .

      Organisms are adapted to cope with a natural range of abiotic conditions, yet anthropogenic climate change may outpace organismal physiological capacities and may also act interactively to result in “ecological surprises’’ (sense, Paine et al., 1969). For example, the cumulative impact of two drivers acting together may interact to be equivalent to their sum, known as an additive effect, less than their additive effect, which is known as an antagonistic interaction, or greater than their additive effect, known as a synergistic interaction (Côté et al., 2016). For decades anti-racist and feminist scholars have provided critical insight into the ways in we must think about isolated phenomena as intersectional due to their complex interactions (Davis, 1983; Crenshaw, 1989). The field of marine biology requires this paradigm shift that embraces the interactions between stressors to grasp the complexity of the future. Combined, these drastic changes in abiotic drivers will continue to act in conjecture with one another and could potentially ameliorate or exacerbate impacts on organismal physiological processes and reverberate the effects of ecological change through ecosystems (Kroeker, Kordas, & Harley, 2017).

## Organism Physiology in Response to Multiple Drivers of Environmental Change

      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 environment, 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 2.89C [2.01–4.07C] 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 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 20C 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. Intertidal organisms are well known for the ability to utilize anaerobic metabolic responses (e.g., metabolic depression) to minimize metabolic costs of dealing with thermal stress, making them an ideal model system to understand the impacts of thermal stress on physiology (Pörtner et al., 2017).

      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 . 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 . 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 .

## Thermal Performance Curves as a Tool to Understand Multiple Drivers of Change on Organismal Physiology

      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 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 .

      Despite the increasing emphasis on multi-driver and multi-species studies, a mechanistic understanding of the nonlinear responses of multiple confounding stressors of future scenarios remains a knowledge gap. (Kroeker, Kordas, & Harley, 2017). The elevated sense of urgency involved with these global threats contributes to the need for a more nuanced understanding of the impact of multiple stressor interactions on organismal and ecosystem processes (Côté et al., 2016). The response of organisms to climate change, survival, and fitness depends on physiological trade-offs, which are essentially changed within the allocation of an organism’s energy for different biological functions and demands for resources. Considering that metabolic processes respond differently to multiple environmental drivers and that physiological systems within and between organisms differ, it is imperative to tease apart the metabolic rates. Organisms may respond to changing abiotic drivers by altering their energetic allocation or energetic intake, such as altering consumption rates or growth (O’Connor 2009). For example, the metabolic theory of ecology predicts an increase in consumption rates with increasing temperature (O’Connor 2009). These alterations within an individual species’ physiological performance are significant because they can scale up to affect ecosystem function (Post et al., 1999). Building a mechanistic understanding regarding 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.

      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.

# Methods

## (a) 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 m, 5 m, 1 m) prior to being introduced into the sump of the mesocosm system. Within the system, recirculating seawater underwent further filtration through three 50 m 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) x 31.75 cm (W) x 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, 0.1C), pH probe (Neptune Systems, Lab Grade Double Junction, measures pH from 4.0 to 12.0 0.1), three flow sensors (Apex, FS25 ” fitting, flow rates from 3-12 GPH (12-24 LP)), and a temperature logger (HOBO TidBit MX2203, 0.2C). 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 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 .

## (b) Species Collection and Maintenance

      For this experiment, black turban snails () (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 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 12-26C 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 (16C), and temperatures were then increased or decreased at a rate of up to 2 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 , 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. was rinsed with fresh water to remove epiphytes prior to feeding.

## (c) Temperature and pH Treatment

      Sea snails were subjected to one of eight temperatures ranging from 12-26C (12C, 14C, 16C, 18C, 20C, 22C, 24C, 26C; 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.

## (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.

## (e) Metabolic Experiment

      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 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 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 () 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 60C for 72 hours and then in a muffle furnace (Fisher Scientific Isotemp Muffle Furnace) set to 450C for 5 hours.

## (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.

# Results

## Statistical Analysis

The AFDW was determined using the following equation:

      Cellular respiration involves the consumption of oxygen (O2) through a process of organismal respiration to produce energy in the form of adenosine triphosphate (ATP) according to the equation:

      To calculate respiration, we first multiplied the moles of oxygen consumed per hour by the molar mass of oxygen, which is approximately , to convert from moles of oxygen to grams of oxygen. Then we used an oxy-joulometric conversion factor derived from , which is , to convert from grams of oxygen consumed per hour to joules of energy expended during cellular respiration.

The calculation can be expressed as follows:

To convert moles of oxygen to grams, we used the molar mass of oxygen:

Substituting the values, we get:

where is the molar mass of oxygen and is the conversion factor derived from and expressed as before conversion.

      After converting the oxygen consumption rates to energy expenditure in joules per hour per snail and performing statistical analysis, we conducted a two-way ANOVA to investigate the effects of temperature treatment and pH treatment on energy expenditure. The ANOVA revealed a significant effect of temperature treatment (), indicating that different temperature treatments had a significant impact on energy expenditure. Although the pH treatment showed some effect on energy expenditure, it was marginally significant (F(1, 44) = 3.9760, p = 0.05237), suggesting a potential influence of pH on energy expenditure. The interaction between temperature and pH treatment was not significant (F(7, 44) = 1.5286, p = 0.18280), indicating that the combined effect of temperature and pH treatment did not significantly impact energy expenditure beyond the individual effects of each factor. Subsequent Tukey multiple comparisons showed significant differences between most temperature treatment pairs. For example, the energy expenditure at 14°C was significantly higher than at 12°C (mean difference = 13.31 J/h, p < 0.001), and the energy expenditure at 22°C was significantly higher than at 20°C (mean difference = 60.21 J/h, p < 0.001). Tukey multiple comparison indicated a significant difference between low and ambient pH conditions, with a higher energy expenditure observed under low pH (mean difference = 5.95 J/h, p < 0.001).

      All statistical analyses were run in the R environment . The and packages were used to fit a series of models to the respiration rate data and build thermal performance curves. The analysis utilized the package in R, which enables a pipeline to fit data to multiple models simultaneously. Thermal performance curves (TPC) were analyzed to investigate the relationship between temperature and performance measure (e.g., metabolic rate) of . The statistical processing of the thermal performance curves involved analyzing the data collected from the respiration chambers and examining statistical graphs of oxygen evolution over time. Regression analysis was employed to explore the relationship between temperature and oxygen evolution and to estimate the parameters of the thermal performance curve (e.g., slope). Specifically, we have utilized techniques such as nonlinear regression to fit thermal performance models to the data and assess the goodness of fit.

      During the regression analysis, it was crucial to examine the distribution of model parameters this involved assessing the normality of the distribution of values and investigating potential outliers or unusual patterns.Additionally, we inspected the distribution of residuals, which are the differences between observed and predicted values, to ensure they were normally distributed and exhibited homoscedasticity. Additionally, diagnostic plots, specifically quantile-quantile (Q-Q) plots, were employed to assess the normality of residuals and identify any potential outliers or systematic patterns embedded within the data. We examined the relationship between predictor variables (e.g., temperature) and fitted values to ensure the model adequately captured the underlying trends in the data. Moreover, we inspected the standard residuals, to identify observations with unusually large or small residuals. Any outlying observations that indicated data errors, measurement inaccuracies, or other anomalies that necessitated further investigation were subsequently excluded from the analysis.

      The thermal performance curve (TPC) model was fitted to the data using nonlinear regression techniques in R, specifically the function. Model fit was assessed with the coefficient of determination (), indicating a strong fit to the observed data. The model selection process involved fitting several models for ectotherm physiology (e.g., Gaussian, Sharpe-Schoolfield) using Akaike Information Criterion corrected for small sample sizes (AICc). The Sharpe-Schoolfield model was chosen as the preferred model for this study due to its goodness of fit, and its physiological relevance to ectotherms based on enzyme kinetics. This model is widely used in ecological and physiological studies to understand how organisms respond to changing environmental temperatures, particularly in the context of climate change and thermal stress.Additionally, bootstrap resampling methods were employed to assess parameter uncertainty and validate the uncertainty of the data following .

The Sharpe-Schoolfield high model describes the temperature dependence of biological rates, such as metabolic rates, enzyme activities, or reaction rates, in response to high temperatures. It incorporates parameters such as activation energy, high temperature deactivation energy, and optimum temperature to characterize the thermal performance curve . The results of the thermal performance curve analysis provide insights into the organism’s temperature-dependent performance and its thermal ecology. The optimum temperature (Topt) indicates the temperature range in which the organism performs optimally, while the thermal limits (CTmin and CTmax) define the species’ thermal tolerance. The thermal sensitivity or breadth parameter (b) reflects the organism’s ability to acclimate or adapt to temperature changes and its vulnerability to thermal stress.

# Discussion

Better understanding the impacts of global climate change on marine ecosystems requires us to think outside the confines of direct impacts (Kroeker et al., 2012) since individual alterations in organismal physiology have much larger implications for communities (Kroeker et al., 2012; Gaylord et al., 2015).These results presented in this thesis highlight the importance of considering the physiological and geochemical interactions between temperature and carbonate chemistry when interpreting species’ vulnerability to OA, as multiple drivers of ecological change may interact in myriad ways. This study also illustrates the use of thermal performance curves as a mechanistic way to tease apart multiple drivers of ecological change to make more accurate predictions of species’ vulnerability to future climate change.Understanding changes in metabolic rates to different magnitudes and combinations of abiotic drivers may elucidate complex changes in physiological mechanisms that are unable to be uncovered using a few treatments (Becker and Silbiger, 2020; Edmunds et al., 2011).

urther, assessing the interactive effects warming and pH using a performance curve approach may help us better understand how performance curves change shape with the inclusion of other abiotic factors, which have been explored through other mechanistic approaches such as the Oxygen and Capacity-Limited Thermal Tolerance concept (Pörtner, et al. 2017). Therefore, performance curves should be utilized through a comparative lens to better understand the role that pH has on thermal performance to create novel approaches to quantify and categorize performance under a range of pH conditions.It is expected that ocean acidification will impose additional energetic costs through physiological stress and therefore narrow the breadth and optimum of the curve (Pörtner 2008; Gaylord et al., 2015). The ability for organisms to respond to anthropogenic-induced acidification depends on species level acid- base regulation and will result in increased energy demand and respiration rates (Pörtner, 2008). Measuring organism responses to a range of pH in the presence or absence of thermal stress may also provide information regarding how temperature may impact performance under differing pH environments.

One possible explanation for this phenomenon is that the increased metabolic rate exhibited by serves as a compensatory response to the adverse conditions induced by ocean acidification. In the case of , it is plausible that the species is augmenting its metabolic rate as a means of upholding vital functions and maintaining homeostasis amidst acidic conditions. Another mechanism could be the upregulation of metabolic pathways that generate ATP, the energy currency of cells, to meet the increased energy demands at higher temperatures. This is consistent with the findings of other studies that have shown elevated metabolic rates in gastropods or decreases in body size associated with increased temperture Ccitation). Turban snails found at lower latitudes of the coastline grow smaller at a faster rate as well as live longer than those found at higher latitudes (Frank, 1975); falling in line with the metabolic theory of ecology and the expected decrease in body size under higher temperatures (Brown et al., 2004). Research by Elahi (2020) illustrates decreasing gastropod shell size over the past few decades and expectations for changes in the future. It is therefore imperative to understand the direct and indirect implications of shrinking shell sizes on organisms.

The present study found that the respiration rate of is highly dependent on temperature, with the highest respiration rate occurring between 20-22°C, consistent with other thermal performance studies of the species (somnero). The present study also found that the effects of OA on respiration rate are highly dependent on temperature. Although high CO increased respiration rate at 20°C, this effect gradually lessened with successive warming to 20°C, illustrating how moderate warming might be able to mediate the effects of OA through temperature’s effects on both physiology and seawater geochemistry. Furthermore, other studies on the energy budget and transfer of indicate that rates of aquatic and aerial respiration differ, with low intertidal individuals respiring at a rate of 75 µl O/hr in water and 43 µl/hr in air cite(paine1969). The large- scale spatial gradient and relatively heterogeneous environment of the Pacific coast, as well as the wide biogeographic distribution of the Tegula genus species specific evolution to particular zonations make them the Darwins finches of thermal ecology. It has been found that has a wider thermal tolerance range compared to its congeners, and , which inhabit lower intertidal and subtidal zones . However, this is precisely what might make more vulnerable to future changes of a few degrees as it already withstands drastic temperature fluctuations within the intertidal.

This study lso discovered that there will be changes in the energy budget of as a result of ocean acidification and warming, which could have consequences on other specie in the intertidal. Slight changes within the energy budget of this ubiquitous species could have downstream consequences in terms of energy transfer, as the rate of annual consumption by is estimated to be 1,071 kcal m yr while the net primary production is estimated to be about 1,167 kcal m yr, with about 60% of energy transferred, illustrating the important role play in both balancing intertidal ecosystems but also in transferring energy throughout the ecosystem cite(paine19710). The macroalgal-herbivore interaction,is of prime importance as it is the primary pathway for energy to move through trophic levels and is predicted to change under projected ocean warming and acidification (O’Connor et al., 2009). Alterations within metabolic rates of performance such as respiration, calcification, and growth attributed to climate change will ultimately affect net ecosystem production, net ecosystem calcification, and food web dynamics.

# Conclusion

# 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.

## Appendix A: additional tables

Insert content for additional tables here.

## Appendix B: additional figures

Insert content for additional figures here.

## Appendix C: code

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

#Converting a .csv bibliography (google scholar) to a .bib bibliography   
  
#path <- here("Bibliography", "bibliography.csv")  
  
#bib <- revtools::read\_bibliography(path, return\_df = FALSE)  
  
#revtools::write\_bibliography(bib,  
#filename = "bibliography.bib",  
#format = "bib")