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PREDICTING ECOLOGICAL CHANGE IN MULTIVARIATE ENVIRONMENTS

A dissertation submitted in partial satisfaction
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

ECOLOGY AND EVOLUTIONARY BIOLOGY

by

Emily M. Donham

June 2022

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Abstract

IMPACTS OF ENVIRONMENTAL CHANGE ON KELP FOREST GRAZERS

by Emily Donham

Natural and anthropogenic environmental changes are impacting marine species worldwide. However, our understanding of how changes to multiple environmental drivers impact the physiology and ecology of organisms is still largely unknown. Kelp forest ecosystems along the coast of California present a unique system to assess how environmental variability (both natural and human-induced) impacts key species of concern. In particular, these biodiverse ecosystems reside within the California Current System, which is characterized by dynamic oceanographic conditions that vary across latitude largely due to differences in the strength and intensity of coastal upwelling. Furthermore, environmental conditions are predicted to change especially rapidly in this region due to accelerated acidification, deoxygenation, and warming. In this study I first use ecologically and economically important grazer taxa to understanding how current and future environmental changes impact the physiology and ecology of marine organisms. Secondly, I conduct a synthesis of multiple driver experiments across ecosystems to assess the generality in interactive effects of warming and ocean acidification. In chapter one, I use *insitu* monitoring of pH, temperature, and dissolved oxygen conditions within a central California kelp forest to further understanding of the relationships between

environmental conditions across seasons. I then use these environmental relationships to undertake a manipulative laboratory mesocosm experiment to assess how upwelling impacts the physiology and ecology of the gastropod, *Promartynia pulligo*, and the echinoderm, *Mesocentrotus franciscancus.* In chapter two, I expand monitoring of pH, temperature, and dissolved oxygen to northern and southern California kelp forests to better understand differences in the coupling of environmental drivers across regions that experience strong versus weak upwelling. I then conduct a laboratory mesocosm experiment to look for signs of local adaptation of red sea urchins, *Mesocentrotus franciscanus,* across regions and compare responses of *M. franciscanus* to region-specific coupled future changes in pH, temperature, and dissolved oxygen. In my final chapter, I zoom out to think more broadly about how multiple environmental drivers interact to alter species responses to warming and ocean acidification. I use a meta-analysis to calculate the frequency of interaction types across seven response variables and eight broad taxonomic groupings. I then assess the relationship between the magnitude of the predicted cumulative effect and the measured cumulative effect to determine when the magnitude of the effect drives the interaction. In this dissertation I show that kelp forest grazers responses to environmental changes vary across species and populations and that broad patterns in how species respond to multiple environmental drivers are likely to be less pronounced than predicted.

Acknowledgements

The land on which I completed my PhD dissertation "is the unceded territory of the Awaswas-speaking Uypi Tribe. The Amah Mutsun Tribal Band, comprised of the descendants of indigenous people taken to missions Santa Cruz and San Juan Bautista during Spanish colonization of the Central Coast, is today working hard to restore traditional stewardship practices on these lands and heal from historical trauma (UC Santa Cruz Land Acknowledgment Statement)."

think differently about my research. I would also like to thank the Kroeker lab

for all their support over the years, from providing feedback, lending a hand in

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adventures (and less last-minute cancellations) in the future!

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Introduction

Marine ecosystems worldwide are threatened by CO_2-driven global changes, such as ocean warming, deoxygenation, and acidification (OA). For the past few decades, researchers have helped improve our understanding of the consequences of global change by conducting laboratory and field experiments assessing the vulnerabilities of species and communities to the threats of global warming, deoxygenation, and OA. Although some general patterns in species responses have emerged, deviations from these patterns are common and likely require both context and more complex experimental designs to gain the mechanistic understanding necessary to link physiological responses to ecological change. ***The aim of my dissertation is to improve our understanding of the consequences of global change on kelp forest ecosystems by utilizing environmental monitoring, laboratory experiments, and meta-analyses to elucidate the eco-physiological underpinnings of kelp forest grazer responses to warming, deoxygenation, and OA.***

To improve our ability to predict the ecological consequences of global environmental change on marine communities, it is necessary that we understand the mechanisms underlying variation in species responses to the combined exposure to warming and OA. Our current predictions of the consequences of global change are limited by the context in which our experiments are conducted, which often manipulate one or two factors at two or

three fixed levels of each factor. In nature, however, environmental conditions

and organisms' responses are often much more complex. For example,

organisms inhabiting kelp forest ecosystems along the coast of California

experience particularly high temporal fluctuations in temperature, dissolved

oxygen (DO) and pH (Frieder et al., 2012; Hirsh et al., 2020; Hofmann et al.,

2011; Koweek et al., 2017). This variability differs in intensity and duration

along the California coast, creating an environmental mosaic of exposure that

could influence how species respond to future environmental changes (Kroeker

et al., 2016).

Given the wide range of environmental conditions organisms naturally

experience, it is necessary to first expand experimental designs beyond factorial

manipulations of current and future conditions expected in temperature and pH

to describe the functional relationships between key environmental change

parameters (e.g., pH, oxygen, temperature) and organismal performance.

Functional relationships help us to understand the shape of relationships (i.e.,

environment-performance) and can provide information about potential

environmental thresholds (i.e., tipping points). While decades of research on

thermal physiology have provided a robust understanding of the functional

relationship between temperature and organismal performance (Kordas et al.,

2011), and oxygen concentration and organismal performance (Somero et al.,

2017), our understanding of the shape of the relationship between organismal

performance and pH is very limited. Moreover, how the relationship between pH and organismal performance changes with temperature and dissolved oxygen concentrations is yet unknown, despite concurrent changes in these drivers associated with global change.

Despite the potential importance of environmental history (e.g. variability, frequency of extreme warming events) in altering species responses to climate change drivers, prior exposure history is rarely incorporated into global change experimental designs and interpretations. Given the dynamic nature of temperature, DO, and pH in the CCS, variability in exposure to these drivers could mediate organisms' responses to future change. Environmental conditions have been shown to be an important driver of local adaptation and/or acclimation in marine ecosystems (Kelly & Hofmann, 2013; Sanford & Kelly, 2011). Therefore, understanding whether species are locally adapted or acclimated to key environmental drivers, as well as how species responses to future conditions are mediated by their environmental history will be critical for understanding variability in responses among populations.

Although there is a great need to improve upon global change experimental designs, meta-analyses can also provide insight into the mechanisms underlying variability in organisms' responses to multiple environmental change drivers. Meta-analytical techniques have been used to identify generalities in species

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responses to OA (Gazeau et al., 2013; Goldenberg et al., 2018; Kroeker et al., 2010, 2013) and warming (Gillooly et al., 2001; Kroeker et al., 2013), but the interactive effects of warming and OA appear more idiosyncratic (Ban et al., 2014; Crain et al., 2008; Darling & Côté, 2008; Przeslawski et al., 2015). It's likely that the complexities in species responses to warming and OA can be explained further by considering conceptual models (e.g., energy limited tolerance). Conceptual models that can help to elucidate generalities in how species respond to multiple environmental change drivers will improve our predictions of future ecological change.

My dissertation work at the University of California at Santa Cruz aims to improve our understanding of the consequences of global change on marine organisms and ecosystems by: 1) Utilizing a coupled multi-factor regression design to understand the responses of kelp forest grazers to upwelling; 2) Measuring intraspecific variation in red sea urchin, *Mesocentrotus franciscanus,* population responses to climate change; 3) Examining the literature to understand the generality in how warming interacts with OA to affect organismal performance.

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CHAPTER 1

COUPLED CHANGES IN PH, TEMPERATURE AND DISSOLVED OXYGEN IMPACT THE PHYSIOLOGY AND ECOLOGY OF HERBIVOROUS KELP FOREST GRAZERS

Emily M. Donham, Lauren T. Strope, Scott L. Hamilton, and Kristy J. Kroeker

CHAPTER 1: COUPLED CHANGES IN PH, TEMPERATURE AND DISSOLVED OXYGEN IMPACT THE PHYSIOLOGY AND ECOLOGY OF HERBIVOROUS KELP FOREST GRAZERS

ABSTRACT

Understanding species' responses to upwelling may be especially important in light of ongoing environmental change. Upwelling frequency and intensity are expected to increase in the future, while ocean acidification and deoxygenation are expected to decrease the pH and dissolved oxygen of upwelled waters. However, the acute effects of a single upwelling event and the integrated effects of multiple upwelling events on marine organisms are poorly understood. Here, we use *in situ* measurements of pH, temperature, and dissolved oxygen to characterize the covariance of environmental conditions within upwelling-dominated kelp forest ecosystems. We then test the effects of acute (0-3 days) and chronic (1-3 month) upwelling on the performance of two species of kelp forest grazers, the echinoderm, *Mesocentrotus franciscanus,* and the gastropod, *Promartynia pulligo*. We exposed organisms to static conditions in a regression design to determine the shape of the relationship between upwelling and performance and provide insights into the potential effects in a variable environment. We found that respiration, grazing, growth, and net calcification decline linearly with increasing upwelling intensity for *M. francicanus* over both acute and chronic timescales. *Promartynia pulligo* exhibited decreased respiration, grazing, and net calcification with increased upwelling intensity after chronic exposure, but we did not detect an effect over acute timescales or

on growth after chronic exposure. Given the highly correlated nature of pH, temperature, and dissolved oxygen in the California Current, our results suggest the relationship between upwelling intensity and growth in the 3-month trial could potentially be used to estimate growth integrated over long-term dynamic oceanographic conditions for *M. franciscanus*. Together, these results indicate current exposure to upwelling may reduce species performance and predicted future increases in upwelling frequency and intensity could affect ecosystem function by modifying the ecological roles of key species.

INTRODUCTION

Temporal environmental variability is a fundamental characteristic of ecosystems worldwide that can impact physiological processes, life history traits, behavior, and even species interactions (Bernhardt et al., 2020; Kroeker et al., 2020). While the physiological and ecological effects of seasonal fluctuations in the environment have received considerable attention (White & Hastings, 2020), it is becoming increasingly clear that higher frequency environmental variability (e.g. episodic, diel, semidiel) is not only common, but may also be crucial in shaping the biology and ecology of species across populations and ecosystems. For example, diurnal fluctuations in temperature can alter growth and life history traits (Kingsolver et al., 2009; Ragland & Kingsolver, 2008) and have the potential to alter behavior and subsequent consumer-resource dynamics (Fey & Vasseur, 2016). Yet, despite the potential importance of natural

environmental variability in ecology, in many cases, the consequences of high frequency or episodic temporal variability in abiotic conditions for ecological traits and ecosystem function are still poorly understood.

Past studies focusing on the underlying physiological responses of organisms to high frequency or episodic, temporal environmental variability demonstrate that many species have a remarkable ability to adapt and/or acclimate to their environment, but the energetic costs of their responses could have important implications for their ecology more broadly. For instance, species at increased risk of episodic, extreme thermal fluctuations maintain higher metabolic rates as well as higher critical temperatures, the point at which metabolic rates begin to rapidly decrease with further increases in temperature (Somero et al., 2017). Conversely, some species can depress (or reduce) metabolic rates as an energy-saving strategy during particularly adverse or stressful conditions (Hui et al., 2020; Liao et al., 2021). Moreover, some species, such as intertidal gastropods, are capable of switching entire metabolic pathways (aerobic vs anaerobic) over diel cycles to cope with periods of exposure to air versus submersion in seawater (Somero et al., 2017; Storey & Storey, 1990). Finally, many organisms possess heat shock proteins (HSPs), which facilitate molecular repair following exposure to extreme temperatures (Tomanek, 2008), however HSPs require time to produce (Tomanek & Somero, 2000) and come at high production costs (Hoekstra & Montooth, 2013). As evidenced in these examples, most previous

research has focused on responses to environmental variability in temperature. Much less is known about how organisms respond to episodic or high-frequency variability in other environmental variables (but see Cornwall et al., 2013; Frieder et al., 2014; Hoshijima & Hofmann, 2019). Regardless of the mechanism, strategies to cope with living in temporally fluctuating environments all likely come with energetic trade-offs (Hofmann & Todgham, 2010; Somero, 2020), and in order to maintain positive growth and reproduction, any increases in energetic costs will need to be balanced by corresponding increases in energetic gains via consumption (Sokolova et al., 2012). Importantly, as anthropogenic environmental change continues to alter mean conditions and variability in the environment, it is unclear whether the costs of an organisms' current strategies to deal with temporal variability will be sufficient to deal with new baselines and extremes.

Complex or non-linear responses of species to temporal fluctuations in their environment presents a unique challenge for global change biology. Despite the prevalence of temporal variability in the environment, most manipulative experiments in global change biology are undertaken in static conditions, in order to aid logistics and interpretability of the thresholds at which species respond to environmental change. Several studies have demonstrated that manipulating the variability around the mean conditions (e.g., in temperature or seawater pH) affects the organismal response to environmental change

(Cornwall et al., 2013, 2018; Frieder et al., 2014; Hoshijima & Hofmann, 2019;

Kingsolver et al., 2009; Melzner et al., 2020; Ragland & Kingsolver, 2008), while

other studies have shown no response (Kwan et al., 2017). In highly dynamic

environments, it is especially challenging to design manipulative experiments to

adequately capture the different types of variability that may affect an

organisms' response (e.g., variability in frequency, duration or intensity of

exposure). In these circumstances, understanding the shape of the relationship

between a static abiotic environment and an organism's performance (i.e.,

response curves) can provide important insight into the emergent physiological

and ecological effects of temporal environmental variability (Bernhardt et al.,

2020; Harley et al., 2017). For example, linear response curves have the

potential to link static conditions measured in a laboratory to variable

conditions in the field, whereas, non-linear response curves measured under

static conditions could result in an over- or under-estimation of responses to

variable conditions in nature due to Jensen's inequality (Bernhardt et al., 2018;

Denny, 2017; Ketola & Saarinen, 2015). Therefore, the shape of a response curve

developed under static conditions determines whether that response curve can

be used to integrate a species' response under temporally fluctuating conditions.

Beyond the complexity associated with temporal variability in environmental

drivers, many organisms are also experiencing concurrent changes in multiple

environmental factors simultaneously (Breitburg et al., 2015). To address this,

studies in global change biology that assess how organisms and ecosystems respond to changes in two or more abiotic factors have become more common (Crain et al., 2008; Harvey et al. 2013; Jackson et al., 2016; Przeslawski et al. 2015; Stockbridge et al. 2020). These studies are useful, but they often lack a sufficient number of levels to model the response curves, especially in temporally fluctuating environments. In these environments, response surfaces may be important to understand the range of responses organisms may mount to temporal fluctuations in abiotic conditions (Harley et al., 2017). Unfortunately, multiple driver regression designs are both logistically complex and often difficult to interpret. Additionally, it can be unnecessarily complex to examine ecological processes across a regression of all combinations of two or more factors given that many of these scenarios are unlikely (or even impossible) to occur in nature (Boyd et al., 2018). Despite these difficulties, response curves or surfaces (i.e. two or more factors) are necessary to improve models of ecological change in the future since they provide a species' response to all values of a suite of environmental drivers that it may experience in a variable environmental regime.

In some systems where environmental drivers are tightly coupled, a response surface can be compressed into more simple forms using techniques such as PCA regression (Graham, 2003). This scenario greatly simplifies both the interpretation of results as well as the experimental design, while maintaining

realism across a continuous response variable. For instance, ocean acidification, warming, and deoxygenation are key issues in Eastern Boundary Upwelling Systems (EBUS) (Chan et al., 2017; Cheresh & Fiechter, 2020; Feely et al., 2008; Hauri, Gruber, McDonnell, et al., 2013), where environmental changes have already been documented to affect marine species and ecosystems (Barton et al., 2012; Boch, 2018; Pinsky et al., 2013). Importantly, temperature, pH, and dissolved oxygen (DO) concentrations show high coherence over diel, semi-diel, and event time-scales in marine ecosystems within EBUS due to the physical forcing of upwelling and internal bores (Booth, 2012; Frieder et al., 2012; Hirsh et al., 2020; Walter et al., 2014). As climate change, ocean acidification, and deoxygenation progress in EBUS, temperature, pH, and DO can be compressed into a single "upwelling" variable for global change experiments. Although this compressed design is not feasible in all systems, where possible, it may provide important insights into the shape of species responses to multiple environmental drivers.

Eastern Boundary Upwelling Systems provide a unique opportunity to examine how temporal variability in coupled environmental variables impacts important physiological and ecological processes. Despite our relatively thorough understanding of the general spatial and temporal scales of upwelling in nearshore environments (Checkley & Barth, 2009; García-Reyes & Largier, 2012), the acute effects of an upwelling event and the integrated effects of

multiple upwelling events on marine organisms are still unclear. One reason for this may be that relatively acute, episodic upwelling events do not occur over sufficient time scales with which to resolve important metrics of species performance, such as growth. Therefore, the impacts of acute upwelling on these fitness-related processes cannot be easily measured in the field. Yet, if instantaneous responses to upwelling conditions, such as metabolism, can be used to approximate performance metrics such as growth, it may be possible to integrate ecological responses measured during longer-term exposure to static upwelling conditions established in the lab over the shorter time scales of a single upwelling event. Thus, while laboratory experiments can be used to assess the general relationship (i.e. linear vs. nonlinear) between the covarying factors associated with upwelling and organismal performance, it is also important to assess whether long-term exposure to static conditions reflects organismal responses to acute, short-term exposure.

Kelp forests are some of the most diverse and productive ecosystems in the world (Steneck et al., 2002). Many factors contribute to the abundance and distribution of kelp forests, one of which are grazing taxa, such as gastropods and sea urchins. While both taxa consume habitat-forming kelp, sea urchins exert much higher grazing pressure per capita than most gastropod species (Sala & Graham, 2002). At high densities, sea urchin grazers can decimate entire kelp forests, transforming them into urchin barrens (Rogers-Bennett & Catton, 2019;

Steneck et al., 2002). In contrast, the per capita grazing rates of many gastropods are much lower than those of sea urchins (Sala & Graham, 2002), but unlike sea urchins that generally graze on drift algae, many gastropods graze directly on live kelp or on epiphytes living on standing kelp biomass. Thus, both taxa can have important effects on the structure and function of kelp forest ecosystems. Furthermore, both gastropods and sea urchins have been shown to be sensitive to environmental changes in temperature, pH and/or DO (Bednaršek, 2021; Dupont et al., 2010; Gazeau et al., 2013; Kroeker et al., 2013), but the effect of temporal variability in all of these drivers associated with upwelling events is not well understood. Due to their important roles within kelp forests and their sensitivity to environmental change (e.g. global warming, ocean acidification and deoxygenation), a better understanding of how kelp forest grazers respond to covarying temperature, pH and DO concentrations will help improve our ability to predict future ecological change.

The aim of our study is to assess how organismal performance shifts in response to concurrent variability in temperature, pH and DO, mimicking conditions currently occurring during upwelling season within kelp forests found along the central California coast. First, we examine the co-variation between seawater pH, DO, and temperature in a shallow kelp forest to better understand the natural environmental variability organisms are currently experiencing in our system. Second, we examine the physiological and ecological responses of two important

kelp forest grazer species, red sea urchins, *Mesocentrotus franciscanus,* and brown turban snails, *Promartynia pulligo* to long-term (3-month) exposure to static temperature, pH, and DO concentrations that approximate a gradient of upwelling intensity*.* In addition, we assess how well the responses measured in the 3-month static exposures to upwelling approximate these species' responses to acute exposure to these same conditions over 72 hours, representing a single upwelling event. We focus on the energetic responses of these species, which represent two key benthic grazer taxa (Echinodermata and Gastropoda), by measuring metabolism, consumption, net calcification and growth to link kelp forest structure and function to temporal environmental variability.

MATERIALS AND METHODS

Collection Site: In order to better understand the covariability of environmental conditions within kelp forests in central California, we deployed autonomous sensors measuring pH, temperature, DO and salinity (SeapHOx, Sea-Bird Scientific) near the seafloor at Stillwater Cove, Carmel (36.5607° N, 121.9459° W). From February 2016 until August 2019, we deployed a SeapHOx on a mooring line, approximately 3 m off the bottom (at ~10 m depth). From September 2019 until October 2020, we secured a SeapHOx at the seafloor (at ~15 m depth) within 20 m of the mooring line and within the same kelp forest. Sensors collected data every 30 minutes with the exception of periods of time in between consecutive deployments when we retrieved the sensor for download

and servicing. We collected discrete water samples for pH and total alkalinity next to the sensor upon deployment and retrieval in the kelp forest, in order to calibrate the pH sensor and assess drift in pH measurements over time. Following best practices, we made spectrophotometric pH measurements on the discrete samples using *m*-cresol purple. We measured total alkalinity using open cell titration (Metrohm, 905 Titrandro) and checked for accuracy using certified reference materials from the lab of Dr. Andrew Dickson (Scripps Institution of Oceanography) at the beginning and end of the day on which samples were processed. We used pH, alkalinity, temperature, salinity and stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson & Millero (1987) to calculate *in situ* pH. These *in situ* pH values were used to calculate calibration coefficients (E*) at 25 °C, for each discrete sample in order to assess drift (Bresnahan et al., 2014). We fit second degree polynomials to E* data over time and then used this model to calculated E* at each pH measurement. During our final deployment (March 9, 2020 - October 15, 2020), pH drifted significantly and no discrete samples were taken due to logistical constraints during this period. Therefore, locally interpolated pH regression was run using the LIPHR package within Matlab (Carter et al., 2018). Using LIPHR, estimated pH was calculated from SeapHOx DO, temperature, and salinity measurements. We ran a de-spiking function in Matlab to remove spike noise from all pH, temperature, and DO data (Mori et al., 2007). Finally, we determined the relationships between pH and dissolved oxygen, pH and temperature, and

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temperature and dissolved oxygen to inform environmental conditions within our mesocosm experiments using linear regression analyses on *in situ* sensor data. We ran separate regression analyses separating sensor data into *upwelling* season (April-September) and *non-upwelling* season (November-March) excluding the months of March and October, which are the typical timing of the spring and fall transitions in central California respectively (Checkley & Barth, 2009).

Using SCUBA, we collected juvenile red sea urchins, *M. franciscanus* (~1.5 - 4 cm test diameter), and brown turban snails, *P. pulligo* (~1.5 - 2.5 cm shell diameter), from the kelp forest (~15 m depth) in Stillwater Cove on August 13, 2019 (Experiment 1) and March 9, 2020 (Experiment 2). Upon surfacing from the dives, we placed individuals into a cooler for transport to Long Marine Laboratory at the University of California Santa Cruz. We held all sea urchins and gastropods in seawater tables supplied with ambient flow-through seawater conditions for one month and three months respectively. During this period, the animals were fed an *ad libitum* diet of *Macrocystis pyrifera*. After laboratory acclimation, we measured initial performance metrics and transported individuals to the laboratory mesocosm facility at the National Oceanographic Atmospheric Administration's (NOAA) Southwest Fisheries Science Center in Santa Cruz, CA.

Mesocosm system: The mesocosm system was supplied with chilled and UV-filtered seawater pumped directly from the intertidal rocky reef off of Long Marine Laboratory. Two 50-gallon sump tanks, one containing "*ambient*" seawater and the other containing "*upwelled*" seawater were mixed to achieve six distinct temperature, DO, and pH treatments in header tanks. We chose ambient sump conditions that reflected the highest coupled pH, DO, and temperature measurements experienced *in situ*. The ambient seawater sump had two 1500 W heaters, which raised the seawater temperature to ~13 °C, a temperature higher than our highest treatment. Seawater pH and DO within the ambient seawater sump were allowed to fluctuate, but hovered around 8.1 pH_T and ~9.0 mg/L. We chose upwelled sump conditions that reflected the lowest coupled pH, DO and temperature measurements experienced *in situ*. We created the *upwelled* seawater sump by continuously bubbling N_2 gas and semi-continuously bubbling pure CO_2 at a rate that kept DO at ~2.5 mg/L and pH_T at ~7.4. We created treatment seawater by mixing seawater from the ambient and upwelled sumps within six five-gallon tanks, hereafter referred to as "headers", each with a gamma lock seal. We used a feedback system to trigger solenoid valves to open and allow water from the upwelled sump to mix with water from the ambient sump whenever pH drifted above a target setpoint programmed for that header. Although the system was only actively controlling pH, the coupled nature of pH, temperature, and DO in our sumps mimicked a natural upwelling system, such that an increase/decrease in pH resulted in a corresponding

increase/decrease in DO and temperature. We fitted each header tank with a small aquarium pump to mix seawater, a DuraFET pH electrode (Honeywell) to measure pH and temperature, and a Vernier goDirect DO probe (Vernier) to measure DO. Both pH and DO sensors measured continuously (every 1 min or 15 min respectively) throughout the experiment. Header tanks supplied water to 3 treatment aquaria (~ 20 gallons), which housed both sea urchins and gastropods (details below).

At approximately noon each day, we measured pH, temperature, DO and salinity within all treatment aquaria using a handheld meter (YSI). We collected discrete water samples for pH and total alkalinity from all header tanks and treatment aquaria every other week for the duration of the experiment (N = 8). We processed water samples using the same methods as those for SeapHOx sensor calibration, but we used them to characterize the entire carbonate system across treatments.

Experiment 1: To better understand the consequences of coupled changes in pH, temperature, and DO on sea urchin and gastropod growth, we reared *M. franciscanus* and *P. pulligo* individuals across a gradient of upwelling conditions for three months (September 2019 – December 2019). Within each replicate treatment aquarium, we placed sea urchins and gastropods (N = 4-8 and N = 9-10 respectively) in individual 0.5 L cages where they were fed *ad libitum* for the

duration of the experiment (except during respirometry and grazing trials). At the beginning and after one month, two months and three months in treatment conditions, we measured wet and buoyant weight, respiration rate, and per capita grazing rate of all individuals.

We measured wet and buoyant weights to calculate growth rates and net calcification over time across treatments. To obtain wet weights, we carefully dried individuals using paper towels and then placed them on a scale, measuring to the nearest 0.001 g. To obtain buoyant weights, we placed individuals in a basket submerged in seawater and attached to the weigh-below of our scale and measured them to the nearest 0.001 g (Davies 1989, Manriquez et al. 2017). Since calcium carbonate is more dense than seawater, the change in buoyant weight over time can be used as a proxy for net calcification. We calculated growth rate (as % change in wet weight) and net calcification (as % change in buoyant weight) from initial and final (3 month) weight measurements as:

$$G \text{ or } C = \frac{Fw - Iw}{Iw},$$

where G is the % change in wet weight, C is the % change in buoyant weight, Fw is the final weight, and Iw is the initial weight.

Forty-eight hours before respirometry trials, individuals were starved to remove the potential influence of digestive state (specific dynamic action) on metabolism. Respirometry chambers were made of polycarbonate and sealed

with a rubber gasket to eliminate the potential for gas exchange. Within each chamber a stir bar continuously mixed seawater to prevent the formation of boundary layers that may impede gas exchange within the chamber. We adhered DO sensor spots (PSt3, PreSens Precision Sensing GmbH) to the lids of all chambers to measure the DO concentration within each chamber. We calibrated chambers daily using a two-point calibration (0% and 100% saturation). After calibration, we sealed individuals in chambers (1 individual per chamber) with their respective treatment seawater. Chambers without individuals were also included to control for any effects of water column processes on DO concentrations. We then submerged chambers in a water bath maintained at the treatment temperature on top of a multi-position magnetic stirring system (2mag MIXdrive). We measured DO seven times over an approximately 30-minute trial. We standardized trials by time since initial DO concentrations differed between treatments. Although DO concentrations were potentially low (by design), responses were linear over the time scale of the incubations, suggesting that declining DO concentrations within each chamber did not alter respiration rates. We fit local linear regressions to the measurements using LoLinR in R (Olito et al. 2017). We corrected the slopes of each linear regression using the average slope of the controls during each assay. We derived a scaling coefficient for each species as the slope of the regression of log(mass) and log(control corrected respiration rate). We then mass-corrected respiration

rates using the mean mass for each species and mass-correction equations from

Steffensen et al. (1994).

Following respirometry measurements, we presented all individuals with a pre-

weighed (wet weight) disc of kelp (~ 7 cm diameter), *Macrocystis pyrifera*,

within individual 0.5 L cages for 24 hours. After ~24 hours, we removed the

remaining kelp disc and reweighed the disc. We calculated mass-corrected

grazing rate from the equation:

$$\frac{W_i - W_f}{M_i * t},$$

where W_i is the initial kelp disc wet weight, W_f is the final kelp disc wet weight,

and M_i is the mass of the individual urchin or snail, and t is time as days.

Experiment 2: To assess whether responses to sea urchin and gastropod

energetics measured during long-term exposure approximate responses during

acute exposure to a static gradient in pH, temperature and DO, we reared *M.*

franciscanus and *P. pulligo* individuals across the same gradient of experimental

conditions used in *Experiment 1* for three days in June, 2020. This experimental

duration mimicked the time scale of an upwelling event currently experienced at

our collection site, based on visual analysis of the time series and past studies

(Frieder et al., 2012; Walter et al., 2014). Forty-eight hours prior to initial

measurements, we starved individuals to remove any potential influence of

digestive state. We then weighed individuals and placed them directly in treatment seawater and measured respiration rates (as outlined in *Experiment 1*) to obtain an "acute" response. Following initial respiration rate measurements, we placed individuals in individual 0.5 L cages in treatment seawater (*N* = 8 and *N* = 10 respectively) and grazing assays were conducted for 24 hours following methods outlined in *Experiment 1*. After the initial grazing assay was complete, we starved individuals for another 48 hours before we conducted final respirometry and grazing assays following the same methods, approximately three days after the initial exposure to the treatments.

Statistical analysis for Experimental Data: We calculated mean and standard deviation in pH, temperature, and DO data from YSI measurements taken over the duration of each experiment. For Experiment 1 and 2, we used these data to conduct principal component analyses using R, to reduce collinearity in our explanatory variables and for use in subsequent regression analyses (Graham, 2003). We used scores from PC1 as fixed factors in linear mixed models testing the effects of our six upwelling treatments on various physiological and ecological processes.

We fit mass-corrected respiration and grazing rates to linear mixed models with *PC1* and *Timestep* (Experiment 1: 1 month, 2 month, 3 month; Experiment 2: 0 hr, 72 hr) as fixed factors and treatment aquaria (A, B, or C) as a random factor

using *lmer* in R. If a significant effect of *Timestep* was detected, Tukey *post-hoc* tests were applied to determine which timesteps differed in each model using *emmeans* in R. Models for experiments 1 and 2 were fit separately. We fit growth and net calcification data to linear mixed models with *PC1* as a fixed factor and *Treatment Aquaria* (A, B, or C) as a random factor. Models of growth and net calcification were only fit from data collected during Experiment 1 at the 3-month time point. In all models, we removed the interaction term between *PC1* and *Timestep* if it was highly non-significant ($p > 0.50$). For responses where a significant effect of *PC1* was detected, partial effects for *Timestep* and *Treatment Aquaria* were removed using the *remef* package in R. This allowed estimation of the equations for the relationship between the response variable and retained principal components by means of linear regressions on the resulting partial residuals of the response variable for each timestep. All code and raw data are available at https://github.com/EmilyDonham/Upwelling_Impacts_Grazers and can be found on the Pangaea data repository.

RESULTS

Environmental conditions: Temperature, pH, and DO varied considerably within Stillwater Cove, CA (Fig. 1, Fig. S1). Across the four years of monitoring, during upwelling season, pH = 7.81 ± 0.10, temperature = 11.69 ± 1.35°C and DO = 5.64 ± 1.32 mg/L (mean ± SD). Outside of upwelling season, pH = 7.89 ± 0.10, temperature = 13.18 ± 1.14°C and DO = 6.14 ± 0.72 mg/L (mean ± SD). All three

oceanographic variables were positively correlated with each other, such that periods of cold water were characterized by low pH and low DO, while warm water events had high pH and high DO (Fig. 1). Interestingly, the strength of the relationships among the three environmental variables were strongest during upwelling season (April-September), and in this season, the association between DO and pH exhibited the highest R-squared value of any pairwise comparison (Fig. S1; Table S1). DO concentrations fell below 4.6 mg/L (considered a biologically relevant threshold for sub-lethal effects, where the effects of low oxygen are first apparent, Vaquer-Sunyer & Duarte, 2008) ~14% of the time. During these low oxygen events, pH = 7.71 ± 0.05 and temperature = 10.48 ± 0.72 °C (mean ± SD).

Experimental conditions: Six distinct treatments were maintained across a gradient of upwelling intensity for the duration of both experiments (Fig. S2, Fig. S3), with treatments 5 and 6 being characterized by upwelling conditions of cold, acidic, and hypoxic water, while treatments 1 and 2 had the opposite conditions, and treatments 3 and 4 represented intermediate conditions. In Experiment 1, pH ranged from 7.79 – 7.49, temperature ranged from 12.17 – 10.33 °C, and DO ranged from 7.64 – 4.69 mg L^{-1}. In Experiment 2, pH ranged from 8.01 – 7.56, temperature ranged from 13.8 – 11.3 °C, and DO ranged from 8.82 – 4.67 mg L^{-1} (Table 1).

As expected, temperature, pH, and DO varied among treatments (Fig. 3). Relationships between mean pH and mean temperature, and mean DO and mean temperature were consistent with *in situ* measurements, indicating that the laboratory conditions approximated those observed in nature (Fig. 2b, 2c). The only discrepancies occurred for DO and pH, such that mean DO conditions were ~2.5 mg L^{-1} and 1.9 mg L^{-1} higher than expected at a given mean pH in Experiment 1 and 2, respectively, than those experienced in the field (Fig. 2a). The PCA analysis included 6 original variables, hence informative PC's would be expected to explain > ~17% of the total variation (100%/6 = 16.66 %). Results of PCA analysis of environmental data from Experiment 1 indicated that PC1 accounted for ~81% of the variation in experimental conditions. PC1 was primarily correlated with the mean and standard deviation of pH and temperature and mean DO. PC2 accounted for an additional ~17% of the variation in the experimental conditions, which was primarily correlated with the standard deviation of DO (Fig. 3a). No other PC explained more than 17% of the variation. Thus only PC1 was retained for subsequent modeling. In Experiment 2, PC1 accounted for ~73% of the variation in experimental conditions. PC1 was primarily correlated with the mean and standard deviation of pH and temperature and mean DO. As above, PC2 only accounted for an additional ~17% of the variation in the experimental conditions and was not used in subsequent modeling (Fig. 3b). In both models, increases in PC1 would approximate the conditions associated with increased *upwelling intensity*, as

higher PC1 reflects treatments that are colder, lower in pH, and lower in DO, but also more variable. Thus, we labeled PC1 as upwelling intensity in the figures and discussion of results to ease interpretation.

Respiration and grazing: In Experiment 1, linear mixed models revealed a significant effect of PC1 (approximating upwelling intensity) on respiration rates of *M. franciscanus* (Table 2). Respiration rates decreased with increased upwelling intensity (Table S2; Fig. 4a). The interaction between PC1 and Timestep was dropped since $p > 0.50$. Linear mixed models revealed a significant effect of PC1 and Timestep on respiration rates of *M. franciscanus* in Experiment 2, but did not detect an interactive effect (Table 2). Respiration rates decreased with acute exposure to increased upwelling intensity (Table S2; Fig. 4b) and were significantly higher at 0 hr compared to 72 hours. We also found significant effects of PC1 and Timestep on respiration rate in Experiment 1 for *P. pulligo* (Table 2). Respiration rates decreased with increased upwelling intensity (Table S2; Fig. 5a), but were significantly higher after one month than after two or three months. We found a significant effect of Timestep on respiration rate in Experiment 2 for *P. pulligo*, but did not detect a significant effect of PC1 (Table 2; Fig. S4a). Respiration rates were significantly higher at 0 hours compared to 72 hours.

We found significant effects of PC1 and Timestep on grazing rates in *M. franciscanus* in both Experiments 1 and 2 (Table 2)*.* Grazing rates decreased with increased upwelling intensity (Table S2; Fig. 4b,c). In Experiment 1, grazing rates were significantly higher after one and three months compared to two months, whereas, in Experiment 2, grazing rates were significantly higher at 0 hours compared to 72 hours. We also found significant effects of PC1 and Timestep on grazing rates in Experiment 1 for *P. pulligo* (Table 2). Grazing rates decreased with increased upwelling intensity (Table S2; Fig. 5b) and were highest after two months. We did not detect any significant effects on grazing in Experiment 2 for *P. pulligo* (Table 2; Fig. S4b).

Growth and net calcification: We found a significant negative effect of PC1 (increased upwelling) on growth and net calcification in *M. franciscanus* over three months in experimental conditions (Table 2; Fig 6a,b). We also found a significant negative effect of PC1 on net calcification in *P. pulligo* (Fig. 5c)*,* but did not detect an effect of PC1 on growth (Table 2; Fig. S4c).

DISCUSSION

Despite some variability in the sensitivity to upwelling between the two species studied here, long-term exposure to low temperature/pH/DO reduced metabolism, grazing, and net calcification in *M. franciscanus* and *P. pulligo,* as well as growth in *M. franciscanus.* We found that the physiological and ecological

responses measured in long-term, static temperature, pH, and DO conditions were linear and remarkably similar to responses following an acute exposure to upwelling conditions for *M. franciscanus*, but not for *P. pulligo*. *Promartynia pulligo* showed negative impacts of upwelling after chronic exposure, but we did not detect an effect of upwelling over shorter, acute timescales. This suggests that the relationship between upwelling intensity and growth in the 3-month trial could potentially be used to estimate growth in more dynamic conditions for *M. franciscanus*, but not for *P. pulligo*. Together, these results indicate that current exposure to upwelling conditions experienced in the California Current could reduce *per capita* and density-mediated grazing pressure in kelp forests (if reduced growth rates affect population demographics and size), with further reductions likely as upwelling frequency and intensity increase with climate change (Bakun, 1990; Wang et al., 2015; Xiu et al., 2018). Understanding how changes in grazing pressure associated with upwelling, now and in the future, scale-up to affect the structure and function of kelp forests remains an important area of research.

Many studies have shown a non-linear relationship between environmental conditions and species performance. Our results suggest that within the current range of upwelling-associated environmental variability in Central California, the two kelp forest grazers studied here exhibit linear responses to coupled changes in temperature, pH, and DO. For variable environments, the mean response is

different from the response at the mean environmental condition when the response curve is non-linear, due to Jensen's Inequality (Denny, 2017; Jensen, 1906). Because the acute responses of *M. franciscanus* mirrored those in the chronic, longer-term experiment, our results therefore suggest that integrating linear responses over natural scales of variability (e.g. semi-diel, diel, episodic) during upwelling season, when temperature, pH and DO are tightly coupled, may well approximate the true response of *M. franciscanus*. Here, we show this relationship for physiological processes, such as respiration rate, important organismal level processes, such as growth, and even ecologically relevant processes, such as grazing. Validating this model with *in situ* and/or laboratory experimental data will be an important next step in assessing its utility for predicting future ecological change within kelp forests. For example, growth rates of individuals in variable environments in the field could be compared with the growth rates predicted by the linear model by integrating growth across *in situ* environmental conditions through time. Alternatively, laboratory mesocosm experiments could test how these responses vary in static and variable treatments with the same mean temperature, pH, and DO levels.

Although chronic responses to upwelling were similar between our two species, we did find different responses to acute upwelling. The different responses to acute upwelling between these two species could be due to a number of physiological mechanisms driven by any combination of environmental drivers

in our study. For example, Ng & Micheli (2020) reported that the effect of ocean acidification and hypoxia (~2.0 mg L^{-1}) on per capita interaction strength (PCIS) between grazers and kelp was primarily driven by hypoxia over a two-day exposure. They also found that the impacts of hypoxia on PCIS was greater for the purple sea urchin, *Strongylocentrotus purpuratus*, and two species of crustaceans, *Idotea resecata* and *Peramphithoe humeralis,* than for the brown turban snail, *Tegula brunnea.* Similarly, a meta-analysis by Vaquer-Sunyer & Duarte (2008) found molluscs to be more hypoxia tolerant than all other taxa in their study. DO conditions in our study never reached hypoxic (Experiment 1 mean DO range: 7.64-4.69 mg L^{-1}; Experiment 2 mean DO range: 8.82-4.67 mg L^{-1}) but did reach the biologically relevant sub-lethal threshold of 4.6 mg L^{-1}, where less tolerant species are first affected at the lower range (Vaquer-Sunyer & Duarte, 2008). The relatively high tolerance of molluscs to hypoxia could explain why we did not see impacts of upwelling driven declines in DO on *P. pulligo* over acute time steps.* DO conditions in our experiment were also higher at a given pH than those measured in the field and therefore may underestimate the true effects of upwelling-driven environmental change, unless low oxygen ameliorates the response to declining pH (Frieder et al., 2014). Secondly, the ability to acid-base regulate is taxon specific and important for maintaining a healthy internal homeostasis (Melzner et al. 2011), and it has been suggested that echinoderms may be more sensitive to low pH than gastropods. An increased ability to buffer changes in pH could further explain why we did not

detect an effect of upwelling on *P. pulligo* in our acute experiment. Further mechanistic work on the physiological responses of these species and other closely related species to the individual and combined changes in temperature, pH and DO will be important to understanding why some species and taxa respond differently to changes in environmental conditions.

Rising energetic costs to maintain homeostasis in a changing environment is likely to have consequences for both individuals and populations. Increases in metabolic rates to fuel rising maintenance costs can divert energy from other important processes (Sokolova et al., 2012). Alternatively, reducing metabolic rates via metabolic depression can be an energy-saving mechanism during times of environmental stress, but also results in reduced energy flow to important processes such as growth and reproduction (Guppy & Withers, 2007; Liao et al., 2021; Storey & Storey, 1990). We found that with exposure to both acute and chronic upwelling for *M. franciscanus* and chronic exposure for *P. pulligo,* respiration rates decreased linearly with increasing upwelling intensity. These responses are generally consistent with how organisms respond to temperature alone (i.e. across a range of non-stressful temperatures, increasing temperature increases metabolism; Brown et al., 2004). Additionally, Low & Micheli (2020) found purple sea urchin, *Strongylocentrotus purpuratus*, metabolism declined significantly during rapid pulses (3-6 hrs) of low DO similar to the levels in our study. Interestingly, they also found that under constant low DO exposure,

metabolic rates initially declined but then returned to normal rates in less than 60 hours. In contrast, we did not observe a return to normal metabolic rates following a similar duration exposure (72 hrs). Potential explanations are that metabolic plasticity is highly species and population specific or that the combined responses to multiple drivers in our experiment could explain these differences. Furthermore, some studies have found no effect of OA (in isolation) on metabolic rates in juvenile and adult sea urchins (Moulin et al. 2014; Uthicke et al. 2014), while others have found evidence of increases in metabolism in response to acidification (Carey et al. 2016). Our study suggests that in combination, the effects of reduced dissolved oxygen concentrations and declining temperature associated with upwelling on metabolic rate may counter any potential increases due to acidification. For marine gastropods, the effects of OA may be more nuanced (Calosi et al. 2017) with studies showing no effect, increases and decreases in metabolic rates in response to acidification (Gazeau et al. 2013). Gastropods may also depress metabolism in response to low oxygen concentrations and have even developed the ability to switch metabolic pathways to deal with declining oxygen concentrations (Storey & Storey, 1990). Therefore, it is not surprising that we saw metabolic rates decline with increasing upwelling intensity in *P. pulligo* even if they were resilient to the low pH exposure. Importantly, changes to metabolic rates in response to environmental variability are likely to impact other processes at the organismal

level, such as growth and reproduction, which will have consequences for populations and even ecosystems.

Highly productive kelp forest ecosystems support a diversity of grazers that play a key role in structuring the benthic community through grazing (Steneck et al., 2002). Although *M. franciscanus* and *P. pulligo* are both ecologically important herbivorous grazers within kelp forests (Graham, 2004; Watanabe, 1984), it is widely recognized that *M. franciscanus* can have substantial ecological impacts on kelp standing biomass through grazing (Harrold & Reed, 1985), while the effect of *P. pulligo* is likely much smaller in magnitude (Sala & Graham, 2002). Increases in grazing pressure due to increased sea urchin densities or changes in behavior have caused phase shifts from kelp forests to urchin barrens (Estes & Duggins, 1995; Pearse, 2006). These collapses in kelp biomass cause massive declines in biodiversity and ecosystem functioning. Conversely, reductions in grazing pressure have been shown to contribute to shifts from productive kelp forests to less productive algal turfs in other kelp forest ecosystems (Falkenberg et al., 2014). Therefore, changes in *per capita* grazing rates as a consequence of environmental change could result in shifts from healthy kelp forest ecosystems to alternate states. Here we show that chronic exposure to upwelling can reduce grazing rates in both *M. franciscanus* and *P. pulligo,* with acute exposure also reducing grazing in *M. franciscanus.* These results are consistent with past studies showing reduced grazing in sea urchins due to reduced oxygen (Low &

Micheli, 2020; Low & Micheli, 2018; Ng & Micheli, 2020) as well as acidification

(Brown et al., 2014; Donham et al., 2021), however, the impacts of pH,

temperature and DO on gastropods are more variable.

Although the general relationship between upwelling and grazing did not change

across time (except during acute exposure for *P. pulligo*) there were differences

in the overall grazing rates with exposure duration. Algal quality and or

palatability may have differed between grazing trials as kelp was collected

approximately 24-48 hours prior to each subsequent grazing trial. Changes in

algal quality and palatability in response to global change have the potential to

alter per capita grazing rates (Falkenberg et al., 2013; Ghedini et al., 2015).

Future work quantifying changes to seaweed quality in response to

environmental change and how these changes directly impact grazing species

will be crucial to better understanding seaweed-grazer dynamics.

Growth and calcification have been shown to be negatively impacted by ocean

acidification (Kroeker et al., 2010). We found that *M. franciscanus* growth and

net calcification were negatively affected by increasing upwelling intensity. In

isolation, acidification has been shown to decrease growth and calcification in

sea urchins (Byrne & Hernández, 2020). Low & Micheli (2018), however, did not

detect a significant effect of DO on growth in the purple sea urchin, *S. purpuratus*,

but did find a significant decrease in spine regrowth (a proxy for calcification). In

our study, all individuals were fed *ad libitum* to maximize growth and simulate healthy kelp forest conditions, however, urchin barrens are a common alternate stable state, and starved urchins within barrens are likely more susceptible to the effects of current and future upwelling than healthy urchins (Murie & Bourdeau, 2021). It is therefore likely that the effects on sea urchins in our study are conservative and future work should focus on how energetic context alters the shape of the relationship between upwelling and species performance.

Furthermore, although we did find a reduction in net calcification as a result of upwelling in *P. pulligo,* we did not see similar effects on *P. pulligo* growth. *P. pulligo* has a thick outer shell that is often covered in red encrusting algae (some of which are calcifiers). Many studies have shown that coralline red algae are particularly susceptible to dissolution in acidic conditions (McCoy & Kamenos, 2015). Since we were unable to quantify the biomass of seaweed on snail shells, we cannot separate weight loss in biofouling taxa from the snails' shells themselves. It will be important to understand whether the decrease in net calcification is due to a reduction in shell calcium carbonate (which has been shown in other gastropods) or due to decreased calcium carbonate of the encrusting calcifiers, as this may be an important mutualism within kelp forests that could be impacted by future environmental change.

Negative relationships between upwelling and growth and/or calcification seen here suggest there may be some seasonality in growth patterns for these species. Since mean temperature, pH, and DO conditions are higher outside of upwelling season, although not explicitly tested, we would expect that growth and/or calcification are higher during these times of the year. Seasonal differences in species performance may be crucial to survival, especially if net performance is negative during certain times of the year. This will become increasingly important as global change is predicted to increase the frequency, intensity, and duration of upwelling events in the future (Bakun et al., 2015).

One of the greatest struggles in global change biology is scaling up results from controlled laboratory experiments to *in situ* conditions. By first describing the natural covariance of environmental drivers at our study site, we were able to more accurately design an experiment to inform future studies of the impacts of current environmental variation on species performance. We found linear relationships between pH and temperature, pH and DO, and temperature and DO across both oceanographic seasons at our study site, with the strongest relationships occurring during upwelling season. Importantly, although the effects of multiple drivers, such as temperature and DO, are often interactive, organisms in upwelling environments generally experience low temperatures at the same time as they experience low DO (and low pH). Interestingly, some organisms' tolerance to hypoxia increases with decreasing temperature

(Deutsch et al., 2015; Penn et al., 2018). Thus, organisms inhabiting upwelling regions of the oceans may be more tolerant to hypoxic events (Chu & Gale, 2017) due to the corresponding low temperatures that occur simultaneously, which are likely to depress metabolic rates and oxygen demands. While our experimental design allowed us to examine the combined effects of these important covarying drivers, it did not allow us to disentangle interactions that could be important in future conditions if global climate change and ocean acidification alter the covariance of environmental drivers (Kwiatkowski & Orr, 2018; Takeshita et al., 2015). For example, if upwelled waters become warmer while also more acidic (Hauri et al., 2013) and less oxygenated (Bograd et al., 2008), then the effects of exposure to low pH/DO could become more pronounced if species tolerance to one environmental factor is dependent on other environmental factors. Past studies have shown that cross-tolerance, when the effect of one stressor prepares an organism to deal with exposure to a different stressor, can be an important factor affecting species tolerance to environmental change (Gunderson et al., 2016). Alterations to the environmental covariance matrix could reduce the efficacy of cross-tolerance as the cue from one stressor that primes a species response to a second stressor may no longer be accurate. Future work that improves understanding of how the covariance matrix between environmental drivers is likely to change in the future, as well as how these changes impact organisms and ecosystems, will provide critical information necessary to link manipulative experiments and field settings.

CONCLUSIONS

Organisms are embedded in dynamic, multivariate environmental regimes. The impacts of this complex variability on organismal performance and ecosystem functioning can be difficult to quantify. Moreover, dynamic, temporal variability in key environmental drivers make it especially challenging to forecast the effects of global climate change when these same drivers are expected to change in the future. Furthering our understanding of how organisms respond to natural environmental variability in multiple drivers is paramount to understanding how species and ecosystems are likely to respond to future environmental change. Here, we demonstrate that chronic exposure to static, reduced pH, temperature, and DO associated with upwelling decreases respiration, grazing, and net calcification in a kelp forest sea urchin and gastropod. For the red sea urchin, *M. franciscanus*, we found that upwelling also reduced growth, and that these responses are consistent over acute and chronic time scales. If upwelling increases in frequency and duration due to climate change, as predicted (Bakun et al., 2015; García-Reyes & Largier, 2010), we might expect to see the negative effects of upwelling on growth and calcification further exacerbated in the future, with unknown interactive effects as background conditions continue to become warmer, more acidic and more deoxygenated due to climate change. Furthermore, these results suggest that at least over a natural range of co-varying environmental variability, species responses could be linear, which greatly simplifies mathematical models used

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for estimating performance in variable environments. Important next steps include expanding the species and taxa used in our experiment to see whether these findings can be applied more broadly, incorporating models of future upwelling conditions, and validating these hypotheses in the lab and in the field. Furthermore, it will be important to understand how these species' responses to environmental drivers impact other important ecosystem properties such as energy flow into higher trophic levels.

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Table 1.1. Mean and standard deviation of environmental conditions within treatment aquaria from YSI measurements (temperature, salinity, DO) and discrete samples (pH and total alkalinity, A_T). pCO_2 and saturation states were calculated using CO2SYS.

Exp	Trt	Temp (°C)	Salinity	DO (mg L⁻¹)	pH_T	A_T (μmol kg⁻¹)	pCO_2 (μatm)	Ω_{Cal}	Ω_{Ara}
1	1	12.17 ± 0.20	34.35 ± 0.29	7.64 ± 0.67	7.79 ± 0.03	2222.7 ± 12.5	748.0 ± 56.3	2.0 ± 0.1	1.3 ± 0.1
	2	11.70 ± 0.22	34.35 ± 0.29	6.89 ± 0.42	7.73 ± 0.01	2223.5 ± 13.4	874.1 ± 21.2	1.7 ± 0.1	1.1 ± 0.0
	3	11.04 ± 0.36	34.35 ± 0.29	5.86 ± 0.33	7.65 ± 0.03	2222.9 ± 12.7	1060.9 ± 59.1	1.4 ± 0.1	0.9 ± 0.1
	4	10.68 ± 0.46	34.35 ± 0.29	5.18 ± 0.32	7.58 ± 0.03	2223.2 ± 13.0	1263.4 ± 75.4	1.2 ± 0.1	0.8 ± 0.1
	5	10.40 ± 0.54	34.35 ± 0.29	4.78 ± 0.33	7.52 ± 0.01	2223.0 ± 12.9	1449.0 ± 31.1	1.0 ± 0.0	0.7 ± 0.0
	6	10.33 ± 0.55	34.35 ± 0.29	4.69 ± 0.33	7.49 ± 0.01	2223.6 ± 13.3	1556.3 ± 26.9	1.0 ± 0.0	0.6 ± 0.0
2	1	13.8 ± 0.38	34.8 ± 0.08	8.82 ± 0.95	8.01 ± 0.11	2255.3 ± 10.0	442.7 ± 120.2	3.3 ± 0.7	2.1 ± 0.4
	2	13.3 ± 0.28	34.8 ± 0.08	7.68 ± 0.60	7.82 ± 0.01	2255.6 ± 10.5	708.0 ± 26.5	2.2 ± 0.1	1.4 ± 0.0
	3	12.6 ± 0.07	34.8 ± 0.08	6.62 ± 0.10	7.75 ± 0.01	2255.7 ± 9.9	846.2 ± 8.7	1.9 ± 0.0	1.2 ± 0.0
	4	11.8 ± 0.21	34.8 ± 0.08	5.58 ± 0.15	7.66 ± 0.02	2255.2 ± 8.4	1053.8 ± 57.8	1.5 ± 0.1	1.0 ± 0.0
	5	11.7 ± 0.02	34.8 ± 0.08	5.43 ± 0.08	7.62 ± 0.00	2255.2 ± 9.3	1165.6 ± 2.4	1.4 ± 0.0	0.9 ± 0.0

6	11.3 ± 0.07	34.8 ± 0.08	4.67 ± 0.56	7.56 ± 0.01	2255.2 ± 9.5	1332.3 ± 39.4	1.2 ± 0.0	0.8 ± 0.0

Table 1.2. Results of mixed model fixed effects. Significance at $p < 0.05$ noted in bold.

Exp	Species	Response	Source	d.f.	F	p-value
1	*M. franciscanus*	Respiration	PC1	1	51.00	**<0.0001**
			Timestep	1	0.49	0.49
1	*M. franciscanus*	Grazing	PC1	1	54.40	**<0.0001**
			Timestep	1	27.80	**<0.0001**
1	*M. franciscanus*	Growth	PC1	1	4.35	**0.0390**
1	*M. franciscanus*	Calcification	PC1	1	4.44	**0.0370**
2	*M. franciscanus*	Respiration	PC1	1	43.10	**<0.0001**
			Timestep	1	10.30	**0.0015**
			PC1*Timestep	1	3.20	0.0747
2	*M. franciscanus*	Grazing	PC1	1	49.26	**<0.0001**
			Timestep	1	8.35	**0.0042**
1	*P. pulligo*	Respiration	PC1	1	40.4	**<0.0001**
			Timestep	1	60.0	**<0.0001**
1	*P. pulligo*	Grazing	PC1	1	29.40	**<0.0001**
			Timestep	1	159.30	**<0.0001**
1	*P. pulligo*	Growth	PC1	1	1.32	0.2500
1	*P. pulligo*	Calcification	PC1	1	17.10	**<0.0001**
2	*P. pulligo*	Respiration	PC1	1	1.33	0.2500
			Timestep	1	15.55	**<0.0001**
			PC1*Timestep	1	1.45	0.2300
2	*P. pulligo*	Grazing	PC1	1	0.05	0.8270
			Timestep	1	1.34	0.2470
			PC1*Timestep	1	3.16	0.0770
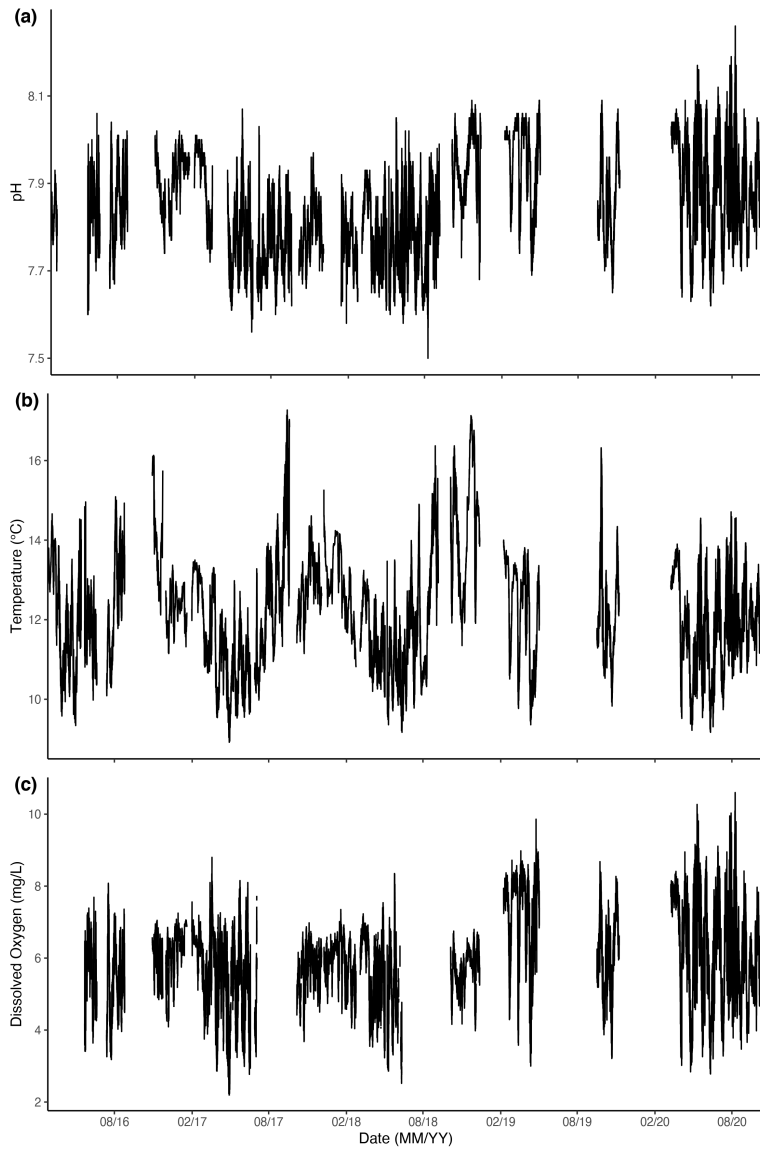
Figure 1.1. Time series of (a) pH, (b) temperature, and (c) DO from SeapHOx sensor deployed at 15m depth within kelp forest at Stillwater Cove, Carmel, CA. SeapHOx was deployed on a mooring ~3m off the seafloor from February 2016-August 2019 and directly on the seafloor from September 2019-October 2020.

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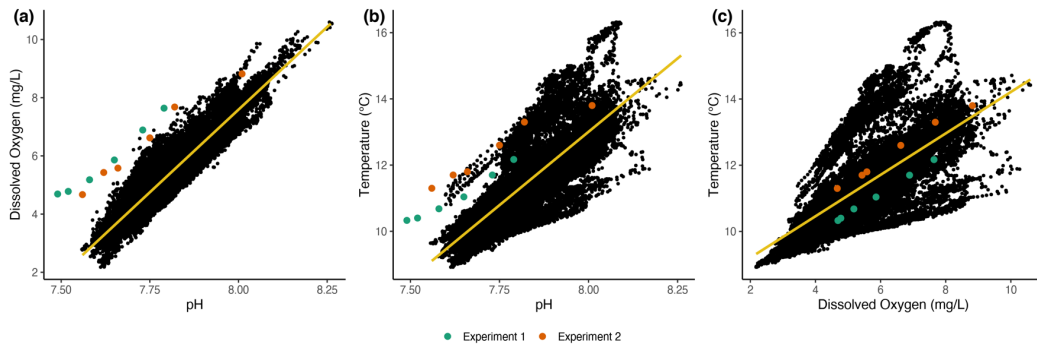
Figure 1.2. Scatterplot of time series data from SeapHOx sensor deployed within kelp forest during upwelling season from 2016-2020 (where data exist), with mean experimental conditions as colored points. (a) Dissolved oxygen as a function of pH with colored points showing mean conditions in Experiments 1 and 2. Line indicates linear fit. (b) Temperature as a function of pH with colored points showing mean conditions in Experiments 1 and 2. Line indicates linear fit. (c) Temperature as a function of dissolved oxygen with colored points showing mean conditions in Experiments 1 and 2. Line indicates linear fit.
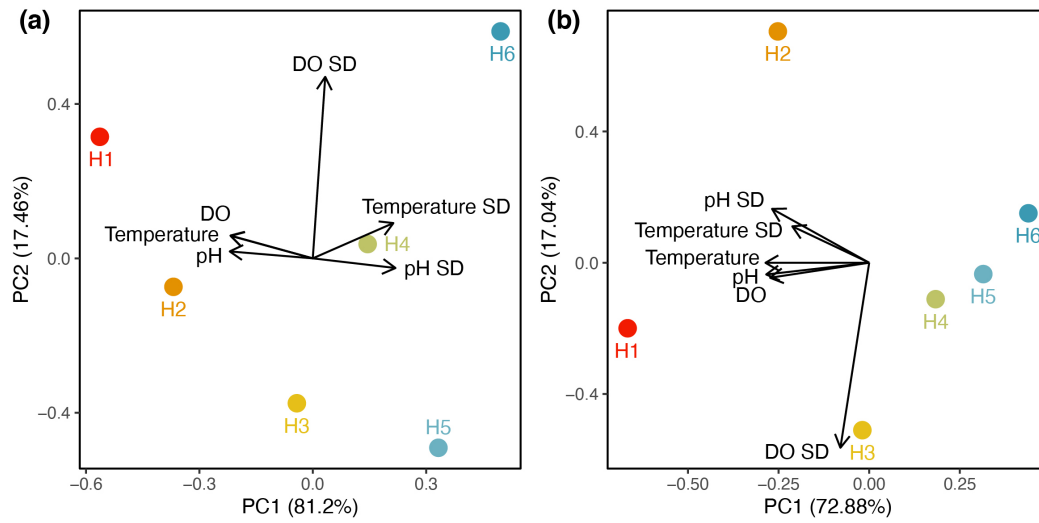
Figure 1.3. PCA biplots from PCA analysis of mean and standard deviation (SD) of pH, temperature and DO from daily YSI measurements within each treatment aquaria for (a) Experiment 1 and (b) Experiment 2. Directions of arrows indicate that values increase in that direction. PC1 was used to model respiration rate, grazing rate, growth and calcification. Numbers represent header buckets.
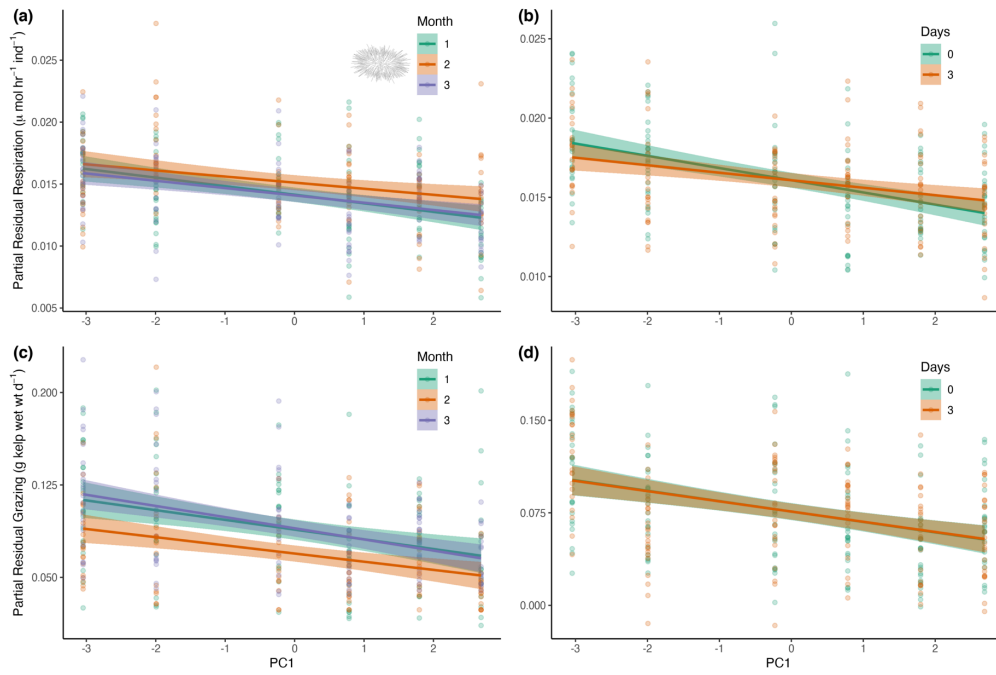
Figure 1.4. Linear models of *M. franciscanus* showing mean centered partial residuals for response variables (a) respiration rate and (c) grazing rate during Experiment 1 and (b) respiration rate and (d) grazing rate during Experiment 2.
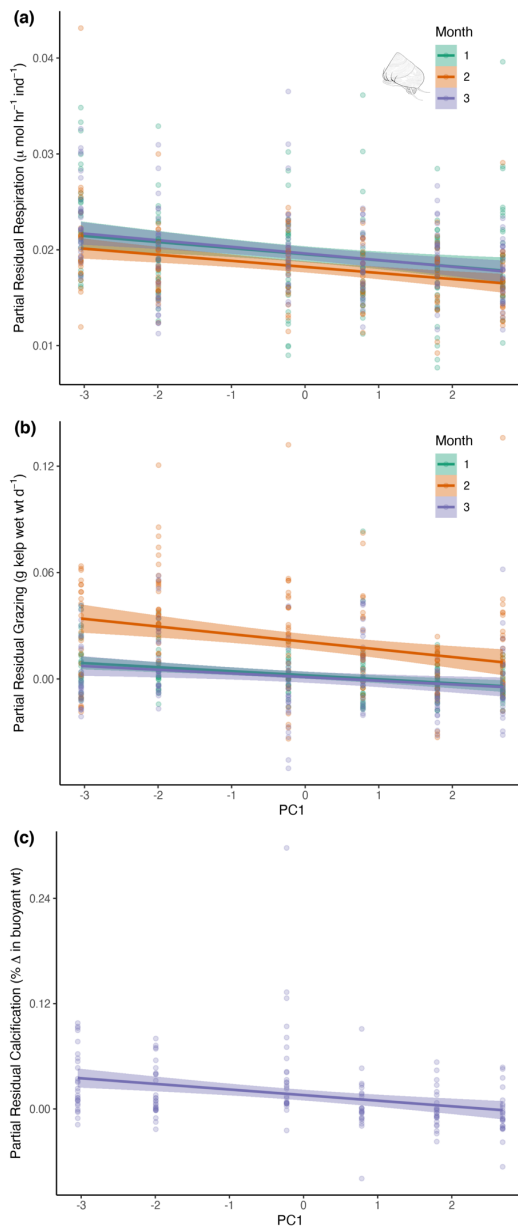
Figure 1.5. Linear models of *P. pulligo* showing mean centered partial residuals for response variables (a) respiration rate, (b) grazing rate, and (c) calcification rate during Experiment 1. Calcification rate is calculated over 3 months following exposure to upwelling conditions.
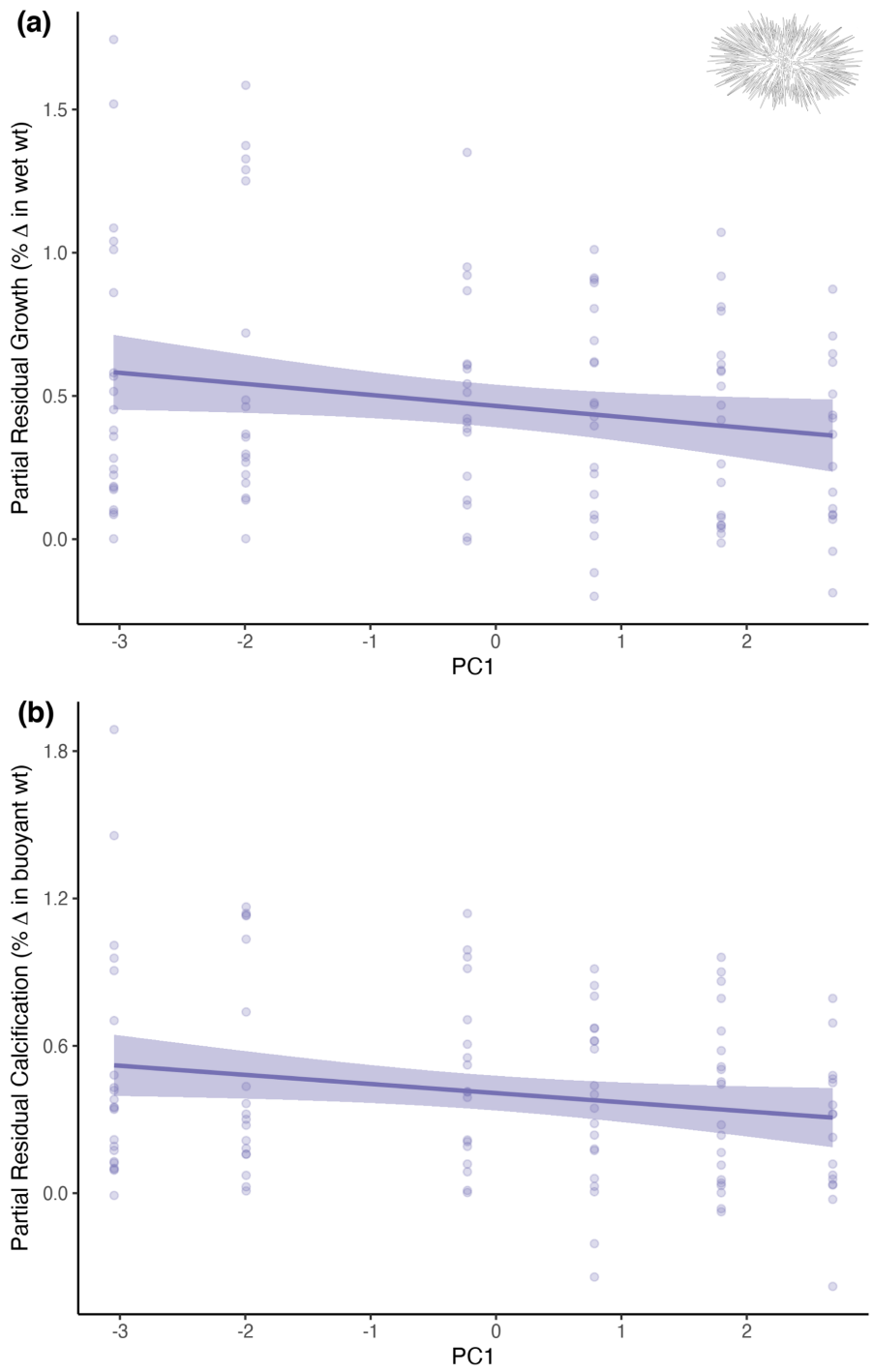
Figure 1.6. Linear models of *M. franciscanus* (a) growth rate and (b) calcification rate after 3 months in upwelling conditions.

CHAPTER 2

ECOLOGICAL AND PHYSIOLOGICAL RESPONSES TO MULTIVARIATE CLIMATE
CHANGE DIFFER ACROSS POPULATIONS

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CHAPTER 2: ECOLOGICAL AND PHYSIOLOGICAL RESPONSES TO MULTIVARIATE CLIMATE CHANGE DIFFER ACROSS POPULATIONS

ABSTRACT

Understanding the capacity for organisms to adapt to environmental change is central to global change biology. Most studies on local adaptation and acclimation to environmental change have focused on one driver (e.g., temperature, pH), thus little is known about the potential for evolutionary rescue to multiple, concurrent changes in the environment. Adaptation or acclimation to change may be particularly challenging for species that have evolved in environments with tightly coupled abiotic drivers, if the covariance structure of drivers associated with global change is altered in addition to the mean. In upwelling systems, seawater pH, dissolved oxygen (DO), and temperature are tightly coupled; however, climate change (and subsequent deoxygenation) and ocean acidification are changing the covariance of these drivers. Here, we assess the evidence for local adaptation/acclimation of the red sea urchin, *Mesocentrotus franciscanus,* to combined seawater pH, DO, and temperature. We reared sea urchins in a common garden laboratory experiment and quantified the responses of two populations that experience differences in upwelling intensity (i.e., strength of covariance) to projected future changes in these conditions. We found evidence for local adaptation/acclimation, with increased survival of populations in their home environment as well as evidence of energetic tradeoffs between growth/net calcification and reproduction (body

condition) across populations in current conditions. Consistent with past work on global change and ocean acidification, we detected increased mortality, decreased growth and decreased net calcification in response to region-specific future conditions. We also found that mortality was ~4x higher in future conditions for weak upwelling populations compared to strong upwelling populations. Together, these results support a growing body of evidence that global change may have differential impacts on marine populations due to local adaptation or acclimation to different environmental regimes.

KEYWORDS

Upwelling, Grazers, Kelp forests, Local adaptation, Acclimation, Population, Physiology

INTRODUCTION

Global climate change is occuring at an unprecedented rate due to anthropogenic activities (e.g. fossil fuel emissions, land use changes). Our current understanding of how climate change will impact species and ecosystems are largely based on studies conducted on a single population of a target species or community, but local adaptation and phenotypic plasticity can significantly alter how species respond to environmental change[1]. Increasingly, studies focused on evolutionary rescue are providing insights into the mechanistic underpinnings of intraspecific variability in response to changes in a single environmental driver

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(e.g., temperature[2], acidification[3-8]). The environmental conditions organisms experience are, however, inherently multivariate, and global change is expected to alter multiple environmental drivers simultaneously. Understanding the potential for species to adapt to multivariate environmental change is a key unanswered question.

Adaptation in the face of multiple environmental changes may be especially complex due to underlying genetic architecture. For instance, pleiotropy can limit adaptation when selection on different traits controlled by the same gene is opposing[9]. This may occur more frequently in environments where multiple abiotic drivers influence fitness, if the number of traits under selection increases with the number of abiotic drivers[10]. Furthermore, in addition to a single gene influencing multiple traits, a single trait can be influenced by multiple genes (i.e., polygenic traits)[11], and in some cases different genes can even produce the same phenotype[12]. Polygenic traits have been shown to be important for tolerance to temperature[12,13], hypoxia[13] and pH[4]. Genetic redundancy in polygenic traits may be especially important in multivariate environments since multiple pathways can lead to the same phenotypic outcomes, allowing for more genetic flexibility. In addition to the importance of pleiotropy and polygenic traits in shaping species adaptive responses to global change, increases in the strength of selection may become more likely as the number of environmental drivers increases[10]. Increased selection intensity could either lead to rapid adaptive

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evolution or further constrain adaptive evolution through reductions in population size, which may prove detrimental if large populations are necessary to maintain rare beneficial alleles[4]. Although it is crucial to further our mechanistic understanding of species' adaptive responses (i.e., genetic changes), measurements of intraspecific variation in traits (due to adaptation, acclimation, or plasticity) across a species' range can provide useful information about a species' capacity to adapt to multivariate change.

Recent work[14] has shown that understanding a species' response to a single dominant environmental driver may be particularly useful in predicting species' responses to changes in multiple environmental drivers. Although this approach may prove useful in some systems, it may also be less accurate in tightly coupled multivariate environments where organisms are prepared for specific co-varying conditions that influence physiological responses. For instance, a species' stress response to one environmental driver can prime an individual for exposure to a second environmental driver due to shared signal pathways (crosstalk) or protective mechanisms (cross-tolerance)[15]. If the environmental signals change rapidly and/or in opposing directions, cross-talk or cross-tolerance mechanisms could become ineffective or even an unnecessary expense. However, there is some evidence that mechanisms to cope with simultaneous changes in multiple stressors may be more general (e.g., heat shock proteins, antioxidants, detoxification enzymes) and therefore could be

advantageous regardless of the identity of any specific environmental factor[16]. Further insights into the relative importance of dominant drivers versus changes to covariance in shaping species responses will be crucial to improving our ability to predict future ecological change.

Due to the relatively recent technological advances that have made high resolution measurements of oceanographic conditions (e.g., pH, dissolved oxygen) in marine systems widespread, there is increasing recognition that small-scale variation in a broad range of environmental drivers exists and can lead to local adaptation and acclimation within marine organisms[17]. Within Eastern Boundary Upwelling systems, marine species experience dynamic oceanographic conditions that vary both spatially and temporally[18–20]. During seasonal upwelling events, cold waters that are reduced in pH and dissolved oxygen (DO) are brought to the surface. Differences in the strength and magnitude of upwelling creates a persistent mosaic of environmental conditions at small spatial scales[19,21]. For example, in the California Current System (CCS), northern and central California experience more frequent and intense upwelling compared to southern California, although "shadow zones" of less intense upwelling also occur within these regions[22]. There is emerging evidence for local selection (which could lead to local adaptation) under exposure to environmental conditions associated with upwelling within the CCS, including species with high pelagic larval durations[4–7,23–25]. It is still unclear, however,

whether local adaptation/acclimation confers greater resilience to future changes in both the mean and covariance in multiple environmental variables.

Climate change and ocean acidification (OA) are expected to progress rapidly in the CCS, resulting in warmer, more acidic and lower DO conditions[26]. These changes in mean conditions may be especially important for species in the CCS, where these same three environmental drivers (i.e., temperature, pH, DO) are negatively correlated with upwelling. Therefore, predicted changes in the mean due to climate change and OA (increases in temperature, but decreases in pH and DO) will also alter their covariance. The covariance between temperature, pH and DO is especially strong during upwelling season[27]. Therefore, regions within the CCS that are more influenced by upwelling are also likely to experience greater deviations in the covariance structure of these three environmental drivers in response to global change compared to regions where environmental conditions are less influenced by upwelling.

We used a network of chemical sensors along the CCS to first characterize the natural covariance of temperature, pH and DO in kelp forests from a region of intense upwelling (northern California) and a region of weak upwelling (southern California)[19,22]. We then assessed the population level differences in performance (i.e., survival, growth, calcification, metabolism, grazing) of juvenile red sea urchins (*Mesocentrotus franciscanus*) consistent with local adaptation or

acclimation across current mean pH, DO and temperature conditions for each region in a common garden laboratory experiment. *M. franciscanus* was used in this experiment since it is an economically important fisheries species [28] and ecologically important grazer[29–31]. *M. franciscanus* is found along the west coast of North America as far south as Baja, Mexico and as far north as Alaska and extending around the Pacific rim to Japan[32]. Despite the potential for high gene flow via extended planktonic larval durations (62-131 days[33]) to limit local adaptation, work on *M. franciscanus* has shown genetic differentiation across populations due to both pre and post settlement selection[32]. Within this same experiment, we tested for population divergence in response to region-specific projected future changes in pH, DO and temperature - where the covariance between these factors is altered compared to the covariance associated with upwelling. We hypothesized that if species are adapted/acclimated to local environmental regimes, then populations from intense upwelling regions are likely to be more vulnerable to global change due to changes in the covariance structure since temperature, pH, and DO are more tightly coupled in nature than populations from regions of weak upwelling, where temperature, pH and DO have been less tightly coupled historically.

REGIONAL DIFFERENCES IN ENVIRONMENTAL REGIMES

Semi-continuous measurements of temperature, ocean pH, and DO between regions of strong (Point Arena, Van Damme) and weak (Catalina Island, Laguna

Beach) upwelling in the CCS showed differences in mean conditions across sites (Fig. 1). Specifically, at Point Arena and Van Damme pH = 7.78 ± 0.14 and 7.72 ± 0.12, temperature = 10.60 ± 1.55 °C and 10.80 ± 0.92 °C, and DO = 5.82 ± 1.74 mg L^{-1} and 6.22 ± 1.61 mg L^{-1} respectively, while at Catalina Island and Laguna Beach pH = 8.02 ± 0.05 and 7.98 ± 0.12, temperature = 17.27 ± 2.31 °C and 15.86 ± 1.46 °C, and DO = 7.73 mg L^{-1} ± 0.45 and 7.47 ± 0.54 mg L^{-1} (mean ± SD) respectively. We also reveal region-specific patterns of environmental covariance between strong and weak upwelling regions (Fig. 1b, c). Although we found significant relationships between pH and DO, pH and temperature, and DO and temperature across all sites, the strength of these relationships differed greatly between regions (Table S1). At sites exposed to strong upwelling, pH and DO were tightly coupled, such that decreases in pH corresponded to decreases in DO (Point Arena: R^2 = 0.81; Van Damme: R^2 = 0.78). Seawater pH and temperature, as well as DO and temperature, showed similar relationships in our strong upwelling region with low pH and DO corresponding to lower temperatures (Point Arena: pH vs Temp, R^2 = 0.58; DO vs Temp, R^2 = 0.55; Van Damme: pH vs Temp, R^2 = 0.47; DO vs Temp, R^2 = 0.37). Although significant, these relationships were weaker at sites experiencing weaker upwelling (Catalina Island: pH vs DO, R^2 = 0.38; pH vs Temp, R^2 = 0.04; DO vs Temp, R^2 = 0.05; Laguna Beach: pH vs DO, R^2 = 0.16; pH vs Temp, R^2 = 0.02; DO vs Temp, R^2 = 0.07). This suggests that in regions experiencing strong upwelling, individuals are exposed to more predictable combinations of environmental conditions (i.e.,

a given pH only occurs for a narrow range of DO concentrations and temperatures).

Juvenile *M. franciscanus* from three replicate populations from these regions of intense upwelling versus relatively weaker upwelling were raised in current and projected future conditions for each region. Current conditions were based on the mean temperature, pH and DO conditions determined from sensor data, while future conditions were based on regional CCS climate projections for the year 2100[34]. Results of PCA analysis of *in situ* and experimental environmental data for the experiments highlight how the experimental treatments aligned with the range in conditions currently experienced in the field. PC1 accounted for 91% of the variability and was primarily associated with temperature, while PC2 accounted for an additional ~8% of variation and is associated with pH and DO (Fig. 2). Importantly, experimental treatment conditions representing current conditions within each region plot within the range of values experienced in the field, while future conditions generally fall outside of these conditions. Notably, future conditions within our weak upwelling region partially overlap conditions currently experienced in the field due to the wide range of pH and DO conditions experienced at a given temperature in this region (Fig. 2). Future conditions within our strong upwelling region deviate from conditions currently experienced in the field more than within our weak upwelling region due to the tighter coupling of pH, temperature and DO.

Individuals within our strong upwelling region experience a much narrower range of temperatures for a given pH and DO combination, resulting in warming creating conditions unlikely to occur in this region at present. Therefore, future changes, due to global change and ocean acidification, will have differential impacts on the covariance of pH, temperature and DO across regions, leading to more novel conditions in strong versus weak upwelling regions.

EVIDENCE FOR LOCAL ADAPTATION/ACCLIMATION

After three months in the common garden lab experiment (representative of current conditions), sea urchins had significantly higher survival in their respective home environments (Table S2). In particular, mortality increased among the populations from weak upwelling when raised in strong upwelling conditions, compared to the populations from strong upwelling conditions (Fig. 3a, Table S3). We also find increased mortality among the populations from strong upwelling when raised in weak upwelling conditions, compared to the populations from weak upwelling conditions (Fig. 3a, Table S3). These results provide evidence in support of our hypothesis that *M. franciscanus* is locally adapted/acclimated to environmental regimes along the coast of California.

Although both populations performed better in their home regimes, mortality was higher in weak upwelling conditions compared to strong upwelling conditions overall, which may be driven by thermal stress. Although we are not

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able to tease apart the effects of any single environmental driver on these results, given that we manipulated temperature, pH and DO in combination to reflect their covariance in nature, it's likely that thermal stress contributed to increased mortality in the current weak upwelling conditions. This interpretation is based on DO concentrations being similar between the two current treatments and pH being higher in the weak upwelling treatment (i.e., higher pH should be less stressful; Table S4). Therefore, sea urchins from weaker upwelling conditions may have higher survivorship in these conditions due to a higher thermal tolerance to cope with the naturally warmer seawater temperatures associated with the region. It is unclear, however, whether there is a cost to increased thermal tolerance in the sea urchins adapted/acclimated to warmer, weak upwelling conditions.

Growth and net calcification also differed across treatments and populations, perhaps due to differences in energetic demands across environmental regimes and populations (Table S2). We reveal significantly higher growth and net calcification in sea urchins originating from our weak upwelling region when exposed to both current weak and strong upwelling conditions, compared to urchins originating from our strong upwelling region (Fig. 3b, c, Table S3). Although sea urchins from weak upwelling conditions have higher growth and net calcification in current conditions overall, the magnitude of the differences in growth differed across environments (Table S3). Growth in sea urchins from

weak upwelling were 350% higher than sea urchins from strong upwelling

conditions in weak upwelling conditions compared to just 34% higher in strong

upwelling conditions. This result is surprising since energetic gains, via

consumption, were greater in weak upwelling conditions than strong upwelling

conditions (Fig. 3e, Table S2). Therefore, it's likely that increases in consumption

were unable to fully offset the increased energetic demands in weak upwelling

conditions and that urchins from weak upwelling sites were better prepared to

compensate for these increased energetic demands.

Among survivors, differences in growth and net calcification across populations

and treatments may also be partially explained by differences in energy

allocation strategies associated with body condition and ecosystem properties in

each region. For instance, we found that metabolic rates were elevated in

individuals from strong upwelling conditions, compared to those from weak

upwelling conditions (Fig. 3d Table S2). These results suggest that energetic

costs are higher for urchins from strong upwelling regions, possibly due to the

increased metabolic demands of maintaining reproductive tissue. Body

condition (gonad:somatic tissue ratio) was greater in sea urchins from the sites

with strong upwelling both initially and after 3 months in experimental

treatments, likely due to factors unrelated to the environmental conditions

manipulated here (Fig. S1a,b, Table S2). Although all sea urchins in our study

were collected from barrens, differences in the duration and extent of barren

history across populations likely led to differences in the initial condition of sea urchins (e.g., barren conditions from regions of weak upwelling are much older[35] and more persistent than those in regions of strong upwelling[36]). To account for these differences, sea urchins were reared in the laboratory for three months and fed weekly. Past studies have shown that gonads of the purple sea urchin, *S. purpuratus,* can recover from starvation after 2-3 months[29]. After 3 months of ad libitum feeding and prior to the start of our experiment, we still found significantly lower body condition in sea urchins from sites in our weak upwelling region. Regardless of the environmental conditions, sea urchins from the weak upwelling sites maintained lower body condition throughout the common garden experiment, but also demonstrated higher growth and net calcification rates.

Together, these results suggest that sea urchins from the different populations may have been allocating energy differently (i.e., prioritizing overall growth and net calcification vs. gonad production). This hypothesized difference in energy allocation could be due to the differences in the duration of time spent in a starved state (noted above), phenology, or ecological factors unrelated to the environmental conditions. For example, differences in the phenology of gametogenesis, which occurs over multiple seasons in sea urchins, could explain differences in gonad production between sites. The timing of egg production and development has been shown to differ across latitude for other marine species

due to differences in temperature regimes[37]. Seasonality can also lead to differences in energy partitioning between growth and reproduction, if attaining a larger size leads to higher over-wintering survivorship[38]. Alternatively, trade-offs between growth and other processes could explain the differences seen here across populations[39,40]. For instance, higher predation rates can select for rapid growth to larger sizes in fishes[41]. It's possible that predation rates on sea urchins from northern CA are significantly lower than in southern CA, and therefore, northern CA sea urchins allocate more energy to reproductive output than somatic growth. Future work assessing the role that environmental and ecological factors (e.g., barren history, phenology, predation) play in shaping energy allocation across populations of sea urchins will be important to understand the mechanistic underpinnings of differences in species responses to environmental change.

LOOKING TO THE FUTURE

Populations of sea urchins from weak upwelling regions were more susceptible to future environmental conditions than sea urchins from strong upwelling regions. After three months in the projected future conditions for each region, we found significant increases in mortality between current and future treatments for both populations (Fig. 4a, Table S5, S6). Mortality increased from 0.0001 to 0.0125 N day^{-1} from current to projected future conditions in the populations from strong upwelling and from 0.0135 to 0.0463 N day^{-1} from the

current to projected future conditions in the populations from weak upwelling conditions. The sharper increase in mortality in the populations from weak upwelling conditions is particularly interesting given that the change in future conditions in each region were of similar magnitude (approximately +2.5°C, -0.2 pH units, -2.0 mgL^{-1}). These results suggest that even locally adapted/acclimated populations are likely to respond differently to the same degree of environmental change, perhaps due to thresholds in tolerance.

Our results indicating higher vulnerability of the warm-adapted/acclimated populations from regions of weak upwelling are in line with past work on range shifts and thermal physiology that suggest additional warming within warm regions of a species range has the potential to push species beyond thermal tolerance limits, leading to localized extinction[42,43]. However, our short-term (83 day) lab experiment does not capture many important aspects of global change that occur over time. For example, recent work by Coleman et al.[44] found that mass mortality of the kelp *Ecklonia* due to a marine heat wave led to "genetic tropicalization," whereby surviving individuals and new recruits had a shift in alleles from cool water types to warm water types. Similarly, Brennan et al.[4] demonstrate shifts in allele frequencies due to differential survival of larval purple sea urchins, *Strongylocentrotus purpuratus,* exposed to extreme pH. These studies suggest that exposure to extreme environmental conditions associated with global change can lead to rapid evolution of more tolerant phenotypes,

which may be beneficial as changes in the mean occur more slowly. We were unable to measure the underlying genetic changes due to differential mortality in our study. Although our work suggests higher vulnerability of the populations near the warm edge of the range for *M. franciscanus* to future change, it is unclear whether red sea urchins will experience a range contraction due to environmental change or adapt to changing conditions via "genetic tropicalization" from warm adapted phenotypes. Future work focused on understanding shifts in underlying allele frequencies could provide insights into the potential of evolutionary rescue of southern populations at risk to future climate change.

Climate change and ocean acidification have been shown to reduce growth and calcification across a wide range of species[45]. Here, we also show that growth and net calcification were significantly reduced in future ocean conditions, but there was no effect of population origin (Fig 4b,c, Table S5). The lack of population effect suggests that growth and net calcification are currently and will continue to be similar across these regions. However, we also found a significant effect of population origin on body condition (Fig. S1c, Table S5), with strong upwelling populations showing higher gonad to somatic tissue ratios than weak upwelling populations. Although it is still unclear why body condition was initially higher in sea urchins from strong upwelling populations, the maintenance of reproductive tissue while maintaining growth rates similar to

sea urchins from weak upwelling conditions suggests that energetic costs may

be elevated for sea urchins from weak upwelling conditions. We did find

elevated grazing rates in individuals from our weak upwelling populations

compared to our strong upwelling populations (Fig. 4d, Table S5). The increased

mortality and lower body condition in weak upwelling populations suggests,

however, that these elevated grazing rates were unable to fully compensate for

the increased energetic demands in weak upwelling conditions. Importantly, sea

urchins were fed bi-weekly in our experiment to ensure sufficient growth, but

access to food is an issue for sea urchins inhabiting urchin barrens in nature.

Therefore, our results likely underestimate the true effects of global change on

sea urchin populations and future work should assess the impacts of sea urchins

across levels of food availability.

CONCLUSION

Differences in environmental conditions that occur across a species range can

lead to local adaptation and or acclimation. Much research on global change in

the ocean, however, does not consider the role that evolutionary processes play

in mediating species responses. These issues may be especially important in

ecosystems with tightly coupled environmental conditions, where changes in the

covariance structure may make it more difficult for species to adapt to climate

change. Here, we find evidence of local adaptation/acclimation to environmental

regimes in red sea urchins, *M. franciscanus*, which likely contributed to the

differential effects of future environmental change across populations. Importantly, this work supports more recent efforts to improve ecological models predicting the effects of climate change and ocean acidification on marine species and ecosystems by incorporating intraspecific variation[8,46].

For the red sea urchin, *M. franciscanus,* we show evidence consistent with local adaptation/acclimation to complex, multivariate environmental regimes, with the populations from warmer and weaker upwelling conditions showing higher survival in their home environment than those populations from strong upwelling conditions and populations from cooler and stronger upwelling conditions showing higher survival in their home environment than those populations from weak upwelling conditions. Despite evidence for local adaptation/acclimation of the populations from the warmer, weaker upwelling conditions, we show that these populations may be more vulnerable to projected future changes. These findings contrast with our hypotheses that populations from intense upwelling conditions would be more vulnerable to future change due to the tight coupling of environmental drivers in these regions and alterations in the covariance structure with future change. Instead, higher vulnerability of the locally adapted/acclimated population from warmer, weaker upwelling suggests that thresholds in tolerance for single drivers may be as or more important than changes in covariance over the range of conditions used here. These results are consistent with previous findings[14] that biotic responses

can be driven by a single dominant environmental driver. Furthermore, our results suggest that using a species response to global change from one population to predict another populations' response may not be appropriate[37]. Across populations, differences in energetic costs and energy allocation strategies likely play an important role in how species respond to future environmental change. Future work linking the molecular and physiological underpinnings of differences in species responses to multivariate environmental change across populations are crucial to gaining a more mechanistic understanding of how and why species abundances and distributions might shift in the future.

MATERIALS AND METHODS

Environmental Monitoring: To determine the mean and covariance in environmental conditions that organisms currently experience within kelp forests along the coast of California, we established an array of monitoring locations for the deployment of autonomous pH, temperature, and dissolved oxygen sensors. We chose two sites in northern California (Point Arena, 38.9460° N, 123.7389° W; Van Damme, 39.2711° N, 123.7948° W) and southern California (Laguna Beach, 33.5421° N, 121.9459° W; Catalina Island, 33.4412° N, 118.4654° W; Fig 1a). Our northern California sites experience stronger upwelling and will be referred to as "strong upwelling" sites, whereas our southern California sites experience weaker upwelling and will be referred to as

"weak upwelling" sites. We collected data continuously (every 10 min) from ~November, 2017 through August, 2021, with the exception of some gaps in measurements due to sensor malfunctioning that typically occurred during the first two years of data collection. Custom built pH and temperature sensors containing the Honeywell DuraFET pH sensors were used for this study[47]. The pH sensors were calibrated by injecting the flowcell with equimolar Tris in artificial seawater solution[48], a standard pH solution for seawater pH[49]. Sensors were calibrated at the time of deployment and recovery of each deployment, and the calibration from the recovery was preferentially used. DO was measured using a MiniDOT, measuring every 10 minutes (Precision Measurement Engineering), co-located to the pH sensor. These sensors were calibrated in DO saturated seawater prior to each deployment[47]. We determined the relationships between pH and temperature, pH and DO and temperature and DO at our sites using linear regression.

Collection Sites: We identified three sites in our strong upwelling region and three sites in our weak upwelling region to collect red sea urchins, *Mesocentrotus franciscanus,* for our experiment examining, 1) evidence for local adaptation to environmental regimes and 2) testing the effects of future environmental change across populations. We collected *M. franciscanus* individuals using SCUBA (~10 m water depth) from strong upwelling sites at Point Arena, CA (38.9460° N, 123.7389° W) on October 7, 2020, Van Damme, CA

(39.2711° N, 123.7948° W) on October 19, 2020, and Noyo reef, CA (39.4283° N, 123.8107° W) on November 5, 2020, and from weak upwelling sites at White Point, CA (33.7125° N, 118.3185° W), Point Vicente, CA (33.7400° N, 118.4140° W), and Hawthorne Reef, CA(33.7470° N, 118.4159° W) on November 18, 2020. We chose strong upwelling sites due to their proximity to two oceanographic monitoring sites (Point Arena, CA and Van Damme, CA) with which we have long-term data to characterize pH, temperature and dissolved oxygen conditions. Since red sea urchins are not as common in southern CA, we chose our weak upwelling sites based on local knowledge of red sea urchin abundances and proximity to existing HOBO (Onset) temperature logger data. After collection, we placed sea urchins in a dry cooler sandwiched between kelp and immediately transported them to Long Marine Laboratory (LML) at the University of California, Santa Cruz. Upon arrival at LML, we immediately placed urchins from different sites into separate water tables and supplied them with flow-through seawater from just off-shore of the marine lab. We fed sea urchins fresh giant kelp, *Macrocystis pyrifera,* once a week until the start of the experiment (~ 3 months).

Mesocosm System: The mesocosm system at Long Marine Laboratory is supplied with ambient UV-filtered seawater. This seawater flows into two large (500 gallon) sumps, a "hot" sump that warms incoming ambient seawater to ~ 24°C via three 9000W heaters (Optima Plus Compact Aquatic Heater, Aqua

Logic, Inc.), and a "cold" sump that is chilled to ~8°C by a water-cooled chiller (Multi Temp Water-Cooled Marine Duty Chiller, Aqua Logic, Inc.). We plumbed seawater from both sumps to a temperature blending valve system (TBS, Aqua Logic, Inc.) where we blended "hot" and "cold" seawater to create four static temperature conditions representing current and future conditions in both northern and southern CA. Each temperature treatment fed three replicate 5-gallon "header" buckets fit with a gamma lock seal containing a DuraFET pH probe and DO sensor (GoDirect Optical Dissolved Oxygen, Vernier). Each header tank supplied flow-through seawater to two replicate "bins" that housed the sea urchins in our study. To manipulate the pH and DO of our treatment water, a third sump was used to create cold, acidic and low DO seawater. This sump was supplied with "cold" seawater from the same "cold" seawater sump used to supply the blending valves. Pure CO_2 was continuously bubbled into the seawater sump, until it reached a desired setpoint of pH = 7.3. The pH of this tank was controlled with a feed-back loop using a DuraFET pH sensor and a custom Labview program that actuated a mass flow controller (MFC, SmartTrak 50; Sierra Instruments) to allow the flow of CO_2 into the sump. DO of the "upwelled" sump was manipulated by continuously bubbling pure N_2 gas at a rate of ~10 L min^{-1}. N_2 gas was supplied via a nitrogen generator (MNG-1010, Compressed Gas Technologies, Inc.). Although we did not control or monitor the dissolved oxygen concentrations in the acidic/low DO sump, preliminary testing suggested that the dissolved oxygen concentration was ~4.0 mgL^{-1}.

All 12 header buckets (3 buckets at 4 temperature levels) mixed seawater from the TBS with small amounts of acidic/low DO seawater using a feedback system. Briefly, the feedback system triggered solenoids to open and allow acidic/low DO seawater in whenever pH drifted above a desired setpoint. Since pH and DO are coupled in this system, our pH-control created four distinct temperature, pH and DO treatments mimicking current and future projected conditions at each location (Strong upwelling current, pH = 7.8, DO = 8.0 mgL^{-1}, temperature = 10°C; Strong upwelling future, pH = 7.6, DO = 6.0 mgL^{-1}, temperature = 13°C; Weak upwelling current, pH = 8.0, DO = 8.0 mgL^{-1}, temperature = 16°C; Weak upwelling future, pH =7.8, DO = 6.0 mgL^{-1}, temperature = 19°C). These temperature and pH treatments represent the mean temperatures measured within the kelp forest at each region during our monitoring period and a +3°C, -0.2 pH unit future treatment based on projected regional warming and acidification by the end of the year 2100[34]. Due to logistical difficulties scrubbing oxygen from our system, DO concentrations are slightly higher than current and future conditions for both regions, but represent conditions currently experienced by organisms within each location and are consistent in direction with expectations (i.e., future conditions are ~2mgL^{-1} lower than current). Between ~ 10:00 - 14:00 every day, we measured pH, temperature, dissolved oxygen, and salinity in each bin using a multimeter (YSI Quatro, Yellow Springs Instruments, Inc.). We collected discrete samples for total alkalinity (TA) and

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spectrophotometric pH from each Header bucket and Bin containing sea urchins every two-three weeks for the duration of the experiment (N = 6 time points). Using best practices[50], we made spectrophotometric pH measurements using *m*-cresol purple (Shimadzu UV- 1800, Shimadzu) and TA measurements using open-cell titration (905 Titrando, Metrohm). Instruments were validated using certified reference materials from the lab of Dr. Andrew Dickson (Scripps Institution of Oceanography) at the beginning and end of each day that samples were processed. We used TA and spec pH measurements from discrete samples, salinity and temperature from YSI measurements and stoichiometric dissociation constants defined by Mehrbach et al.[51] and refit by Dickson & Millero[52] to calculate the entire carbonate system across treatments and to calibrate DuraFET electrodes within Header buckets.

To assess the potential of adaptation/acclimation to local environmental regimes and understand the effects of future environmental change on sea urchins, we reared *M. franciscanus* individuals in a common garden experiment exposing individuals to current and future regimes for 83 days (February 13, 2021 – May 15, 2021). Within each replicate treatment bin, sea urchins (N = 1-2 for each site from weak upwelling, N = 3 for each site from strong upwelling) were placed in individual 0.5 L cages where they were fed ~1 g of kelp twice a week for the duration of the experiment. If kelp was present in the cage at the time of feeding, which was usually the case, it was replaced by fresh kelp.

Survival: We recorded the deaths of sea urchins across treatments and populations for the duration of the experiment. We opened all cages daily and visually assessed each individual urchin. If an urchin died, we immediately removed it from the experiment. For each day of the experiment, we calculated the number of surviving urchins.

Growth and Net Calcification: Before being placed into their respective experimental treatments and after 83 days in treatment conditions, we wet weighted and buoyant weighted each urchin to calculate a relative growth and net calcification rate. Due to the large number of urchins in the experiment, we measured all individuals from just one site at a time and placed them in the system on the same day. Therefore, the experiment's start days and end days were staggered across sites over approximately one week and no anomalies in environmental conditions occurred during these time periods that may confound the results.

We measured wet weights by first carefully patting each sea urchin with a paper towel to remove large water droplets. We then placed sea urchins on a scale and measured their weight to the nearest 0.001 g. To obtain buoyant weights, a proxy for calcified biomass[53], we placed sea urchins in a basket connected by monofilament to the bottom of weigh-below balance. The basket (with sea

urchin) was fully submerged in seawater and measured to the nearest 0.001 g.

We calculated the relative growth rate, RGR, as:

$$RGR = \left(log\left(\frac{WW_F}{WW_I}\right)\right) * 100,$$

where WW_I and WW_F are the initial and final (after 83 days) wet weights

respectively. We calculated relative net calcification rate, RCR, as:

$$RCR = \left(log\left(\frac{BW_F}{BW_I}\right)\right) * 100,$$

where BW_I and BW_F are the initial and final (after 83 days) buoyant weights

respectively.

Gonad:Somatic Tissue: Since gonad production can be a proxy for body

condition in sea urchins, we were interested in assessing the impacts of

environmental conditions on the gonadal to somatic tissue ratios. At the

beginning and end of the experiment, we dissected sea urchins to separate

gonad tissue from the remaining somatic tissue using forceps. At the beginning

of the experiment, we sacrificed individuals from each site to understand

differences at the outset of our experiment. Gonad and somatic tissues were

placed into foil packets and dried in the drying oven at 80 °C for 24 hours. Foil

packets with dried tissue were weighed to 0.001 g before being placed in a

muffle furnace at 550 °C for 8 hours to obtain ash free dry weights (AFDW).

Following combustion, foil packets were reweighed, tissue weight (gonad or

somatic) was calculated as the change in weight before and after combustion (AFDW), and the gonad to somatic tissue ratio, G:S, was calculated as:

$$G{:}S = \frac{G_{AFDW}}{S_{AFDW}},$$

where G_{AFDW} is the AFDW of gonad tissue and S_{AFDW} is the AFDW of somatic tissue.

Grazing and Metabolism: After 83 days of exposure to our treatment conditions, we measured the grazing and metabolic rates of sea urchins to assess whether future environmental conditions alter the balance between energetic costs (metabolism) and energetic gains (grazing) and to assess the potential of local adaptation/acclimation to regional environmental conditions. 48 hours before grazing assays, we removed kelp from sea urchin cages and starved individuals to reduce the potential effects of digestive status on metabolism measurements. To measure standard metabolic rates of individuals, we followed methods outlined in Donham et al. (accepted). Briefly, we placed individual sea urchins into polycarbonate respirometry chambers with seawater from their respective treatment. In addition, we used control chambers (without urchins) to measure the effects of water-column processes on changes in DO over time. We sealed chambers from the external environment and used a stir bar within each chamber to continuously mix seawater and a dissolved oxygen sensor spot (PSt3, PreSens Precision Sensing GmbH) to measure DO within the chamber

using a fiber optic oxygen reader (Fibox 4, PreSens Precision Sensing GmbH). We placed sealed chambers on a multi-position magnetic stirring system (2mag MIXdrive) submerged in a water bath maintaining the respective treatment conditions with 5 to 8 sea urchins (in southern CA assays) or 6 to 15 sea urchins (in northern CA assays) and 3 control incubations run simultaneously. Differences in sample sizes were due to differences in initial samples sizes between regions/sites and differential mortality across treatments. We measured dissolved oxygen concentrations 7 times over an ~30 min incubation and used local linear regression to fit measurements of DO as a function of time using LoLinR in R[54]. All incubations were approximately linear over the duration of the incubations and were not allowed to fall below 3.75 mgL^{-1}. We corrected the slopes (metabolic rate) of each sea urchin with an average of the slopes of controls that were run in the same assay. SMR was mass-corrected using the mean mass of all individuals and mass-correction equations from Steffanson et al.[55].

Following metabolic assays, we returned sea urchins to their respective treatments and presented them a pre-weighed disc of kelp (~7 cm diameter). After 24 hours, we removed and reweighed the remaining kelp disc. We calculated the mass-corrected grazing rate as the change in wet weight of kelp after 24 hours, divided by the mass of the individual sea urchin.

Statistical Analyses:

We ran two sets of models for each response variable to assess: 1) the potential of local adaptation to environmental regimes across *M. franciscanus* populations and 2) differences in the impacts of future environmental change across populations. We fit the number of surviving individuals to linear mixed models with *Time*, *Treatment*, *Population*, and *Mean weight* (calculated from initial weight at each time step) as fixed effects and *Site* nested in *Population* as random effects. Mean initial weight was used to control for any effect of size-dependent mortality. If a significant three-way interaction was found, contrasts were conducted on slopes of regressions calculated using *emtrends* in R to test whether 1) survival in current local conditions differ from the distant populations survival in the current local treatment (i.e. strong upwelling populations in current strong upwelling conditions versus weak upwelling populations in current strong upwelling conditions; weak upwelling populations in current weak upwelling conditions versus strong upwelling populations in current weak upwelling conditions), 2) survival in current local conditions differ from future local conditions (i.e. strong upwelling populations in current strong upwelling conditions versus strong upwelling populations in future strong upwelling conditions; weak upwelling populations in current weak upwelling conditions versus weak upwelling populations in future weak upwelling conditions).

We fit relative growth and net calcification rates to linear mixed models with *Population*, *Treatment* and *Weight* as fixed effects and *Header* nested in *Treatment*, *Site* nested in *Population*, and *Bin* nested in *Header*, which was nested in *Treatment* as random effects. We log-transformed the covariate of *Weight* to linearize the relationship between relative growth and net calcification rates and weight. If significant effects of *Population*, and *Treatment* were found, contrasts were conducted on estimated marginal means calculated using *emmeans* in R.

We fit initial gonad:somatic tissue ratio to linear mixed models with *Population* and *Weight* as fixed effects and *Site* nested within *Population* as a random effect. Finally, we fit final gonad:somatic tissue ratio to linear mixed models with *Population*, *Treatment* and *Weight* as fixed effects and *Header* nested in *Treatment*, *Site* nested in *Population*, and *Bin* nested in *Header* nested in *Treatment* as random effects. Since *Weight* was a covariate in these models, we removed non-significant interactions with *Weight* and re-ran statistical models.

We fit grazing and metabolic rate to linear mixed models with the same fixed and random factors for growth and calcification, excluding the fixed effect of *Weight* which was accounted for by mass-correction of grazing and metabolic rates. We log transformed grazing rate to meet assumptions of normality. Since zeros were present in our non-transformed grazing data we first transformed grazing rates using the equation, $G_t = log\ log\ (G_r + C)$, where G_r is the non-

transformed grazing rate, G_t is the transformed grazing rate and C is a constant added so that grazing rates are greater than 0. We chose a value C equal to 10% of the mean to have little effect on G_r since values of G_r ranged between 0 and 1. All models were fit using *lmer* in R.

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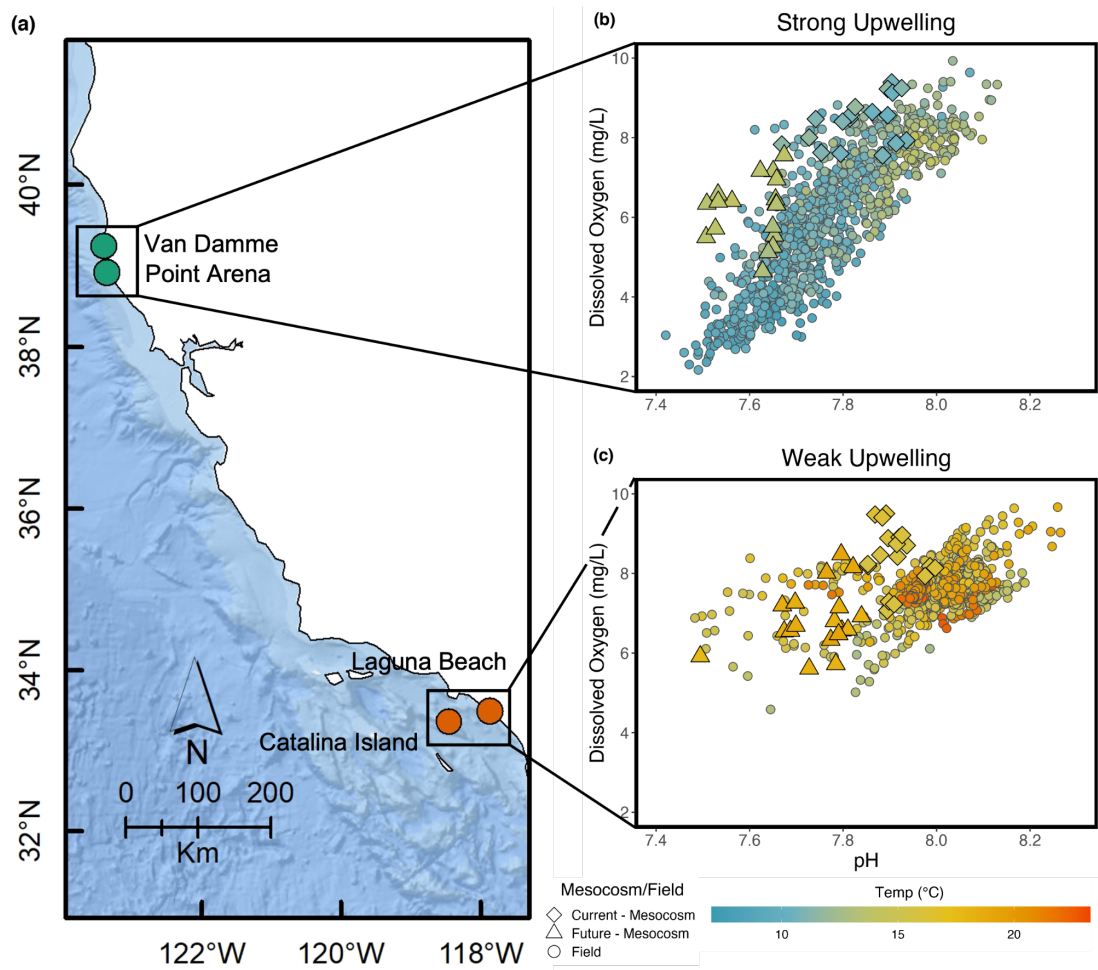
Fig 2.1. Locations of sensor moorings along the coast of California are shown in (a). Diamond and triangle symbols indicate discrete sample measurements within experimental mesocosms for current and future treatments respectively, while circles indicate daily mean conditions in the field. Scatterplot of time series data from oceanographic sensors deployed at ~15 m depth within kelp forests with daily mean experimental conditions as colored points. Data are from, (b) two sites (Van Damme and Point Arena) exposed to strong upwelling; and (c) two sites (Laguna Beach and Catalina Island) exposed to weak upwelling.
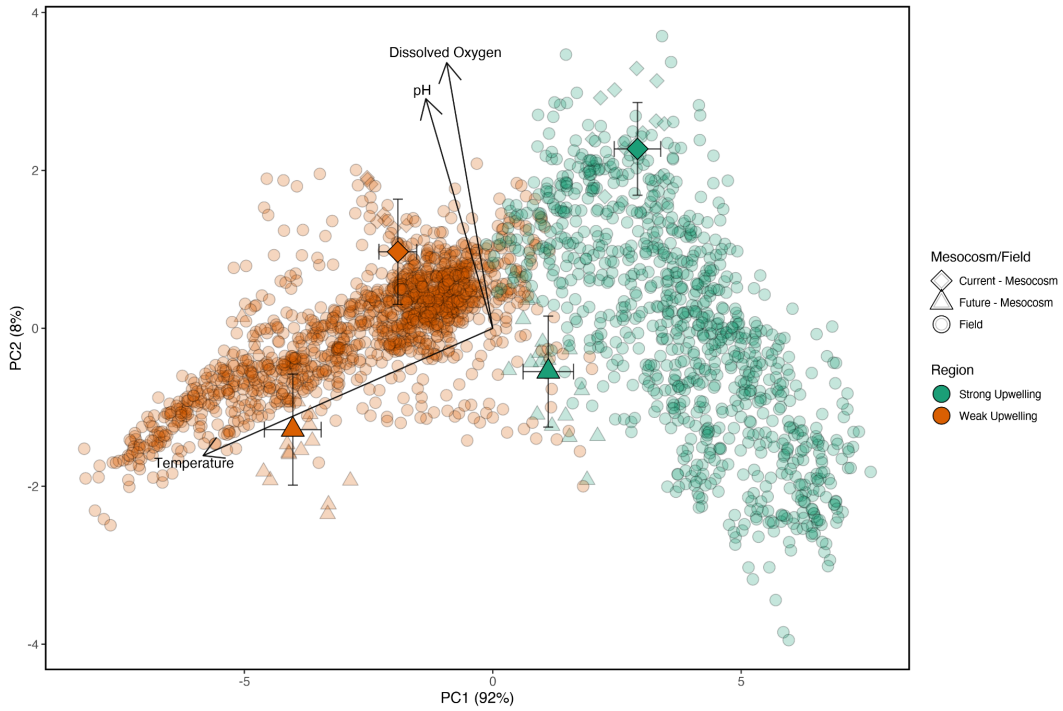
Fig 2.2. PCA plot of *in situ* environmental (field) and laboratory (mesocosm) experimental conditions. Green symbols represent strong upwelling (cooler temperature) conditions, while orange indicates weak upwelling (warmer temperature) conditions. Diamond and triangle symbols indicate discrete sample measurements within experimental mesocosms for current and future treatments respectively, while circles indicate daily mean conditions (pH, DO, temperature) in the field. Large symbols with error bars indicate mean ± SEM of PC scores for current and future experimental treatments for each region.
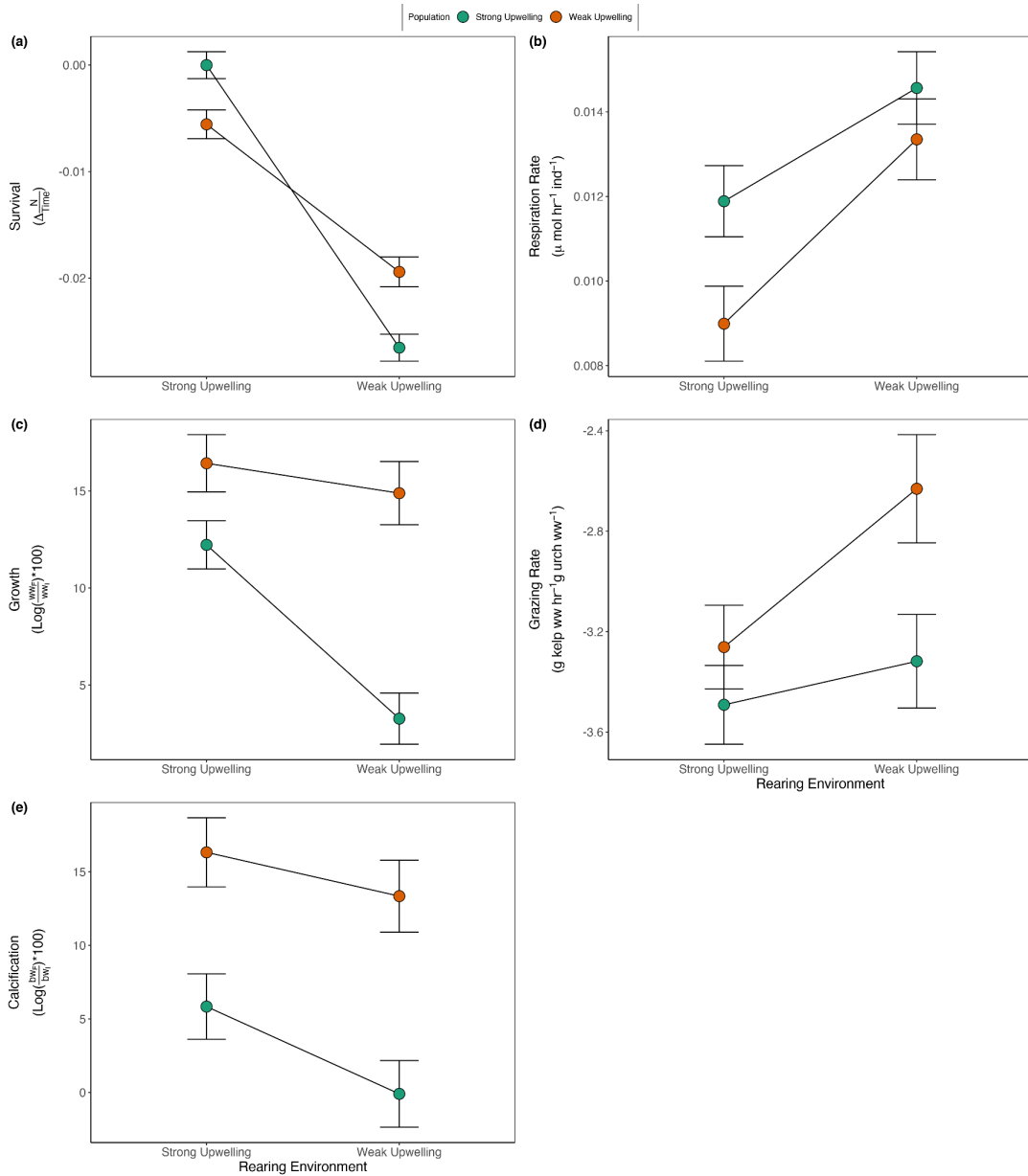
Fig 2.3. Species performance across sea urchin populations reared for 3 months in a common garden experiment with, (a) Survival; (b) Respiration rate; (c) Growth; (d) Grazing rate; (e) Calcification of sea urchins from strong upwelling and weak upwelling regions reared at current conditions for both strong and weak upwelling regions. Points represent mean and error bars indicate standard error.
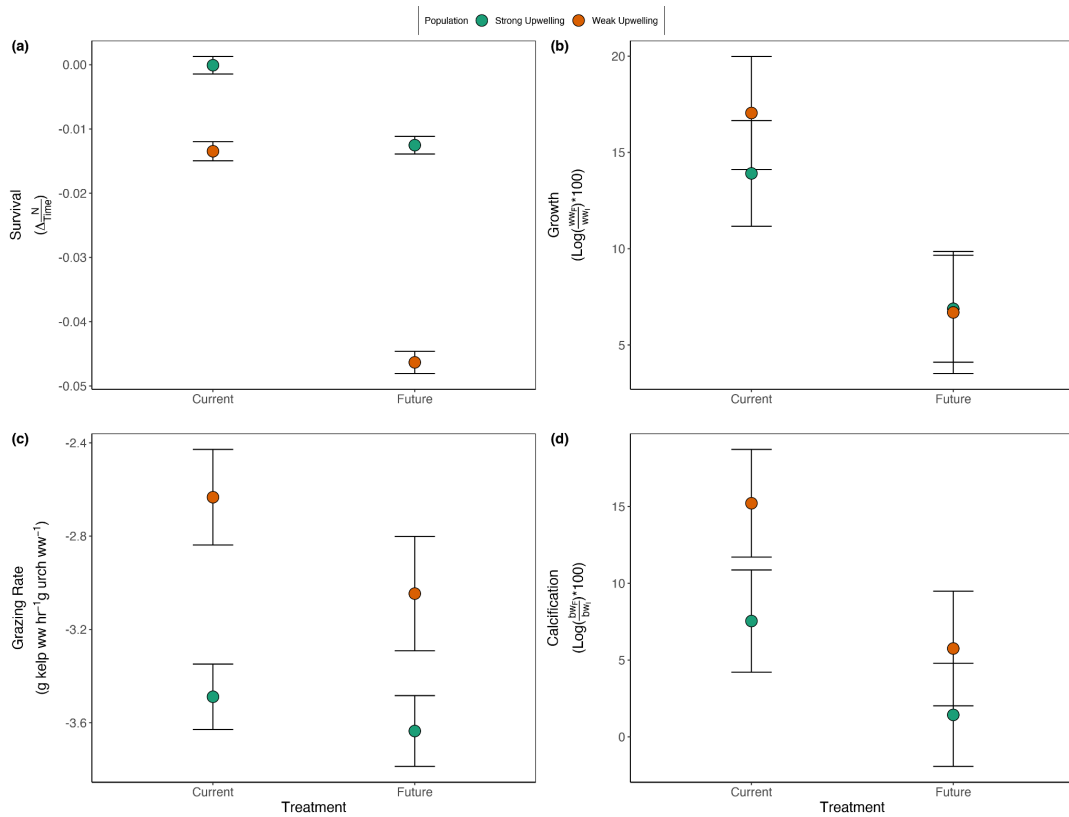
Fig 2.4. Species performance across sea urchin populations reared for 3 months in global change experiment with, (a) Survival; (b) Growth; (c) Grazing rate; and (d) Calcification of sea urchins from strong upwelling and weak upwelling regions reared at region-specific current and future conditions. Points represent mean and error bars indicate standard error.

CHAPTER 3

RETHINKING INTERACTIVE EFFECTS IN WARMING AND OCEAN ACIDIFICATION RESEARCH

CHAPTER 3: RETHINKING INTERACTIVE EFFECTS IN WARMING AND OCEAN ACIDIFICATION RESEARCH

ABSTRACT

Understanding species responses to multiple environmental drivers is especially important in light of rapid and ongoing anthropogenic climate change. In particular, "ecological surprises" present unique challenges to biodiversity and ecosystem functioning. Improving our ability to predict when and why these interactive effects between multiple climate change drivers occur will greatly improve our ability to manage and conserve these resources. Warming and ocean acidification (OA) present an ideal system to further our understanding of interactive effects of multiple drivers due to our robust, general understanding of the individual effects of each driver, as well as a large number of factorial studies to query for generalities. Here, we conduct a systematic review of 258 studies on the effects of warming and OA on organismal performance across a wide range of marine taxa. We first calculated the predicted cumulative effect (CE_P, based on effects of each driver in isolation) to better understand the interactive effects across multiple performance metrics (i.e., development, survival, growth, reproduction, calcification, photosynthesis, and metabolism). We find that overall, when CE_P was positive (denoting predicted increases in response variables), 11% of observations had no interactive effect, 63% were less pronounced, and 25% were more pronounced. When CE_P was negative, 9% of observations had no interactive effect, 63% were less pronounced, and 28%

were more pronounced. We also find strong linear relationships between CE_P and the observed cumulative effect, CE_O, although the slopes of these relationships were commonly less than 1, indicating the species' responses are often less pronounced than expected based on the CE_P. The prevalence of less pronounced effects across the wide range of species and responses tested here, indicates that species responses to multiple drivers are likely to be less pronounced than expected, perhaps due to underlying limits on performance.

INTRODUCTION

Anthropogenic activities are already causing dramatic changes to ecosystems worldwide. Although our understanding of the impending biological impacts of the rapidly accelerating changes in the environment have expanded substantially over the past two decades, our understanding of the emergent effects of global change is still limited by the potential for interactions between multiple drivers of change [i.e., a natural or anthropogenic pressure that causes a biological response, such as nutrient addition or hypoxia (Kroeker et al., 2017)]. Often referred to as multiple stressors, we use "drivers" here since the term "stressor" implies a harmful (negative) response, while driver is more inclusive (i.e., organismal response can be negative or positive). Much organismal research has focused on the effects of one or two drivers of change at a time. Yet, most human-influenced ecosystems are experiencing changes in two or more environmental drivers simultaneously (Breitburg et al., 1998; Halpern et al.,

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2008). Interactions among these drivers can cause ecological effects that are much more or less pronounced than expected based on the biological effects of the individual drivers (Folt et al. 1999). Unfortunately, our ability to predict the outcome of these interactions is quite limited (Crain et al., 2008; Darling & Cote, 2008; Jackson et al., 2016; Yue et al., 2017).

Past studies that have searched for generality in the interactive effects of multiple drivers have used a framework that defines a cumulative effect that is greater than the sum of individual effects in isolation as *synergistic* and an effect that is less than the sum of the individual effects as *antagonistic* (Crain et al., 2008; Folt et al., 1999). This framework requires that the cumulative effect be defined relative to the sign (positive or negative) of the individual effects in isolation (Folt et al., 1999). This framework can become problematic when the sign of the effect differs between driver pairs (e.g., one driver decreases growth, while a second driver increases growth). For example, the cumulative effect may be less than expected relative to the effect of one environmental driver, but greater than expected relative to the second environmental driver. Furthermore, the outcome (for the organism) of a synergism when the individual effects are both negative is very different than the outcome for a synergism when the individual effects are both positive. To address these issues, Piggott et al. (Piggott et al., 2015) developed a more detailed classification scheme that adds a response direction to each interaction type to yield five different interaction

types (additive, + synergistic, - synergistic, + antagonistic, and - antagonistic). Regardless of the classification scheme, however, antagonisms, synergisms and additive (no interaction) effects are often equally likely to occur based on meta-analyses (Crain et al., 2008; Piggott et al., 2015), and therefore don't appear to be particularly adept in predicting interactive outcomes (Orr et al., 2020). Therefore, although considering interactive effects in terms of antagonisms and synergisms may still be useful, it's clear that an alternate framework is necessary to improve our understanding of interactive effects.

Since the primary goal of most climate change studies is to better understand the physiological and ecological impacts of environmental change, a framework focused on the outcome of the cumulative effects for the organism (i.e., positive/beneficial or negative/detrimental) may prove especially useful for understanding when and why certain interactions between multiple drivers occur. In other words, the sign of the predicted cumulative effect describes whether concurrent changes in multiple drivers are predicted to be net beneficial or detrimental for an organism related to underlying tolerances. At the physiological level, differences in performance across an environmental gradient can be partially explained by energy-limited tolerance (Sokolova, 2013). In the energy-limited tolerance model, environmental stress reduces aerobic scope (the fraction of energy available after basal metabolic costs) via alterations to a suite of physiological processes (e.g. increased basal metabolism, oxygen supply

limitation, (Portner & Knust, 2007). A reduction of aerobic scope due to environmental stress limits the availability of energy for physiological processes (leading to decreased performance), while an increase in aerobic scope associated with non-stressful or beneficial environmental changes would increase energy availability (leading to increased performance). Reduced aerobic capacity may cause more pronounced negative interactive effects if there is less aerobic capacity to mount an energetically expensive stress response, while increased aerobic capacity may lead to more pronounced positive interactive effects as the scope for growth is widened.

Alternatively, there are limits to species' performance that could lead to less pronounced negative interactive effects or less pronounced positive interactive effects (Fig. 1). If, for instance, each environmental driver in isolation elicits a high magnitude positive effect on performance, the predicted cumulative effect may be greater than is physiologically possible for the organism. Negative responses may also lead to similar less pronounced negative interactive effects as mechanisms, such as cross-tolerance and cross-talk (Sinclair et al., 2013), also exist to prepare organisms for exposure to concurrent environmental drivers. Moreover, there may also be a lower limit in some physiological responses as well (e.g., mortality). Utilizing the sign of the predicted cumulative effect to test for generality in interactive effects removes some of the confusion surrounding

the former classification of interactive effects (i.e., synergisms/antagonisms) while linking the interaction outcomes to physiological underpinnings.

Standard meta-analysis techniques that calculate the mean effect of a moderator of interest across a broad range of studies may miss important relationships between the magnitude of effects and the outcome of the interaction. Instead, understanding how the magnitude of the predicted cumulative effect relates to the observed cumulative effect could provide further insights into the conditions in which interactive effects occur. For instance, high magnitude predicted cumulative effects that are negative may result in more pronounced (exacerbating) interactive effects if organisms are pushed beyond physiological thresholds. Alternatively, low magnitude predicted cumulative effects may result in no interaction since it's unlikely for small magnitude effects to produce large deviations. Therefore, it's likely that both the magnitude and sign of the cumulative effect are important for predicting multiple driver interactions.

Ocean warming and ocean acidification (OA) are global drivers that are likely to affect marine organisms worldwide. Given the extensive body of literature examining the cumulative effects of these drivers on marine organisms, it is an ideal system to explore new frameworks for the interactive effects of multiple drivers. While our understanding of how organisms will respond to ocean warming and/or OA has increased tremendously over the past few decades

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(Kroeker et al., 2013), our ability to predict their combined effects is still limited. In isolation, OA and warming can have positive or negative effects on marine organisms (Kroeker et al., 2010). In general, OA has negative effects (e.g., increased mortality, decreased growth, etc.) on a wide range of marine calcifiers [e.g., coralline algae (Cornwall et al., 2021), corals (Chan & Connolly, 2013), mollusks (Gazeau, 2008), echinoderms (Bednaršek, 2021; Dupont et al., 2010)] that rely on calcium carbonate to build their shells and skeletons. Conversely, OA can be beneficial (e.g., enhanced growth, increased survival) for some marine primary producers [e.g., seaweeds, seagrass (Harley et al., 2012; Koch et al., 2013)] that are able to utilize increased CO_2 and bicarbonate, HCO_3^-, to increase photosynthesis. Therefore, it appears that the effects of acidification may be, at least in part, an energetics problem, where increased energy allocation to the maintenance of calcified structures results in negative outcomes for calcifiers, while decreased costs/increased energy for processes such as photosynthesis results in positive outcomes for primary producers. The effects of warming, however, are largely dependent on where a species currently resides on its species-specific thermal tolerance curve. Organismal performance increases with increasing temperature until it reaches a thermal optimum, T_{opt}. Increased temperature beyond this optimum leads to a decline in performance. Therefore, if warming results in a temperature that is cooler than T_{opt}, the effect of warming should be positive, while warming that results in a temperature warmer than T_{opt}, should have a negative effect on the organism. Although the shape of the

relationship between temperature and performance is well defined (Brown et al., 2004), less is known about the shape of the relationship between pH and performance. Given the abundance of information about the effects of OA and warming on marine species and that these two environmental drivers are most commonly used in multiple driver experiments in marine ecosystems (Harvey et al., 2013; Kroeker et al., 2013), OA and warming are an ideal study system to further our understanding of interactive effects in ecological research.

Here, we test whether considering a species' predicted cumulative effect can improve our ability to predict its response to the combined effects of warming and OA by assessing: 1) the frequency of different interaction types in Warming*OA research, 2) how the magnitude of the predicted cumulative effect influences the outcome of the interaction, and 3) whether interaction types differ across taxa and response variables.

METHODS:

Literature review and data extraction

We searched the literature for factorial studies published from January 1, 2013 - July 2, 2019 that report the effects of temperature and OA on marine organismal performance. We used the Ocean Acidification International Coordination Centre (OA-ICC) ocean acidification database to conduct our literature search for the relevant keywords: ocean acidification and temperature. The OA-ICC database is

updated weekly with relevant studies on OA which are mined from searches for keywords (i.e. ocean acidification, acidification, acid, acidic, pH, CO2, "carbon dioxide" ocean) using Google, Biogeosciences, ScienceDirect, WebOfScience, and Frontiers. We screened 837 publications for relevance and determined 212 met our criteria for extraction. We only considered fully factorial experiments where the warming treatment was an increase in temperature between 1.5-6 °C, the acidification treatment corresponded to an ~0.2-0.4 pH unit decrease and the control conditions approximated present day conditions at the given location of the study. These treatment thresholds were chosen since we were interested in modeling species responses to climate change and OA, and these conditions most closely approximate the predicted future conditions based on SSP5-8.5 (IPCC, 2021). Furthermore, responses needed to represent one of seven response variable categories (i.e., calcification, photosynthesis, metabolism, growth, development, survival, reproduction). For studies that measured a given performance metric at multiple temperature and/or pH conditions, we chose the treatment combinations closest to a 2°C increase in temperature and a 0.4 pH unit decrease. We extracted the mean, error and sample size values for all factorial OA and warming treatments (i.e. Control, $X_{Control}$; OA only, X_{OA}; Warming only, X_{warm}; OA*Warm, $X_{Warm*OA}$) from figures using WebPlotDigitizer (Rohatgi, 2021) as well as tables, text or supplementary data. We merged our database with a data set [built with the same methods and criteria (Kroeker et al., 2013)]

that extracted studies published prior to January 1, 2013. This combined data set

had 258 studies.

Data analysis

To better understand the prevalence of different interaction types, we calculated

the mean effect size as the log-transformed response ratio of means [$lnRR$;

(Hedges et al., 1999)] and the sampling variance ($\hat{s}^2$) for the interaction between

warming and OA for our seven response variables. We calculated the log

response ratio of the interaction, $lnRR_{Int}$, as:

$$lnRR_{Int} = ln(\frac{X_{warm*OA}}{X_{OA}}) - ln(\frac{X_{warm}}{X_{control}}),$$

where $X_{warm*OA}$ is the cumulative response of an organism under warming and

acidified conditions, X_{warm} is the response of an organism under warming

conditions, X_{OA} is the response of an organism under acidified conditions, and

$X_{control}$ is the response of an organism under control (ambient) conditions

(Morris et al., 2007). We calculated the sample variance for the interaction, $\hat{s}^2$, as:

$$\hat{s}^2 = \frac{s_{warm}^2}{\bar{X}_{warm}^2 N_{warm}} + \frac{s_{OA}^2}{\bar{X}_{OA}^2 N_{OA}} + \frac{s_{warm*OA}^2}{\bar{X}_{warm*OA}^2 N_{warm*OA}} + \frac{s_{control}^2}{\bar{X}_{control}^2 N_{control}},$$

where s^2 is the variance for each treatment, $\bar{X}$ is the mean performance for each

treatment, and N is the sample size for each respective treatment (Morris et al.,

2007). Since it is not possible to log transform a negative value, negative

response ratios were removed ($N = 2$ publications). We weighted individual

mean effect sizes by multiplying by the inverse of the sampling variance. Due to

the weighting by variance, any publications that did not include an estimate of error of the mean were excluded from further analyses ($N = 3$ publications). We then calculated the predicted cumulative effect, CE_P, as the log of the response ratio:

$$CE_P = ln(\frac{X_{warm} + X_{OA} - X_{control}}{X_{control}}),$$

where X_{warm} is the response of an organism under warming conditions, X_{OA} is the response of an organism under acidified conditions, and $X_{control}$ is the response of an organism under control (ambient). We removed an additional $N = 3$ publications where $\frac{X_{warm} + X_{OA} - X_{control}}{X_{control}} < 0$, therefore precluding our ability to log transform the response ratio. To assess whether the interactive effect differed due to the sign of the predicted cumulative effect (i.e., if the effect was predicted to be beneficial or detrimental from an organism's perspective), we classified CE_p as positive or negative depending on whether CE_p was > or < 0. We then calculated the frequency of different interaction types (i.e., more or less pronounced; Fig 1), when CE_p was > 0 (positive) and CE_p was < 0 (negative) determined by whether *lnRR_Int* and confidence intervals were greater than zero, less than zero, or included zero (no effect) for *CE_p (+)* and *CE_p (-)*. When *CE_p* is negative, a value less than zero indicates a *more pronounced* effect, while a value greater than zero indicates a *less pronounced effect*. However, when *CE_p* is positive, a value less than zero indicates a *less pronounced* effect, while a value greater than zero indicates a *more pronounced* effect (Fig. 1).

Finally, to better understand the relationship between the magnitude of the response to warming and OA and the outcome of the OA*warming interaction, we modeled the relationship between CE_P and the *observed cumulative effect, CE_O*. We calculated CE_O as:

$$CE_O = ln(\frac{X_{warm*OA}}{X_{control}}),$$

where $X_{warm*OA}$ is the response of an organism under warming*OA conditions. We first fit CE_O to linear mixed models with fixed factors of CE_P and *response variable* (i.e., growth, development, photosynthesis, metabolism, calcification, reproduction, and survival), and a random factor of *Publication* to account for multiple studies within publications. Since we found a significant interaction between CE_P and *response variable,* we ran additional analyses to assess differences in the relationship between CE_O and CE_P across taxa and response variable. Given differences in the taxa represented across our six response variables, we first subset our data by response variable. For each response variable, we pooled species into eight separate taxa [calcified metazoans (corals and bryozoans), echinoderms, mollusks, calcified algae, crustaceans, fish, fleshy algae, non-calcified unicellular protists]. We then fit CE_O to linear mixed models for each response variable with fixed factors of CE_P and *Taxa*, and a random factor of *Publication.* If a significant *Taxa* or *Taxa*$*CE_P$ effect was found, we reran separate linear mixed models for each taxa with fixed factors of CE_P and random

factor of *Publication*. We excluded the random effect of *Publication* in models that did not have any studies with multiple data points. We extracted the slopes and confidence intervals of the mixed effects models using *emtrends* within the emmeans package in R and classified slopes that were significantly different from 1, based on non-overlapping 95% confidence intervals. A slope less than one indicates that cumulative effects are *less pronounced* than predicted from the effects of individual drivers in isolation. Conversely, a slope greater than one indicates that cumulative effects are *more pronounced* than predicted from the effects of individual drivers in isolation. If the slope was not significantly different than 1, this indicated that there was *no interactive effect*. We only included taxa with observations from three or more studies and greater than five total observations for a given response variable. Therefore, all taxa are not represented for each response.

RESULTS:

Frequency of cumulative effects. Our review of 258 studies, yielded 979 unique measurements across the eight broad taxonomic categories exposed to factorial manipulation of warming and OA. Regardless of the sign of the predicted cumulative effect, CE_P, we found higher prevalence of less pronounced effects, followed by more pronounced effects, and then finally no interaction (Fig. 2). When CE_P was positive, 63% were less pronounced, 25% were more pronounced, and 11% of observations had no interactive effect. When CE_P was

negative, 63% were less pronounced, 28% were more pronounced, and 9% of observations had no interactive effect.

Relationship between magnitude of predicted response and cumulative effect. We found a significant interaction between CE_P and *response variable* (Table 1), which led to additional analyses for each response variable. We found a significant effect of CE_P, but no effect of *Taxa* or CE_P*Taxa for survival, photosynthesis, and metabolism indicating that CE_O depends on the magnitude of CE_P (Table 1, Fig. 3). In all cases, the slope of the relationship was less than 1, indicating that the interactive effect is less pronounced than predicted (Table 2). For response variables of development, growth, and reproduction, we found a significant interaction between CE_P*Taxa (Table 1) such that the relationship between CE_O and CE_P differs across taxa (Figs. 4, 5, 6). For development, calcified metazoans (Fig. 4a), echinoderms (Fig. 4b), mollusks (Fig. 4c) and fleshy algae (Fig. 4e) all show less pronounced interactive effects (Table 2), while fish show no interactive effect (i.e., slope = 1; Fig. 4d). For growth, calcified metazoans (Fig. 5a), mollusks (Fig. 5c), crustaceans (Fig. 5d), fleshy algae (Fig. 5f), and non-calcified unicellular protists (Fig. 5g) all had slopes < 1 (indicating less pronounced effects), while echinoderms (Fig. 5b) and fish (Fig. 5e) had slopes = 1. For reproduction, we found echinoderms (Fig. 6b) with a slope = 1, while we did not detect an effect of CE_P on calcified metazoans (Fig. 6a). Finally, we found significant effects of CE_P and *Taxa* for calcification. Therefore, we did not detect

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differences in the slopes across taxa (i.e., calcified metazoan, calcifying algae, mollusk) for calcification, but mean effect sizes did differ (Table 1, Fig. 7). We found that across taxonomic groups, interactive effects were less pronounced for calcification (Table 2).

DISCUSSION:

Multiple drivers have the potential to interact in complex ways that may be either beneficial or detrimental to an organism. Here, we show that the combined effects of warming and ocean acidification are most often less pronounced than would be expected given the individual effects of each driver in isolation. We found that greater than 60% of all observations were classified as less pronounced across a wide range of taxa and response variables. This general pattern is striking and was consistent across the directions (e.g., positive, negative) of the predicted response. Furthermore, we find evidence of strong linear relationships between the predicted cumulative effect and the observed cumulative effect across all but one taxa * response variable combination. These linear relationships were categorized as less pronounced (slope <1) than predicted in 15 out of the 19 regressions for different taxa and response variables. Together, these results suggest that although cumulative effects appear to be less pronounced overall, accurately predicting a species cumulative effect requires information about the magnitude of the predicted cumulative effect, the response variable of interest, and in some cases taxa.

Past syntheses have shown a range of prevalences of different interaction types. Although our classification system is not directly comparable to the additive model used in most ecological meta-analyses (i.e., using terms antagonisms, synergisms), in a large portion of our dataset (i.e., when the signs of each individual driver are the same) less pronounced effects are categorically similar to antagonisms, while more pronounced effects are categorically similar to synergisms. Unlike past studies, we found that the majority of observed cumulative effects were less pronounced than expected based on the individual drivers. One reason for this may be that an additive model is inappropriate for comparing across many different types of response variables. For example, bounded responses such as mortality or survival are biased towards antagonisms under an additive model (Folt et al., 1999; Orr et al., 2020), thus leading to differences in the biases of response variables being compared within a single meta-analysis. In addition, it's likely that many physiological responses behave like bounded response variables given that most of these responses have bioenergetic limitations that could be more constrained than the assumptions of an additive model. Therefore, null model selection should be considered carefully as it not only impacts the prevalence of different interactive outcomes (Schäfer & Piggott, 2018), but also the validity of comparisons made across different response types.

The dominance of less pronounced interactive effects in our meta-analysis may be surprising, given the popular narrative of the high prevalence of synergisms (classified here as more pronounced interactive effects) resulting from the interaction between multiple environmental drivers. Synergisms are of particular concern due to their unpredictable behavior and potentially large negative impacts on marine species and ecosystems. We found that more pronounced effects were the next most likely interactive effect, behind less pronounced, although only about a third as likely to occur. This suggests that although more pronounced interactive effects do occur, they are not as likely as previously thought. Côte et al. (2016) also found that synergisms are not common across their meta-analyses survey, and that their overstated prevalence is a combination of powerful storytelling and issues with researchers' understanding of the terminology. The smaller proportion of more pronounced interactive effects seen here also supports the notion that perhaps physiological responses are more bounded than previously recognized (i.e., responses can only occur over a restricted range). Ultimately, these results suggest that although multiple driver impacts may be greater than the impacts of a single driver, they are not commonly greater than expected.

Multiple driver studies have previously considered mean effect sizes to understand interactive effects. However, the magnitude of an effect size is likely to play an important role in whether certain interactions arise. In our study we

show that the slope of regressions between the predicted cumulative effect and the observed cumulative effect are less than one in most instances. We show that at small magnitude effect sizes (values closer to zero), the deviation from one is smaller than at large magnitude effect sizes. In other words, as the magnitude of the predicted cumulative effect (either positive or negative) increases, the magnitude of the interactive effect is even greater, with the interaction being less pronounced in our analyses. These results are consistent with a bioenergetic framework that suggests limits on performance exist at both extremes and act to dampen both positive and negative effects. These strong linear relationships between the predicted cumulative effect and the observed cumulative effect across a wide range of performance metrics and taxa suggest that less pronounced effects are more likely to occur regardless of the magnitude of the effect, though the magnitude of the predicted cumulative effect is important in driving the magnitude of the deviation of the observed cumulative effect.

CONCLUSIONS:

Species are exposed to multivariate environmental changes that are further exacerbated by anthropogenic climate change. Concerns about the prevalence of "ecological surprises" as a result of the interactive effects of multiple environmental drivers, have led to increased interest in multiple driver research (Boyd et al., 2018; Orr et al., 2020). Here, we show that the combined effects of warming and ocean acidification are most often less pronounced than expected

based on the individual drivers. This suggests that the effects of multiple environmental drivers are often dampened compared to the effects of each environmental driver in isolation. This trend was consistent across a wide range of taxa (including both calcifying and non-calcifying organisms) and response variables. This suggests that the interactive effects of warming and OA may be less threatening to marine species than previously thought, although negative effects overall are common. Although far less frequent, we did find a significant number of cumulative effects that were more pronounced than expected. Future work focused on studies of more pronounced interactive effects will be crucial to understand when and why ecological surprises arise, especially since these interactions are likely to have a disproportionately large impact on biodiversity and ecosystem functioning.

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Table 1. Results of linear mixed model fixed effects. Significance at $p < 0.05$ noted in bold.

Response	Source	d.f. num	d.f. den	F	p-value
All	**CE_p**	**1**	**690.57**	**768.09**	**<0.0001**
	Response	**7**	**656.49**	**3.75**	**0.0005**
	CE_p*Response	**7**	**726.45**	**3.06**	**0.0035**
Development	**CE_p**	**1**	**65.04**	**575.17**	**<0.0001**
	Taxa	4	20.07	1.34	0.2903
	CE_p*Taxa	**4**	**64.74**	**2.89**	**0.0290**
Survival	**CE_p**	**1**	**32.44**	**69.36**	**<0.0001**
	Taxa	4	25.28	1.33	0.2846
	CE_p*Taxa	4	27.23	2.24	0.0910
Photosynthesis	**CE_p**	**1**	**56.33**	**108.64**	**<0.0001**
	Taxa	3	42.05	0.40	0.7554
	CE_p*Taxa	3	58.23	1.32	0.2777
Calcification	**CE_p**	**1**	**51.35**	**116.39**	**<0.0001**
	Taxa	**2**	**22.26**	**5.67**	**0.0103**
	CE_p*Taxa	2	43.16	0.55	0.5804
Growth	**CE_p**	**1**	**213.03**	**153.38**	**<0.0001**
	Taxa	7	105.87	1.05	0.3987
	CE_p*Taxa	**7**	**229.92**	**3.21**	**0.0029**
Metabolism	**CE_p**	**1**	**88.54**	**53.20**	**<0.0001**
	Taxa	5	63.45	1.29	0.2788
	CE_p*Taxa	5	92.07	1.73	0.1362
Reproduction	**CE_p**	**1**	**9.97**	**14.70**	**0.0033**
	Taxa	1	6.28	0.17	0.6914
	CE_p*Taxa	**1**	**9.97**	**13.75**	**0.0041**

Table 2. Estimated marginal means of linear trends (slope) from linear mixed effects models. If a significant effect of taxa was found, lines were fit to individual taxa. Slope estimates that are less than 1 (less pronounced) are in orange. Slope estimates that are equal to 1 (no interaction) are in green.

Response	Taxa	Intercept	Slope	lower CI (slope)	upper CI (slope)	F	p-value
Development	Calcified Metazoan	-0.114	0.51	0.30	0.71	38.80	<0.0001
	Echinoderm	-0.178	0.75	0.65	0.84	319.00	<0.0001
	Mollusk	-0.044	0.73	0.53	0.93	77.10	<0.0001
	Fish	0.146	0.86	0.71	1.01	210.00	<0.0001
	Fleshy Algae	-0.275	0.75	0.58	0.93	180.00	0.0008
Survival	All	-0.309	0.73	0.54	0.91	69.36	<0.0001
Photosynthesis	All	0.006	0.63	0.51	0.75	108.64	<0.0001
Calcification	Calcified Metazoan	-0.156	0.45	0.29	0.61	34.90	<0.0001
	Calcifying Algae	-0.109	0.44	0.18	0.71	18.60	0.0016
	Mollusk	0.174	0.58	0.42	0.74	76.00	0.0001
Growth	Calcified Metazoan	0.002	0.61	0.44	0.79	65.70	<0.0001
	Echinoderm	0.166	1.04	0.85	1.22	202.00	<0.0001
	Mollusk	-0.137	0.70	0.60	0.81	180.00	<0.0001
	Crustacean	-0.117	0.43	0.26	0.61	27.30	<0.0001
	Fish	0.291	0.95	0.77	1.12	157.00	<0.0001
	Fleshy Algae	0.160	0.47	0.32	0.62	43.90	<0.0001
	Non-calcified unicellular protists	0.195	0.72	0.61	0.83	186.00	<0.0001
Metabolism	All	0.284	0.59	0.43	0.76	53.20	<0.0001
Reproduction	Calcified Metazoan	n.s.	n.s.			0.01	0.9200
	Echinoderm	-0.132	0.95	0.22	1.68	23.30	0.0085
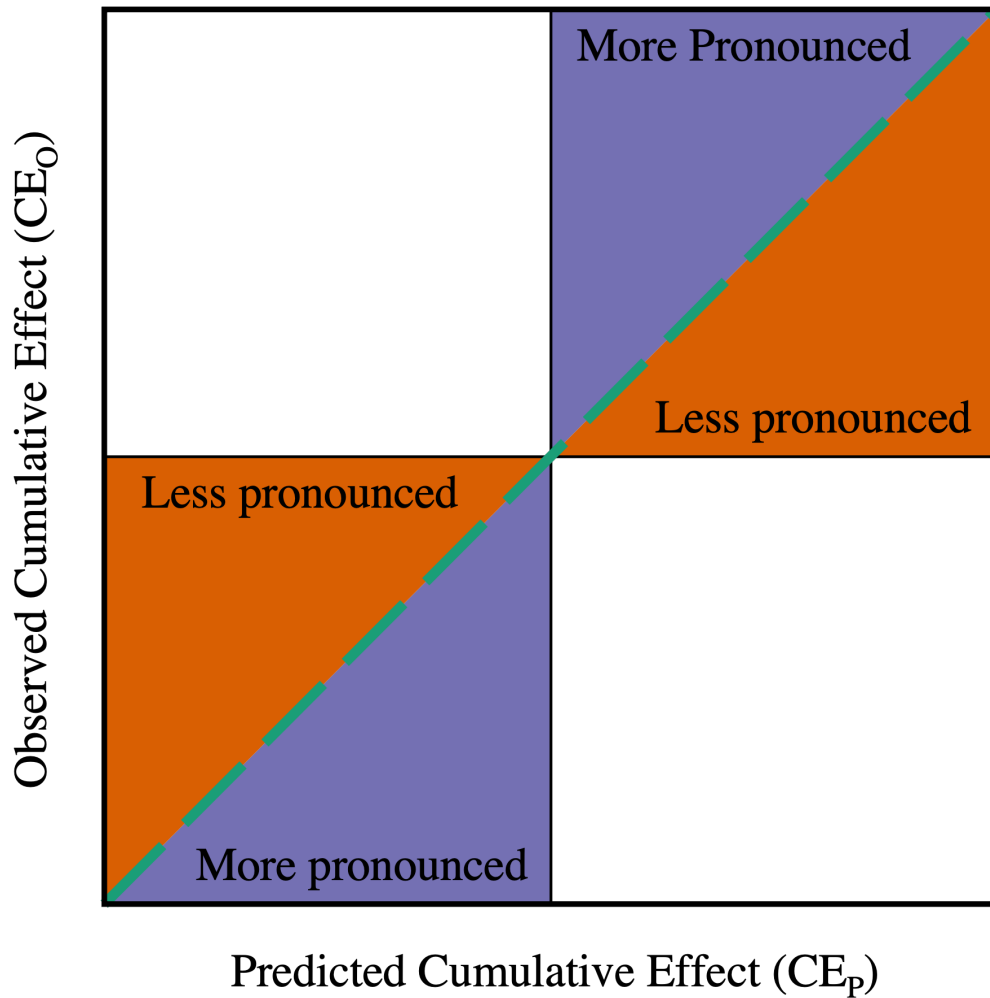
Figure 1. Schematic of multiple environmental driver interactive effects. Purple triangles represent effects that are more pronounced, while orange triangles represent effects that are less pronounced. The green dashed line represents a 1:1 relationship between the predicted cumulative effect, CE_P, and the observed cumulative effect, CE_O, which would indicate the lack of an interactive effect.
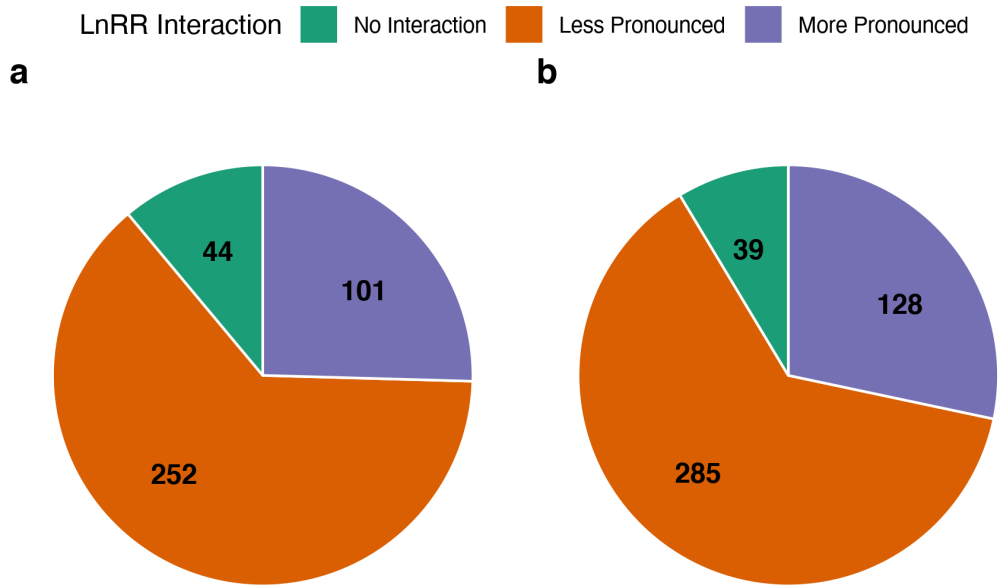
Figure 2. Pie charts of proportion of different interaction types; a) if the predicted cumulative effect is *positive*; b) if the predicted cumulative effect is *negative*. Numbers indicate the number of data points for each response type.
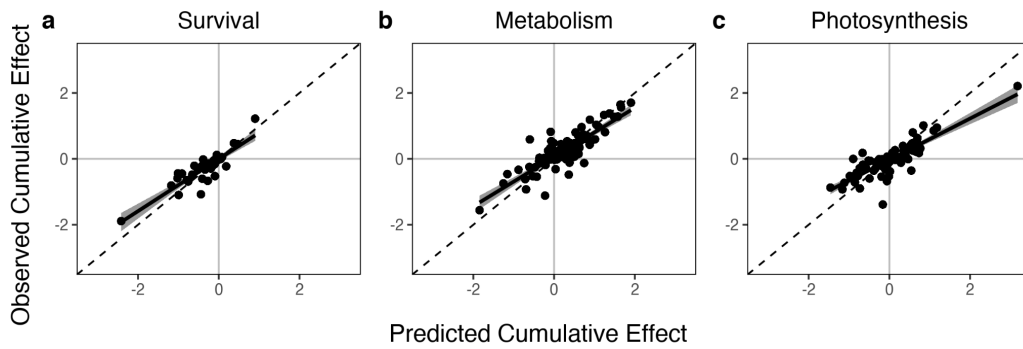
Figure 3. Linear relationships between the predicted cumulative effect and observed cumulative effect for, a) survival, b) metabolism, c) photosynthesis. Dashed line indicates a slope equal to 1. A slope less than 1 (below the 1:1 line) indicates a less pronounced cumulative effect than predicted, while a slope greater than 1 (above the 1:1 line) indicates a more pronounced cumulative effect.
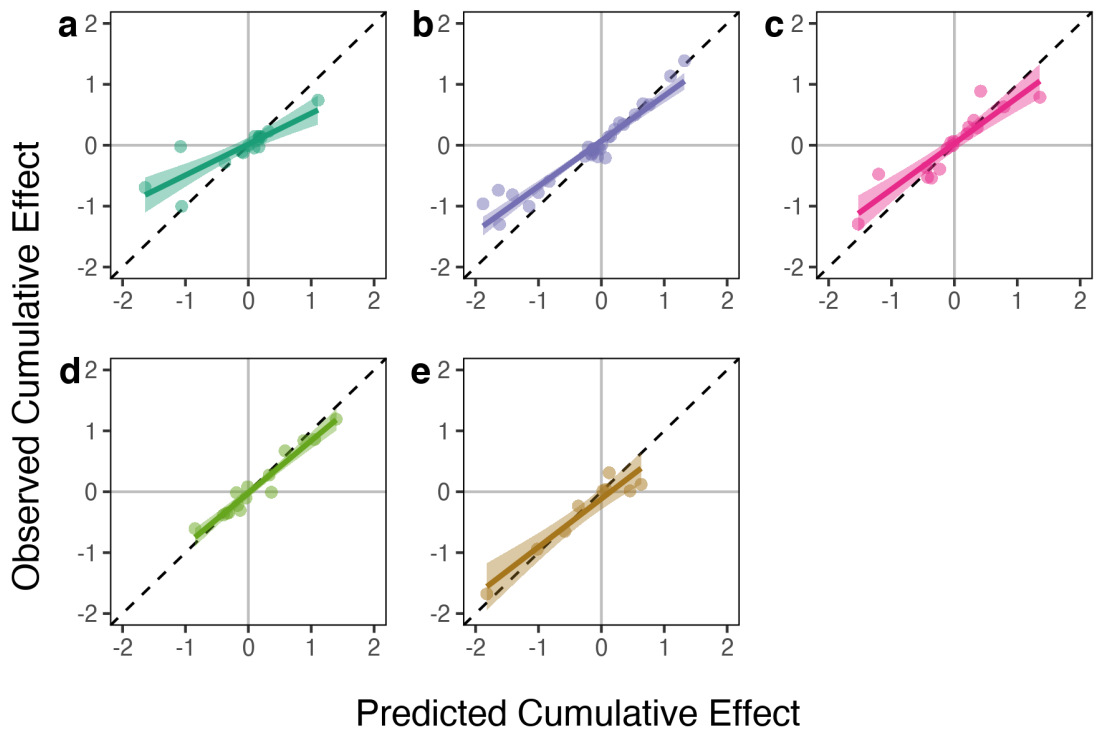
Figure 4. Linear relationships between the predicted cumulative effect and observed cumulative effect for development in, a) calcified metazoans, b) echinoderms, c) mollusks, d) fish, e) fleshy seaweeds. A slope less than 1 (below the 1:1 line) indicates a less pronounced cumulative effect than predicted, while a slope greater than 1 (above the 1:1 line) indicates a more pronounced cumulative effect.
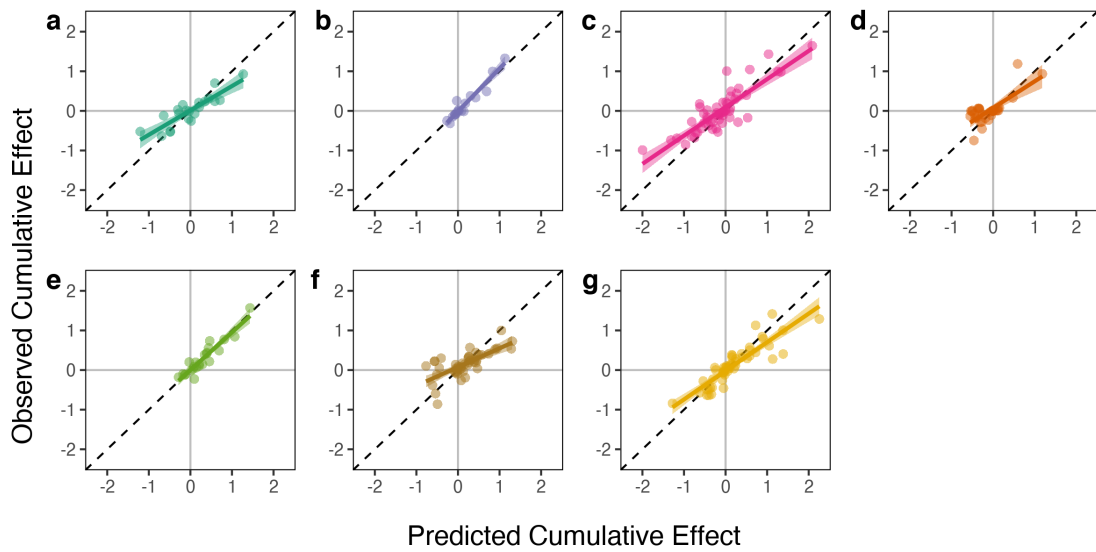
Figure 5. Linear relationships between the predicted cumulative effect and observed cumulative effect for growth in, a) calcified metazoans, b) echinoderms, c) mollusks, d) crustaceans, e) fish, f) fleshy seaweeds, g) non-calcified unicellular organisms. A slope less than 1 (below the 1:1 line) indicates a less pronounced cumulative effect than predicted, while a slope greater than 1 (above the 1:1 line) indicates a more pronounced cumulative effect.
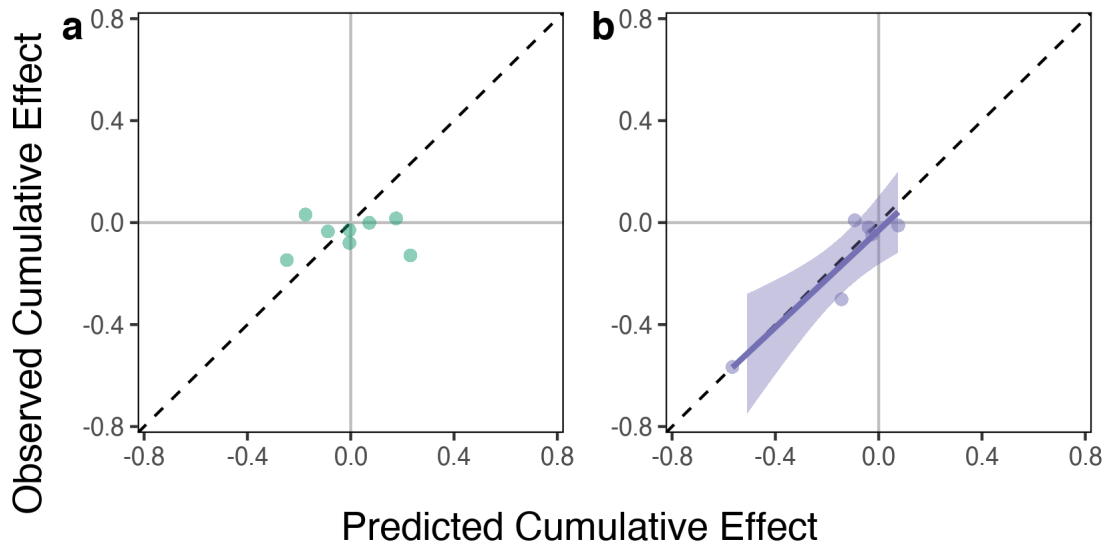
Figure 6. Linear relationships between the predicted cumulative effect and observed cumulative effect for reproduction in, a) calcified metazoans and c) echinoderms. A slope less than 1 (below the 1:1 line) indicates a less pronounced cumulative effect than predicted, while a slope greater than 1 (above the 1:1 line) indicates a more pronounced cumulative effect. No regression line is plotted for calcified metazoans due to the lack of a statistically significant relationship between the predicted cumulative effect and observed cumulative effect.
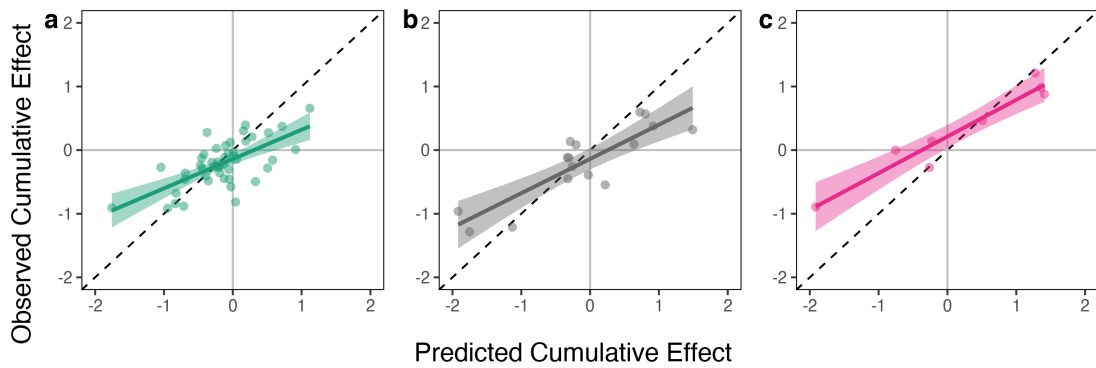
Figure 7. Linear relationships between the predicted cumulative effect and observed cumulative effect for calcification in, a) calcified metazoans, b) calcifying algae, c) mollusks. A slope less than 1 (below the 1:1 line) indicates a less pronounced cumulative effect than predicted, while a slope greater than 1 (above the 1:1 line) indicates a more pronounced cumulative effect.

Conclusion

Marine organisms inhabit highly variable environments. Prior exposure to environmental conditions shape the physiology and ecology of species and populations and has the potential to impact responses to accelerated climate change and ocean acidification. In chapter 1, I first assess how natural environmental variability driven by coastal upwelling impacts the physiology and ecology of kelp forest grazers. I find that the two species studied here respond differently to acute upwelling events, with the gastropod, *Promartynia pulligo,* showing resilience to acute upwelling, yet negative impacts over longer exposure. The echinoderm, *Mesocentrotus franciscanus,* responded similarly over both acute and chronic exposure and had lower performance with increasing upwelling intensity. Differences in the responses of our two species raise questions about why these results occurred. Since we only examined one species for each broad taxonomic group, it's unclear whether these patterns hold across all echinoderm and gastropod species. Alternatively, behavioral differences in these two species could have resulted in differences in stress responses brought on by upwelling. For instance, *M. franciscanus* lives primarily at the benthos and feeds on a variety of macroalgal sources, whereas *P. pulligo* lives and feeds on the giant kelp *Macrocystis pyrifera* and travels from the benthos to the kelp canopy.

In chapter 2, I show that populations of *M. franciscanus* are locally adapted across two regions characterized by strong versus weak upwelling. Importantly, populations from weak upwelling regions are more negatively impacted by region-specific future environmental changes than populations from strong upwelling regions. This suggests that species are likely to respond differently to global change drivers across a species range and using a species response to climate change in one location to inform how a species may respond in a second location may be inaccurate. Across populations of red sea urchins we also found striking differences in body condition and energy allocation. Future work should assess the underlying causes of these differences as they are likely important for how organisms respond to future environmental changes.

Chapters 1 and 2 improved our understanding of the variability in species' and population responses to global change and suggest that prior exposure history and species'-specific tolerances both play a key role in mediating species responses. Yet, in nearly all ecosystems, multiple environmental drivers are changing at the same time. Understanding broad scale patterns in how species' responses to concurrent change in multiple drivers is crucial to improving our ability to forecast ecological change across ecosystems. In chapter 3, I show that across eight taxonomic groups (spanning both calcifying and non-calcifying organisms) and seven response variables, species responses are most often less pronounced than would be predicted by their responses to individual

environmental drivers. Contrary to the popular notion that more pronounced effects are common when species are exposed to changes in multiple environmental drivers, we find these phenomenon to be infrequent compared to less pronounced interactive effects. These results suggest that although species' responses are often negative when exposed to warming and ocean acidification, they may be less detrimental than previously thought. I further show that the magnitude of the effect size matters, such that a larger magnitude effect size leads to a greater deviation from the 1:1 line (no interactive effect) compared to smaller magnitude effect sizes. These results are in line with a more mechanistic understanding of the physiological feedbacks that maintain organismal homeostasis. When species responses to environmental drivers result in an increase in performance, there are still limits to how positive performance can be. While, when species response to environmental drivers result in a decrease in performance, species general stress responses may be able to ameliorate some of the negative effects of environmental stress.

Appendix 1: Supplementary material for Chapter 1

Table S1. Results of linear models between environmental variables (temperature, DO, and pH) from oceanographic sensor data deployed within kelp forest. Significance at $p < 0.05$ noted in bold.

Season	Response	Factor	Estimate	s.e.	t	p-value	R^2
Upwelling	DO	Intercept	-83.3112	0.1958	-425.50	**<0.0001**	0.88
		pH	11.3627	0.0250	454.30	**<0.0001**	
	Temp	Intercept	-57.9099	0.3041	-190.40	**<0.0001**	0.64
		pH	8.8645	0.0388	228.20	**<0.0001**	
	Temp	Intercept	7.9553	0.0225	353.10	**<0.0001**	0.47
		DO	0.6258	0.0039	161.00	**<0.0001**	
Non-upwelling	DO	Intercept	-31.0091	0.3451	-89.86	**<0.0001**	0.36
		pH	4.7072	0.0437	107.71	**<0.0001**	
	Temp	Intercept	-35.7575	0.5981	-59.79	**<0.0001**	0.24
		pH	6.1922	0.0757	81.76	**<0.0001**	
	Temp	Intercept	10.9947	0.0673	163.47	**<0.0001**	0.05
		DO	0.3481	0.0108	32.12	**<0.0001**	

Table S2. Results of linear models with partial residuals of response variables versus PC1. Significance at $p < 0.05$ noted in bold.

Experiment	Timepoint	Species	Response	Factor	Estimate	s.e.	t	p-value	R^2
1	1 month	*M. franciscanus*	Respiration	Intercept	0.0141	0.0003	48.00	**<0.0001**	0.17
				PC1	-0.0007	0.0001	-4.76	**<0.0001**	
1	2 month	*M. franciscanus*	Respiration	Intercept	0.0151	0.0003	49.32	**<0.0001**	0.09
				PC1	-0.0005	0.0002	-3.28	**0.0014**	
1	3 month	*M. franciscanus*	Respiration	Intercept	0.0141	0.0003	53.25	**<0.0001**	0.15
				PC1	-0.0006	0.0001	-4.52	**<0.0001**	
2	0 hr	*M. franciscanus*	Respiration	Intercept	0.0161	0.0002	66.49	**<0.0001**	0.23
				PC1	-0.0008	0.0001	-6.44	**<0.0001**	
2	72 hr	*M. franciscanus*	Respiration	Intercept	0.0161	0.0002	69.64	**<0.0001**	0.11
				PC1	-0.0005	0.0001	-4.14	**<0.0001**	
1	1 month	*M. franciscanus*	Grazing	Intercept	0.0888	0.0042	21.28	**<0.0001**	0.12
				PC1	-0.0079	0.0021	-3.82	**0.0002**	
1	2 month	*M. franciscanus*	Grazing	Intercept	0.0693	0.0033	20.90	**<0.0001**	0.13
				PC1	-0.0066	0.0016	-4.10	**<0.0001**	
1	3 month	*M. franciscanus*	Grazing	Intercept	0.0898	0.0034	26.30	**<0.0001**	0.20
				PC1	-0.0091	0.0017	-5.40	**<0.0001**	
2	0 hr	*M. franciscanus*	Grazing	Intercept	0.0760	0.0035	21.71	**<0.0001**	0.14
				PC1	-0.0084	0.0017	-4.87	**<0.0001**	
2	72 hr	*M. franciscanus*	Grazing	Intercept	0.0760	0.0033	23.09	**<0.0001**	0.15
				PC1	-0.0083	0.0016	-5.07	**<0.0001**	
1	3 month	*M. franciscanus*	Growth	Intercept	0.4652	0.0375	12.42	**<0.0001**	0.04
				PC1	-0.0385	0.0185	-2.09	**0.0390**	
1	3 month	*M. franciscanus*	Calcification	Intercept	0.4075	0.0358	11.39	**<0.0001**	0.04
				PC1	-0.0372	0.0176	-2.11	**0.0370**	

					Estimate	SE	t	p	R^2
1	1 month	*P. pulligo*	Respiration	Intercept	0.0195	0.0004	46.50	**<0.0001**	0.05
				PC1	-0.0006	0.0002	-3.04	**0.0027**	
1	2 month	*P. pulligo*	Respiration	Intercept	0.0182	0.0003	61.31	**<0.0001**	0.10
				PC1	-0.0006	0.0001	-4.31	**<0.0001**	
1	3 month	*P. pulligo*	Respiration	Intercept	0.0196	0.0003	57.59	**<0.0001**	0.10
				PC1	-0.0007	0.0002	-4.14	**<0.0001**	
1	1 month	*P. pulligo*	Grazing	Intercept	0.0021	0.0010	2.08	**<0.0001**	0.11
				PC1	-0.0023	0.0005	-4.55	**<0.0001**	
1	2 month	*P. pulligo*	Grazing	Intercept	0.0210	0.0022	9.42	**<0.0001**	0.09
				PC1	-0.0043	0.0011	-3.90	**0.0001**	
1	3 month	*P. pulligo*	Grazing	Intercept	0.0011	0.0016	0.65	0.5143	0.04
				PC1	-0.0021	0.0008	-2.62	**0.0098**	
1	3 month	*P. pulligo*	Calcification	Intercept	0.0157	0.0031	5.06	**<0.0001**	0.10
				PC1	-0.0064	0.0015	-4.16	**<0.0001**	
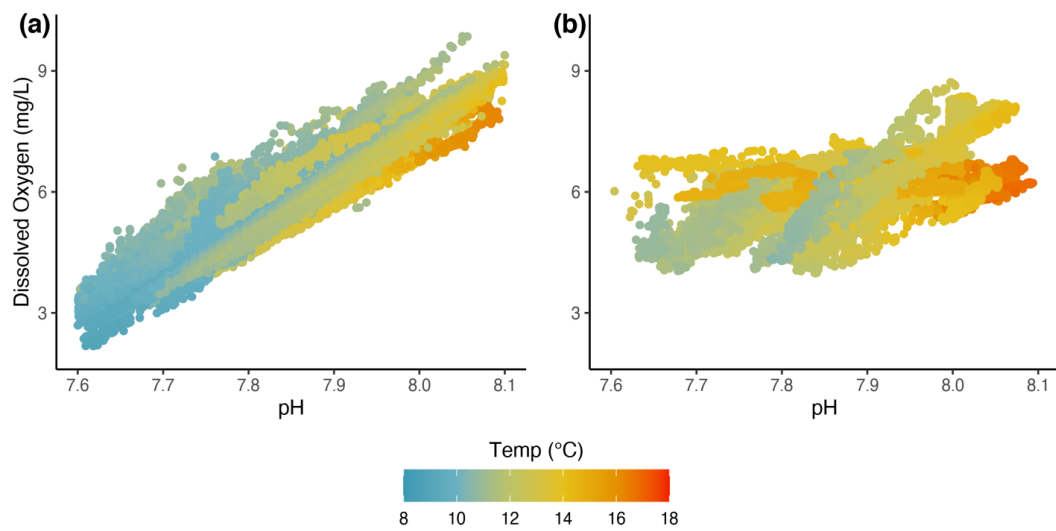
Figure S1. Scatterplot of time series data from SeapHOx sensor deployed within kelp forest at Stillwater Cove, Carmel, CA during (a) upwelling season (April-September); (b) non-upwelling season (November-March) from 2016-2020, where data were available.
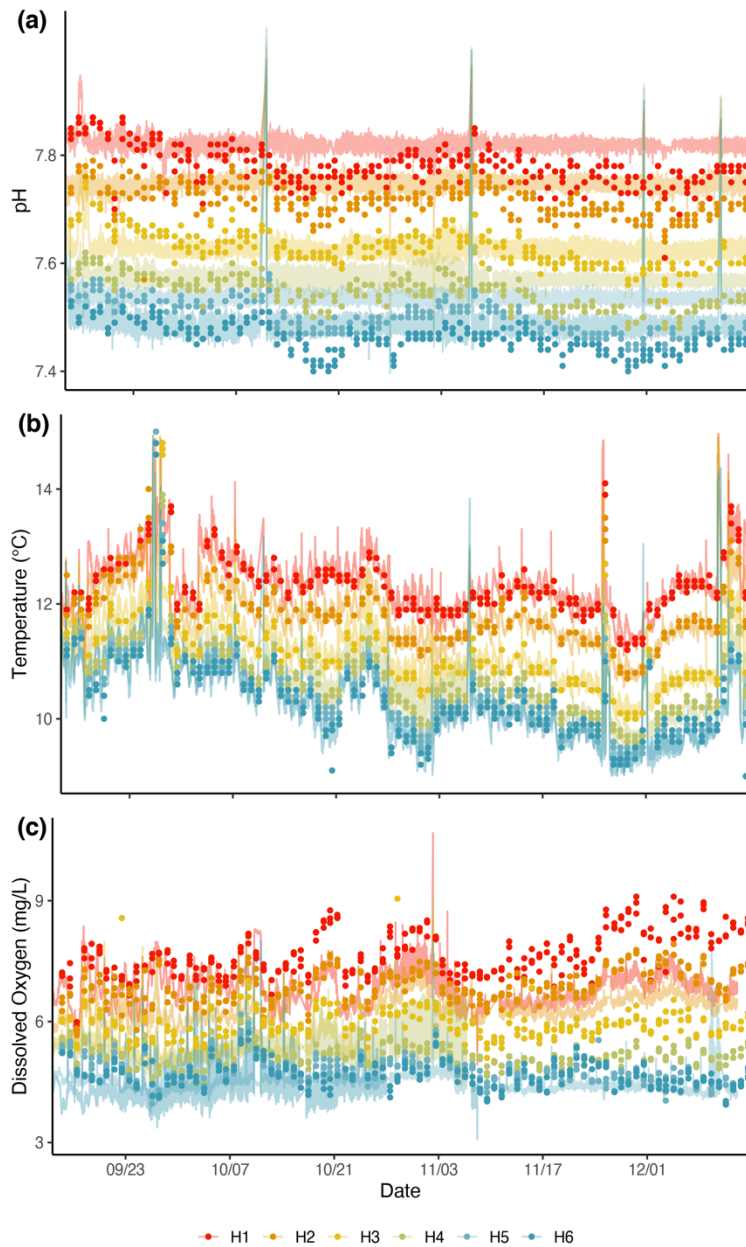
Figure S2. Time series of pH, DO and temperature from Durafet (a, b) and Vernier GoDirect Optical DO (c) probes within header buckets for the duration of Experiment 1. Headers (H1, H2, H3, H4, H5, H6) represent a gradient in upwelling intensity with H1 representing conditions with the least amount of upwelling and H6 representing conditions with the greatest upwelling.
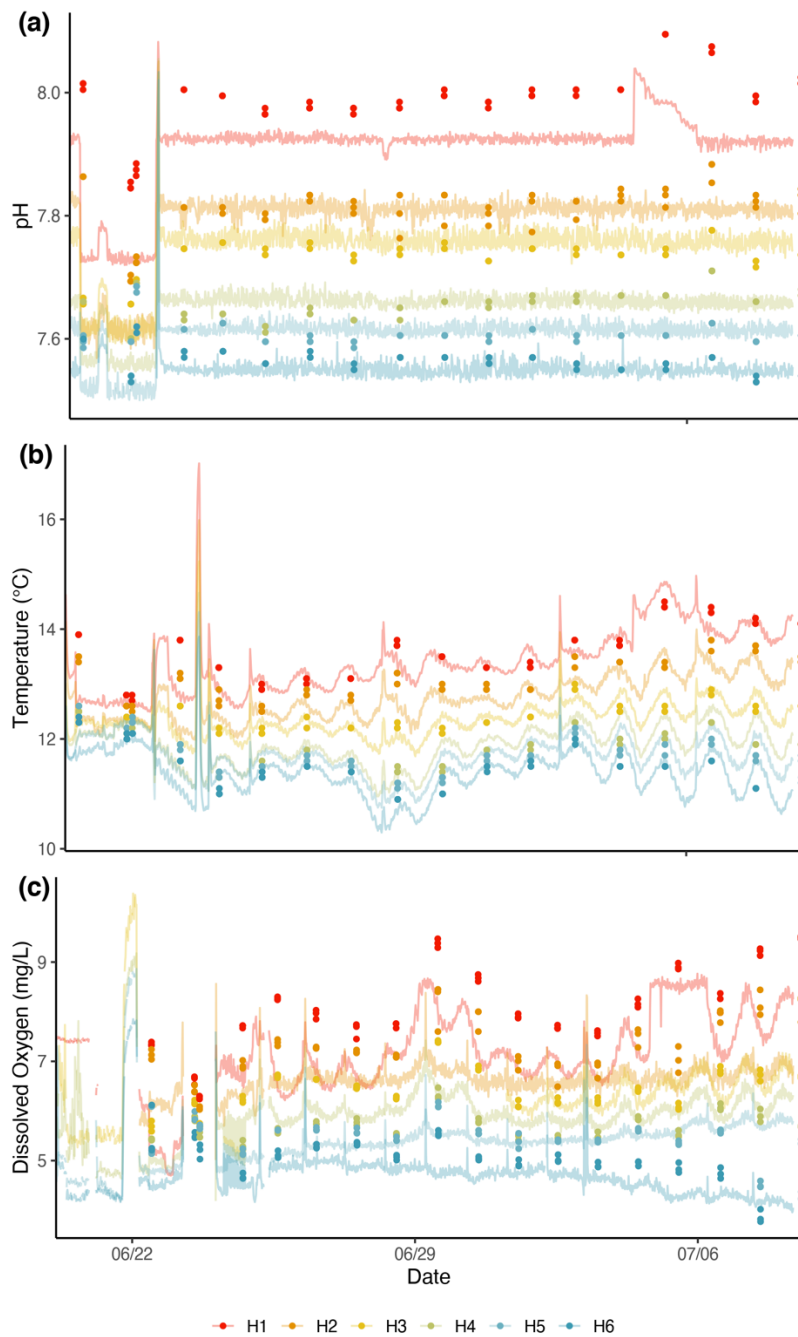
Figure S3. Time series of pH, DO, and temperature from Durafet (a, b) and Vernier GoDirect Optical DO (c) probes within header buckets for the duration of Experiment 2. Headers (H1, H2, H3, H4, H5, H6) represent a gradient in upwelling intensity with H1 representing conditions with the least amount of upwelling and H6 representing conditions with the greatest upwelling.
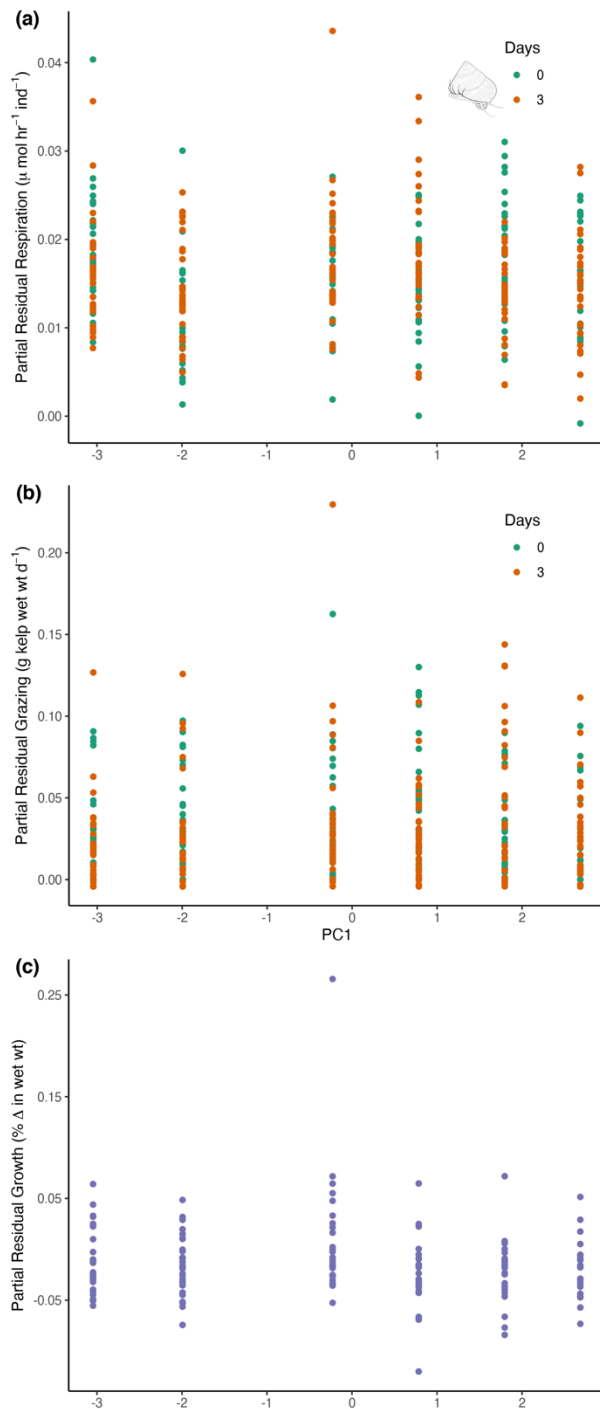
Figure S4. *Promartynia pulligo* mean centered partial residuals for response variables (a) respiration rate and (b) grazing rate after 0 and 72 hours in upwelling conditions from Experiment 1, and c) growth rate after 3 months in upwelling conditions from Experiment 2.

Appendix 2: Supplementary material for Chapter 2

Table S1. Results of linear models between environmental variables (temperature, DO and pH) from oceanographic sensor data deployed within kelp forests. Significance at $p < 0.05$ noted in bold.

| Site	Response	Factor	Estimate	s.e.	t	p-value	R^2
Point Arena	DO	Intercept	-81.0113	1.6654	-48.65	**<0.0001**	0.8099
		pH	11.1559	0.2139	52.15	**<0.0001**	
	Temp	Intercept	-55.1078	2.022	-25.02	**<0.0001**	0.5824
		pH	8.4424	0.2829	29.84	**<0.0001**	
	Temp	Intercept	6.74438	0.14364	46.95	**<0.0001**	0.5517
		DO	0.66305	0.02365	28.04	**<0.0001**	
Van Damme	DO	Intercept	-84.2005	3.0634	-27.49	**<0.0001**	0.7775
		pH	11.709	0.3967	29.52	**<0.0001**	
	Temp	Intercept	-29.6354	2.6991	-10.98	**<0.0001**	0.473
		pH	5.2366	0.3495	14.98	**<0.0001**	
	Temp	Intercept	8.6426	0.1855	46.59	**<0.0001**	0.3655
		DO	0.3473	0.0289	12.02	**<0.0001**	
Catalina Island	DO	Intercept	-34.8515	1.8872	-18.47	**<0.0001**	0.3775
		pH	5.3123	0.2354	22.57	**<0.0001**	
	Temp	Intercept	89.717	11.889	7.546	**<0.0001**	0.04133
		pH	-9.037	1.483	-6.094	**<0.0001**	
	Temp	Intercept	25.838	1.3268	19.474	**<0.0001**	0.04648
		DO	-1.1078	0.1712	-6.469	**<0.0001**	

	DO	Intercept	-6.885	1.4075	-4.892	**<0.0001**	0.156
		pH	1.7991	0.1764	10.197	**<0.0001**	
Laguna Beach	Temp	Intercept	30.5726	4.1169	7.426	**<0.0001**	0.02069
		pH	-1.8439	0.5161	-3.573	**0.0004**	
	Temp	Intercept	10.5585	0.832	12.691	**<0.0001**	0.06683
		DO	0.7107	0.1111	6.394	**<0.0001**	

Table S2. Results of mixed model fixed effects for common garden experiment. Significance at $p < 0.05$ noted in bold.

Response	Source	d.f.	F	p-value
Growth	Population	1	28.77	**0.0190**
	Treatment	1	14.97	0.0046
	Weight	1	509.80	**<0.0001**
	Pop:Trt	1	9.54	**0.0024**
Net Calcification	Population	1	16.47	**0.0172**
	Treatment	1	9.46	**0.0358**
	Weight	1	594.65	**<0.0001**
	Pop:Trt	1	1.62	0.2057
Grazing	Population	1	3.84	0.1059
	Treatment	1	8.78	**0.0036**
	Pop:Trt	1	2.18	0.1418
Metabolism	Population	1	15.52	**0.0190**
	Treatment	1	5.67	0.0680
	Pop:Trt	1	2.25	0.1369
Mortality	Population	1	369.84	**<0.0001**
	Treatment	1	0.88	0.3491
	Weight	1	45.22	**0.0025**
	Time	1	0.28	0.5956
	Time:Trt	1	236.83	**<0.0001**
	Time:Pop	1	0.35	0.5565

	Pop:Trt	1	72.51	**<0.0001**
	Time:Trt:Pop	1	23.61	**<0.0001**
G:SOM	Population	1	0.51	0.5032
	Treatment	1	0.07	0.7982
	Weight	1	57.62	**<0.0001**
	Pop:Trt	1	0.03	0.8527
	Pop:Weight	1	45.06	**<0.0001**
G:SOM - Initial	Population	1	0.87	0.3712
	Weight	1	34.52	**<0.0001**
	Pop:Weight	1	16.31	**0.0002**

Table S3. Results of pairwise contrasts for common garden experiment. Significance at $p < 0.05$ noted in bold.

Response	Comparison	Estimate	s.e.	d.f.	t	p-value
Growth	Northern North Current - Southern North Current	11.6000	2.0000	13.40	5.83	**0.0001**
	Northern South Current - Southern South Current	4.2100	1.8200	9.66	2.31	**0.0443**
	(Northern North Current - Southern North Current) - (Northern South Current - Southern South Current)	-7.4200	2.4100	136.00	-3.08	**0.0025**
Calcification	Northern North Current - Southern North Current	10.5000	3.1200	5.01	3.36	**0.0201**
	Northern South Current - Southern South Current	13.4000	3.2200	5.65	4.17	**0.0067**
	(Northern North Current - Southern North Current) - (Northern South Current - Southern South Current)	-2.9600	2.3300	136.00	-1.27	0.2067
Mortality Rate	Northern North Current - Southern North Current	0.0056	0.0019	1003.00	3.01	**0.0027**
	Northern South Current - Southern South Current	-0.0071	0.0019	1004.00	-3.81	**0.0001**

Table S4. Mean (± standard error) of environmental conditions within mesocosm bins containing animals. Salinity, temperature and dissolved oxygen are from daily YSI measurements within bins (averaged by treatment each day n = 93) and carbonate chemistry parameters within bins (averaged by treatment each day n = 6) measured from discrete water samples.

Trt	Temp	Salinity	DO (mgL^{-1})	A_T (μmol kg^{-1})	DIC_T ($mmol$ kg^{-1})	pH_T	HCO_3^- ($mmol$ kg^{-1})	CO_3^{2-} ($mmol$ kg^{-1})	CO_2 ($mmol$ kg^{-1})	pCO_2 (μatm)	Ω_{Cal}	Ω_{Arag}
North Current	10.93 ± 0.05	34.73 ± 0.02	8.34 ± 0.08	2266 ± 4	2158 ± 13	7.84 ± 0.07	2038 ± 15	91.54 ± 4.98	28.48 ± 2.11	671.9 ± 51.3	2.18 ± 0.30	1.39 ± 0.08
North Future	13.34 ± 0.04	34.74 ± 0.02	5.99 ± 0.07	2268 ± 4	2222 ± 11	7.61 ± 0.06	2114 ± 11	61.80 ± 3.07	46.59 ± 3.05	1188.8 ± 80.6	1.48 ± 0.07	0.94 ± 0.05
South Current	15.86 ± 0.08	34.75 ± 0.02	8.53 ± 0.07	2268 ± 4	2102 ± 9	7.91 ± 0.04	1956 ± 12	125.81 ± 4.52	20.16 ± 0.91	555.1 ± 25.0	3.01 ± 0.11	1.93 ± 0.07
South Future	18.53 ± 0.12	34.77 ± 0.02	6.84 ± 0.07	9 ± 4	2152 ± 14	7.75 ± 0.03	2024 ± 18	99.09 ± 5.84	29.40 ± 2.67	872.4 ± 76.6	2.37 ± 0.14	1.53 ± 0.09

Table S5. Results of mixed model fixed effects for global change experiment. Significance at $p < 0.05$ noted in bold.

Response	Source	d.f.	F	p-value
Growth	Population	1	0.15	0.7165
	Treatment	1	27.83	**0.0008**
	Weight	1	408.56	**<0.0001**
	Pop:Trt	1	1.04	0.3393
Net Calcification	Population	1	1.72	0.2561
	Treatment	1	17.62	**0.0023**
	Weight	1	379.11	**<0.0001**
	Pop:Trt	1	0.83	0.3866
Grazing	Population	1	13.27	**0.0095**
	Treatment	1	2.45	0.1202
	Pop:Trt	1	0.55	0.4581
Metabolism	Population	1	2.79	0.1514
	Treatment	1	0.03	0.8711
	Pop:Trt	1	0.00	0.9930
Mortality	Population	1	529.23	**<0.0001**
	Treatment	1	0.40	0.5261
	Weight	1	16.21	**0.0157**
	Time	1	313.30	**<0.0001**
	Time:Trt	1	240.77	**<0.0001**
	Time:Pop	1	240.88	**<0.0001**

	Pop:Trt	1	13.80	**0.0002**
	Time:Trt:Pop	1	50.94	**<0.0001**
G:SOM	Population	1	0.51	0.5032
	Treatment	1	0.07	0.7982
	Weight	1	57.62	**<0.0001**
	Pop:Trt	1	0.03	0.8527
	Pop:Weight	1	45.06	**<0.0001**

Table S6. Results of pairwise contrasts for climate change experiment. Significance at $p < 0.05$ noted in bold.

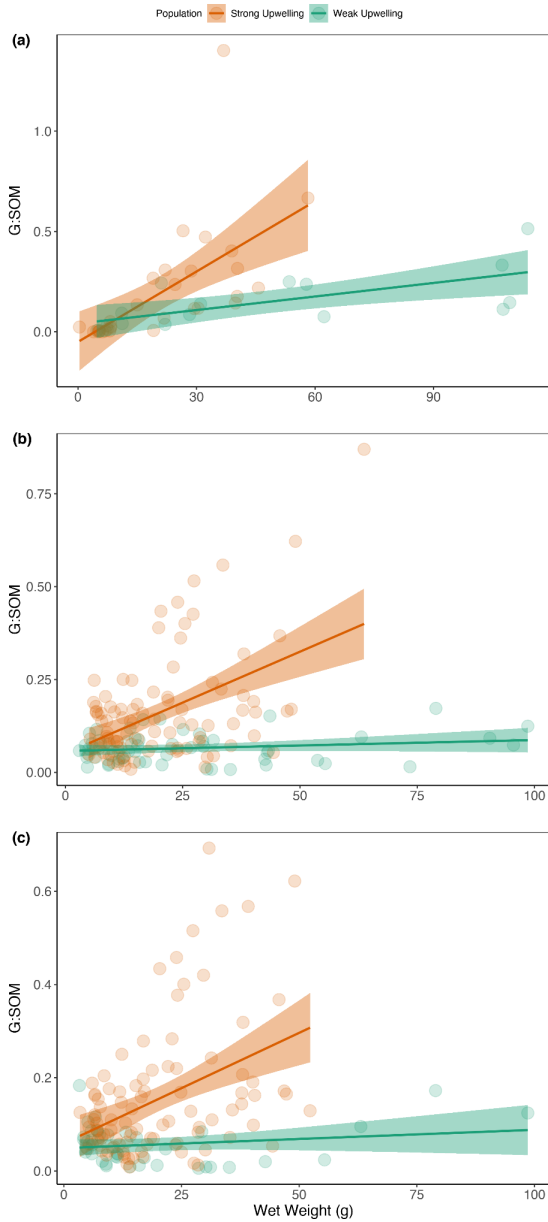| Response	Comparison	Estimate	s.e.	d.f.	t	p-value
	Northern North Current - Northern North Future	0.0124	0.0019	1003	6.41	**<0.0001**
Mortality Rate	Northern North Current - Southern North Current	0.0329	0.0021	1003	15.33	**<0.0001**
	(North Current - North Future) - (South Current - South Future)	-0.0204	0.0029	1003	-7.14	**<0.0001**

Fig S1. Shows relationship between weight and gonad to somatic tissue ratio (body condition), a) initially; b) after three months in common garden current conditions; and c) after three months in region-specific current and future conditions. Orange circles and lines indicate individuals from the strong upwelling population and green circles and lines indicate individuals from the weak upwelling population.
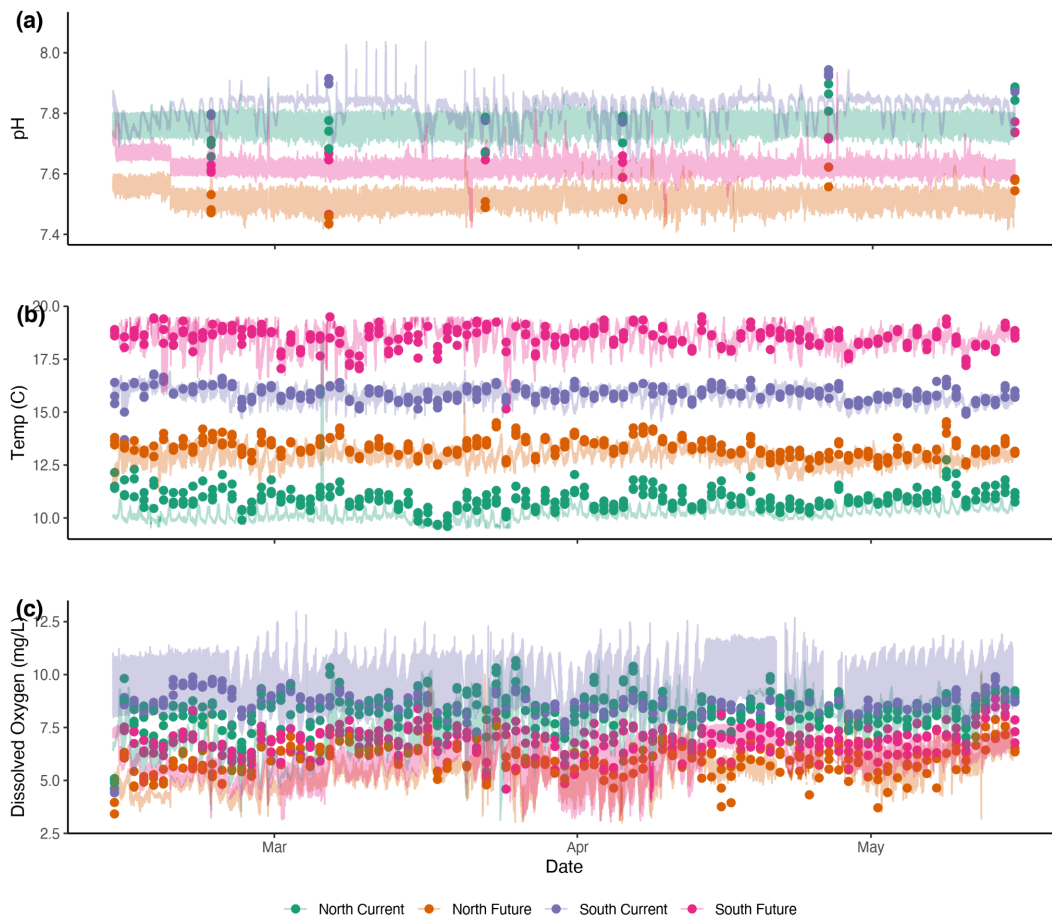
Fig S2. Experimental conditions within the mesocosm for the duration of the 83-day experiment. Lines represent Durafet (temperature and pH) and Vernier (dissolved oxygen) measurements within header buckets occurring every 15 min. Points in (a) represent spectrophotometric pH measurements in header buckets (YSI pH measurements were not reliable). Points in (b) and (c) represent YSI measurements (temperature and dissolved oxygen) in experimental bins taken daily (~10:00-14:00).