UNIVERSITY OF CALIFORNIA

Santa Barbara

Studies of early life history stages of marine invertebrates in the context of coastal pH and oxygen variability

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution and Marine Biology

by

Umihiko Hoshijima

Committee in charge:

Professor Gretchen Hofmann, Chair

Professor Libe Washburn

Professor Erika Eliason

June 2018

The dissertation of Umihiko Hoshijima is approved.

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Libe Washburn

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Erika Eliason

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Gretchen Hofmann, Committee Chair

May 2018

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VITA OF UMIHIKO HOSHIJIMA

Education

2013-2018 **Ph.D. Candidate** – Ecology, Evolution and Marine Biology
University of California Santa Barbara

2009-2013 **B.S. Biological Sciences** – University of California Santa Cruz
Honors in the Major; Minors in Chemistry, Electronic Music

Professional Experience

2013-2018 **Graduate Research.** Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara.
Advisor: Professor Gretchen Hofmann

2016-2018 **Graduate Research**. Santa Barbara Coastal Long-Term Ecological Research (SBC-LTER) project. PI: Dr. Dan Reed

2013 **Lab and Field Technician**. Department of Ecology and Evolutionary Biology, University of California, Santa Cruz. Advisor: Professor Mark Carr

2010-2013 **Lab and Field Technician**. National Marine Fisheries Service, NOAA, Santa Cruz, CA. Advisor: Dr. Sean Hayes

2012 **NSF - REU Internship**. Shannon Point Marine Center, Western Washington University, Washington

2007 **Lab Technician**. The Scripps Research Institute, San Diego, CA.

Grants in Support of Research

2015 **Santa Barbara Coastal LTER Supplemental Equipment Grant**
Physiological responses of kelp forest organisms to low pH and O_2.
($7,555)

2014 **California Sea Grant** – *Global Chance Ecophysiology of egg masses and juveniles of the kelp forest fish,* Scorpaenichthys marmoratus. Co-written with PI, G. Hofmann. ($24,999)

2014 **UCSB Associated Students Coastal Fund** – *Sensor deployments in kelp forests measuring carbon dioxide and oxygen levels to assess the impacts of a changing ocean.* ($1,414)

Fellowships and Awards

2014-2017 **National Science Foundation** Graduate Research Fellowship (GRFP)
2014 **Continuing Student Graduate Fellowship**. Department of Ecology, Evolution and Marine Biology, UC Santa Barbara. ($6,000).
2013-2014 **Graduate Fellowship** Department of Ecology, Evolution and Marine Biology, UC Santa Barbara.
2012 **National Science Foundation** Research Experience for Undergraduates (NSF-REU) recipient. Shannon Point Marine Center, Western Washington University, Washington.

Peer-Reviewed Publications

Umihiko Hoshijima, Juliet M. Wong, and Gretchen.E. Hofmann. Additive effects of pCO_2 and temperature on the respiration rates of the Antarctic pteropod, *Limacina helicina antarctica. Conservation Physiology* (2017). doi: 10.1093/conphys/cox064

Kevin M. Johnson, **Umihiko Hoshijima**, Cailan S. Sugano, Alice T. Nguyen, and Gretchen E. Hofmann. Shell dissolution observed in *Limacina helicina antarctica* from the Ross Sea, Antarctica: paired shell characteristics and in situ seawater chemistry. Biogeociences Discussions (2016). doi: 10.5194/bg-2016-467

Brent B. Hughes, Kamille K. Hammerstrom, Nora E. Grant, **Umihiko Hoshijima**, Ron Eby, Kerstin Wasson. Trophic cascades on the edge: fostering seagrass resilience via a novel pathway. *Oecologia* (2016). doi: 10.1007/s00442-016-3652-z

Emily B. Rivest, Margaret O'Brien, Lydia Kapsenberg, Chris C. Gotschalk, Carol A. Blanchette, **Umihiko Hoshijima**, and Gretchen E. Hofmann. Beyond the Benchtop and the benthos: dataset management planning and design for time series of ocean carbonate chemistry associated with Durafet® - based pH

sensors. *Ecological Informatics* (2016). doi: 10.1016/j.ecoinf.2016.08.005

Manuscripts in Preparation and Review

Hoshijima, U. and Hofmann, G.E. Spatiotemporal variability of seawater chemistry and dissolved oxygen in a kelp forest environment and linkages to *in situ* transgenerational effects in the purple sea urchin, *Strongylocentrotus purpuratus*. In preparation – Frontiers in Marine Science (abstract accepted for special issue: *Spatial and Temporal Variability of Seawater Chemistry in Coastal Ecosystems in the Context of Global Change*).

Hoshijima, U. and Hofmann, G.E. A time series of year-round pH from the Ross Sea, Antarctica: inter and intra-annual trends. (In preparation).

Wong, J.M., Leach, T., Kozal, L., **Hoshijima, U.,** Hofmann, G.E. Transgenerational effects in an ecological context: Conditioning of adult sea urchins to upwelling conditions alters the progeny's response to differential pCO2 levels (In preparation).

Hoshijima, U., Dilly, GF, Rivest, EB, Kapsenberg, L., Nguyen, A.T., Johnson, K.M., Kroeker, K.J., Sanford, E., Rose, J.M., Blanchette, C.A., Chan, F., Chavez, F.P., Gaylord, B., Helmuth, B., Hill, T.M., McManus, M.A., Menge, B.A., Nielsen, K.J., Raimondi, P.T, Russell, A.D., Washburn, L., and Hofmann, G.E.. Exploring local adaptation to ocean acidification in *Mytilus californianus* during simulated upwelling events (in preparation).

Johnson, K.M., Wong, J.M., **Hoshijima, U**., Sugano, C., and Hofmann, G.E. Seasonal transcriptomes of the Antarctic pteropod, *Limacina helicina antarctica* (in preparation).

Presentations of Research

Hoshijima, U., Hofmann, G.E. Ocean acidification in Santa Barbara and Antarctica. **Keynote Speaker**. Next Generation Science Standards: Local Phenomena Summer Institute, June 20, 2017.

Hoshijima, U., Hofmann, G.E. Marine sensor networks informing biological experiments at Mohawk Reef (poster). Santa Barbara Coastal Long-term Research Symposium, November 2016.

Bachhuber, S.M., Wong, J.M., **Hoshijima, U**., Johnson, K.M., Sugano, C.S., Hofman,

G.E. Transgenerational impacts of pH and temperature on early stage purple sea urchins, *S. purpuratus* (poster). Western Society of Naturalists, November 2016.

Hoshijima, U. How Antarctic pteropods (swimming snails) will cope with future ocean change. Sunday Science Lecture Series, McMurdo Station, Antarctica. October 29, 2016.

Hofmann, G.E., Johnson, K.M., **Hoshijima, U.**, Wong, J.M. Antarctic Pteropods (*Limacina helicina antarctica*) as a Sentinel Organism for the Impact of Ocean Acidification. Oceans in a High CO_2 world, April 2016.

Hoshijima, U. and Hofmann, G.E. Metabolic Effects of CO_2 and Temperature Stress on *Limacina helicina antarctica*. Graduate Student Symposium, UC Santa Barbara. February 2, 2016.

Hofmann, G.E., K.M. Johnson, **Hoshijima, U**, Wong, J.M., and Sugano, C. Pteropods, little marine snails, as an indicator of climate change. Sunday Science Lecture Series, McMurdo Station, Antarctica. November 22, 2015.

Hofmann, G.E., **Hoshijima, U**., Bachhuber, S. The Value of Long-term Oceanographic Data Sets for Global Change Ecology (poster). Western Society of Naturalists, November 2015.

Hoshijima, U. and van Alstyne, K. NSF-REU final presentation, Shannon Point Marine Center, Anacortes, WA. August 2012.

Teaching

Instructor (4 workshops) – *Scientific Programming in R.* UC Santa Barbara, CA. April 2015, April 2016, April 2017, May 2018). Designed lesson plans for "Intro to R", "Data Manipulation with reshape2, dplyr, and pipes", and "Data visualization with ggplot2".

Teaching Assistant - *Global Change Biology* (EEMB 155). UC Santa Barbara (Summer 2015).

Lab Teaching Assistant – *The Diversity of Life* (EEMB 3L). UC Santa Barbara (Spring 2014).

Workshops Attended

Climate Engagement Program –Professor Kristy Kroeker, UC Santa Cruz, CA. September 11-14, 2017.

*The Civic Scientist – how to become an effective public communicator***.** Richard Hayes, *The Union of Concerned Scientists.* April 28, 2017.

Autonomous pH Sensors – Best Practices. Scripps Institute of Oceanography, San Diego, CA. August 4-8, 2014.

Scientific Programming Python Workshop, UC Santa Barbara, CA. January 30-31, 2014.

Field Research

Field Team Leader, Antarctic Scientific Diver – United States Antarctic Program (USAP); McMurdo Station, Antarctica (6 weeks in 2016). PI: Professor Gretchen Hofmann.

Research Assistant, Antarctic Scientific Diver – USAP; McMurdo Station, Antarctica (11 weeks in 2015 and 2014). PI: Professor Gretchen Hofmann.

Channel Islands Research Cruise for Acidification Studies (CIRCAS), Santa Barbara, CA; **NOAA R/V Shearwater** (2 days, 2016; 2 days, 2015; 5 days, 2014). PI: Professor Gretchen Hofmann.

NOAA SWFSC Salmon Cruise, California Current; **F/V Frosti** (6 days, 2011). Chief Scientist: Dr. Sean Hayes.

Research Diving

- 300+ dives, 150+ hours logged
- Experience in low visibility, ice diving (McMurdo Station, Antarctica), drysuit diving.
- AAUS: Scientific diving authorization maintained at UC Santa Barbara (100ft) with drysuit authorization.
- UCSB Diving Control Board representative, 2017-present

- NAUI: Advanced Open Water, Rescue Diver, Teaching Assistant, EANx, and Master Diver certifications.
- PADI: Open Water certification.
- First Aid, CPR, DAN O_2 Certifications maintained 2012-present.

Student Advising

2017-present Maddie Housh (lab assistant/technician)

2016-2017 Margarita McInnis (lab assistant)

Public Education and Outreach

Guest Lecturer, *Graduate Professional Development* (EEMB 510), UC Santa Barbara. April 24, 2018.

Live music and Antarctic Diving. World Oceans Day Event, Santa Barbara, CA. June 3, 2017.

On Thin Ice: Exploring global change biology in the Antarctic with art and science. Public lecture series, Sierra Nevada Aquatic Research Laboratory, March 2, 2017. https://youtu.be/CyRiDlIeBaw?t=10m43s

Diving Under Antarctic Ice: Researching seawater pH dynamics and marine biology in one of the last true marine wildernesses. UC Santa Barbara SCUBA club, February 7, 2017.

Under the Ice. INT91: Early start program for high school students. July 27, 2016.
Global Change Biology and Antarctic Diving. Science "Pub Talk" Series hosted by the Santa Barbara Natural History Museum. July 11, 2016.

Antarctica: On Thin Ice. World Oceans Day Event, Santa Barbara, CA. June 4, 2016.
To Antarctica and Back Again: Researching seawater pH dynamics and marine physiology in one of the last true marine wildernesses. Paradise Dive Club Lectures Series, Santa Barbara, CA. May 31, 2016.

Guest Lecturer, *Graduate Professional Development* (EEMB 510), UC Santa Barbara. April 15, 2016.

Career Panelist. *Biology Mentoring and Engagement* (MCDB 13), UC Santa Barbara. April 12, 2016.

Career Panelist. *Processes in Aquatics Systems* (EEMB 142B), UC Santa Barbara. February 12, 2016.

Guest Lecturer. *Exciting Developments in Biology Research* (INT 94ES), UC Santa Barbara. January 29, 2016.

Global Change Biology and Antarctic Diving. Marine Science course, La Jolla High School, CA. January 5, 2016.

Antarctic Research and Coldwater Diving. Paradise Dive Club Lectures Series, Santa Barbara, CA. August 2015.

Guest Lecturer, *Intro to Oceanography* (Earth 3), UC Santa Barbara. June 6, 2015.
Kelp Forests on Acid: Local Climate Change Mitigation in a Changing Ocean. Grad Slam competition, UC Santa Barbara. April 2015. **Semifinalist.**

Global Change Biology: From California to Antarctica. Marine Science course, La Jolla High School, CA. January 2015.

Academic Service

Co-Chair, Gordon Research Seminar on Ocean Global Change Biology, 2018.

Skype a Scientist: video conferencing with K-12 science classrooms (2017-present).

Chair, Cohort representative and Webmaster. Graduate Student Advisory Committee (GSAC). UC Santa Barbara. 2013-Present, Chair (2016-17), Vice Chair (2015-16) and Webmaster (2017).

Contributor and panelist for the NOAA Channel Islands Marine Sanctuary Condition Report, 2016.

Mentor for junior high school students and families. Family Ultimate Science Exploration (FUSE), Center for Science and Engineering Partnerships, UC Santa Barbara. Leading science activities for junior high school students and families. 2016.

Mentor for undergraduate biology students. Graduate Biology Mentorship Association (BIOME), UC Santa Barbara. Panelist at 5 events geared towards undergraduate exposure to different career paths. 2014-2016.

In The News

Shedding New Light on the Mysteries of Antarctica's Long, Dark Winter. Sarah Laskow, Atlas Obscura. May 15, 2018. https://www.atlasobscura.com/articles/when-is-winter-in-antarctica

Sisters of the Blue (Podcast/Radio Show). KCSB 91.9FM. July 12, 2017. https://www.sistersoftheblue.com/radio/2017/11/9/l7colo43nmt2fznnkkwdngthd5b0bm

The Dissolving Sentinels of the Southern Ocean. Michael Lucibella, The Antarctic Sun (NSF US Antarctic Program). http://antarcticsun.usap.gov/science/contentHandler.cfm?id=4207

Pteropods and B-134. Shaun O'Boyle (NSF Antarctica Artists and Writers Program). https://popantarctica.wordpress.com/2015/11/17/pteropods-b-134/

Asians Doing Everything. http://asiansdoingeverything.tumblr.com/post/128280622030/name-umihiko-hoshijima-location-santa-barbara-ca

UCSB Hofmann Lab Examines Effects of Ocean Acidification on Sculpin. https://thebottomline.as.ucsb.edu/2015/03/ucsb-hoffman-lab-examines-effects-of-ocean-acidification-on-rockfish

Graduate Student Umi Hoshijima Takes on Bitter Cold and Lightning-quick Penguins. UCSB GradPost. http://gradpost.ucsb.edu/headlines/2014/12/1/graduate-student-umi-hoshijima-takes-on-bitter-cold-and-ligh.html

ABSTRACT

Studies of early life history stages of marine invertebrates in the context of coastal pH and oxygen variability

by

Umihiko Hoshijima

One challenge of global change biology is understanding the fate of organisms under future pH change in variable coastal environments (Shaw *et al.*, 2013). Coastal pH dynamics can vary at the global scale (Hofmann *et al.*, 2011), as well as at finer regional scales (Frieder *et al.*, 2012), and this mosaic of pH exposure will likely play a large role in mediating the fate of coastal organisms to near-future change.

Through this dissertation, I deploy oceanographic sensors to categorize these changes at a variety of scales, and pair these results with biological experiments designed to investigate the response of juvenile life stages to current and future extremes. I deployed and retrieved moorings, conducted time series data analysis, deployed caged outplant experiments, spawned and cultured sea urchin embryos, and conducted respirometry on macrozooplankton.

This dissertation consists of 4 chapters: **1)** deploying sensors to investigate fine-scale spatiotemporal variation in a Southern California kelp forest, **2)** conducting a transgenerational experiment to the biological performance of offspring when adults are conditioned inside of a kelp forest environment, **3)** gathering a long-term time series of ocean pH in Antarctica by deploying oceanographic sensors under the fast ice, and **4)** investigating the metabolic response of juvenile Antarctic pteropods to current and near-future extremes of temperature and pH.

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I. Introduction

Ocean acidification (OA), dubbed the "other CO_2 problem" (Doney *et al.*, 2009), has become a major focus in our attempt to understand the response of marine organisms to future ocean change. OA is a multi-faceted stressor – it increases the partial pressure of CO_2 (pCO_2) in seawater (1.90 μatm yr^{-1}), lowers the pH by forming carbonic acid in (H_2CO_3) in seawater (-.0018 pH units yr^{-1}), and subsequently alters the carbonate system by decreasing the amount of carbonate (CO_3^{2-}) (Feely *et al.*, 2009). Not only does the depletion of CO_3^{2-} decrease the buffering capacity of seawater, but it also interferes with the biomineralization of calcium carbonate ($CaCO_3$) crystal forms such as aragonite and calcite by decreasing their saturation state (-.012 yr^{-1}, -.00758 yr^{-1}, respectively), a process which has already had biological consequences in certain systems (Bednaršek *et al.*, 2017b, 2012a).

OA was initially identified as an open ocean issue, with most of the data coming from large-scale oceanographic cruises (Dore *et al.*, 2009). However, with the advent of autonomous sensors that facilitated the collection of pH data at nearshore locations, it has since come to light that pH along coastlines is very location-specific, shows different patterns across a number of types of coastal ecosystems, and was revealed to be heterogenous at a variety of spatial scales (Hofmann *et al.*, 2011). In these coastal marine ecosystems, biotic and abiotic factors drive pH swings that can indeed occur on a variety of timescales at magnitudes relevant in the context of global change (Kapsenberg and Hofmann, 2016). Understanding these dynamics and their

drivers at a range of spatiotemporal scales is critical to assessing the fate of future marine communities – a venture important in generating informed management decisions to protect coastlines vulnerable in the future (McLeod *et al.*, 2009).

Conversely, it may be equally important to understand the nature of potential refuges from anthropogenic OA; that is, some coastlines naturally have more basic pH values, and thus may function to locally buffer the effects of ocean acidification in the future. Potential sources of this buffering capacity may include submerged underwater vegetation (SUVs), areas of high standing photosynthetic biomass such as kelp forests and seagrass beds that dramatically alter local pH dynamics (Frieder *et al.*, 2012). Photosynthesis by these SUVs has received attention in the context of carbon sequestration, commonly referred to as "blue carbon" (McLeod *et al.*, 2011). However, more recently, SUVs have been recognized for their potential to alter ocean pH on a variety of timescales, creating refuges from future acidification (Nielsen *et al.*, 2018).

In the context of studying sites with natural variation in pH, one of my study systems was the California Current System (CCS), a coastal region characterized by episodic upwelling. Here, upwelling of deep, CO_2-rich seawater drives a decrease in the mean pH of water masses, which are subsequently altered by local biotic conditions to create a dynamic pH system (Chan *et al.*, 2017a; Kapsenberg and Hofmann, 2016). In general, this makes the CCS a unique opportunity to use environmental variation as a "natural experiment" to observe tolerance of organisms to today's highly variable conditions (Hofmann *et al.*, 2014; Koweek *et al.*, 2017). However, upwelling events bring not only a decrease in pH, but is also coupled with

low oxygen content and low temperature (Chan *et al.*, 2017a; Frieder *et al.*, 2012). This creates a unique set of circumstances that will likely increase in frequency and magnitude in the near future (Wang *et al.*, 2015). Particularly in the CCS, investigating the fate of marine organisms to future environmental change may mean trying to understand the physiological costs and consequences of being in an increasingly high CO_2 (hypercapnic), low O_2 (hypoxic), and cold environment for punctuated times during the spring upwelling season (McAfee *et al.*, 2015).

To address the biological consequences of physical variation in the CCS, I chose to work in the kelp forest environment, a dominant ecosystem type where upwelling signatures interact with the ability of this macrophtye, *Macrocystis pyrifera (Agardh, 1823)*, to transform the physicochemical characteristics of the local seawater (Frieder *et al.*, 2012; Koweek *et al.*, 2017). For the study organism, I chose to investigate the early life history stages of the purple sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857), an ecologically important grazer in the kelp forest ecosystem (Pearse, 2006). In these dynamic kelp forest environments, sea urchins are a key algal herbivore that can cause large-scale regime shifts in the community (Filbee-Dexter and Scheibling, 2014). With their calcium carbonate skeletal rods as a critical part of their larval morphology, larval purple urchins in California have been a subject of study and a species of concern under future OA (e.g. Kapsenberg *et al.*, 2017; Kelly *et al.*, 2013; Wong *et al.*, 2018; Yu *et al.*, 2011). With looming ocean acidification often expected to outpace evolution, mechanisms that drive rapid adaptation – e.g. epigenetics and transgenerational effects – have become a focus of attention in the

research community for their ability to alter phenotype over very few generations (Hofmann, 2017; Marshall, 2008). Recent work has shown the potential for larval gene expression to change based on the environmental exposure of the parental generation of sea urchins (Wong *et al.*, 2018), further solidifying the possibility that mechanisms that alter seawater conditions for organisms, such as SUV, may play a large role in the fate of marine communities in the near future.

Although the CCS exhibits strong seasonal effects with the frequency of seasonal upwelling events, other coastal systems look starkly different on a seasonal scale. For example, in McMurdo Sound, Antarctica, a region in the Pacific sector of the Southern Ocean, the seasonal cycles observed over two years' worth of data showed a large seasonal cycle of pH (~0.4 pH unit range) linked to seasonal light availability and the resulting net photosynthesis and respiration (Kapsenberg *et al.*, 2015). These pH changes were also accompanied by modest temperature fluctuations. Continuing to understand these seasonal dynamics in polar seas will be critical to understanding how organisms in these subzero waters will cope with future ocean change.

In the Antarctic, these large seasonal changes in carbonate chemistry are of particular interest in the context of zooplankton that spend long periods developing on the plankton. *L. h. antarctica,* a dominant member of the macrozooplankton in the Southern Ocean, overwinter as juveniles, exposed to low pH during the winter, and begin to grow and mature rapidly in the summer months (Lischka and Riebesell, 2012). As such, these pteropods are overwintering in the coldest, most food-poor, most low-pH times of the year, and it is critical to understand the fate of such

ecologically key species in response to near-future change, while considering in tandem the timing of their reproduction and life history, and its relationship with the abiotic environment.

Statement of the Problem

The overarching goal of my dissertation research was to understand the physiology of early developmental stage invertebrates in response to both present-day abiotic conditions, and to future environmental extremes that are projected in various scenarios for increases in anthropogenic atmospheric CO_2. This was accomplished with paired field and laboratory components in two distinct marine ecosystems: the temperate kelp forests of California, and in coastal polar seas, here McMurdo Sound in the southern Ross Sea. My general approach was to deploy autonomous sensors *in situ* to capture natural variability, and these observations where then used to plan experiments with an ecological context.

To gather these environmental data, I deployed sensors at two locations and collected data for two times series: (1) a long-term time series in McMurdo Sound, Antarctica during research with the U.S. Antarctic Program, and (2) a short-term time series that was developed with dense sensor deployments at kelp forest study sites near Santa Barbara, CA, a project that conducted in collaboration with the Santa Barbara Coastal Long Term Ecological Research program. I coupled each of these sensor deployments with a biological experiment designed to test the capacity of present-day organisms to respond to current and future extremes of environmental

conditions. In the Antarctic system, I used the distinct seasonal pH changes described in my long-term pH time series to investigate how juvenile thecosome pteropods cope with exposure to current and future extremes of pCO_2 and temperature in tandem. In the California system, I designed and executed an experiment to test how the environmental conditioning/experience of adult purple sea urchins within a kelp environment changed the traits of their progeny.

***Chapter II**: Spatiotemporal variability of seawater chemistry and dissolved oxygen in a kelp forest environment*

In this Chapter, I investigated the variation of pH, dissolved oxygen, and temperature around a kelp forest at Mohawk Reef, a study site that is offshore of Santa Barbara, CA. I deployed oceanographic sensors at paired sites, inside the kelp forest and outside of the kelp forest, in order to ascertain the effect that the presence of kelp had on the pH and oxygen regimes of the seawater in that area of the kelp bed. Through subsequent deployments, I was able to assess the dynamics of oxygen and pH both between the inside and outside kelp forest sites, as well as vertically in the water column inside of the kelp forest.

I found stark differences in the oxygen and pH regimes between these sites, which would indicate that organisms around kelp forests would experience different conditions in their benthic environment depending on the presence of kelp.

Furthermore, I found that changes in the physical environment also existed in the water column, even in the relatively shallow depths of the onshore site.

The work in this Chapter laid the groundwork for the experiment detailed in Chapter 3, where I investigated the effect of this environment on purple sea urchins in a transgenerational context.

Chapter III: *Transgenerational effects in a kelp forest environment: The influence of in situ adult conditioning on egg quality and the performance of early life history stages of the purple sea urchin,* Strongylocentrotus purpuratus

In this Chapter, I applied the differences in coastal water chemistry observed in Chapter 2 to design a coupled field and laboratory experiment that inquired about how the environmental exposure of adult sea urchins can mediate the resilience of their offspring to pCO_2 stress. For this, I placed cages of sea urchins, co-located with sensors, inside and outside of a kelp forest environment at Mohawk Reef for 4 months in 2017 to allow them to undergo gametogenesis *in situ*. Then, in the laboratory, the progeny of these urchins were raised under two pCO_2 treatments designed to bracket a range of conditions that early stages might experience during development in the plankton.

This experiment is novel for being, to my knowledge, the first caged outplant experiment to attempt to understand the affect that underwater macrophytes, and their ability to transform characteristics of seawater, can have on the

transgenerational plasticity of organismal response to variation in abiotic conditions. In this experiment, I found that females held inside of the kelp forest generated fewer, more protein-rich eggs, while females held outside of the kelp forest generated more numerous, less protein-rich eggs. When raised in culture in the laboratory, the progeny of adults conditioned outside the kelp forest had a pCO_2 effect on body size, while progeny of adults conditioned inside of the kelp forest did not. It is clear that the environmental exposure of adults during their 4-month outplant not only affected measurable traits in the egg, but also affected the response of their progeny to stress.

***Chapter IV**: A multi-year dataset of ocean pH from McMurdo Sound, Antarctica*

In this Chapter, I deployed oceanographic pH sensors in McMurdo Sound, Antarctica. This series of deployments is novel for being the first set of ocean pH measurements made through the austral winter under seasonal fast ice in the Ross Sea due to the restrictions of earlier shipboard measurement techniques. These deployments encompassed high-frequency pH data over 5 years and 3 different sites in the Ross Sea region, which allowed me to resolve both inter-site and inter-annual variation in the time series. In the most salient finding, I found evidence of modest ocean acidification over the 5-year period under study, and found differences in the seasonal timing of pH variation that coincides with known current flows in the area. Through these deployments, I also compiled the inherent difficulties of conducting fieldwork and diving operations in a remote, overhead, subzero environment.

Chapter V: *Additive effects of pCO_2 and temperature on respiration rates of the Antarctic pteropod Limacina helicina antarctica*

In this Chapter, I exposed juveniles of the Antarctic thecosome pteropod, *Limacina helicina antarctica*, to a suite of pCO_2 and temperature conditions mirroring the natural variation measured in Chapter 4, as well as pCO_2 conditions that mimicked future, ocean acidification conditions. This involved the fabrication of a novel system for maintaining these macrozooplankton in a flow-through system with pCO_2 and temperature control. Through these studies, I found that temperature and pCO_2 stress interact additively to increase the metabolism of the Antarctic pteropod. This published dataset is the first look into the stress response of juvenile pteropods in the Ross Sea (Hoshijima *et al.*, 2017).

II. Spatiotemporal variability of seawater chemistry and dissolved oxygen in a kelp forest environment

Abstract

In the face of global change, there has been a renewed interest in marine carbon sinks, often termed blue carbon. In addition to sequestering carbon, marine macrophytes have the potential to locally modulate the effects of ocean acidification (OA) and hypoxia through net photosynthesis. However, while this phenomenon has been widely discussed for seagrasses and kelp forests, it has not been rigorously detailed in many coastal environments.

Here, I deployed high-frequency pH and oxygen sensors inside and outside of the Mohawk Reef kelp forest, as well as at various depths (surface, benthos, and midwater) at these sites. I found that pH and dissolved oxygen (DO) varied with depth, as well as varying between sites inside and outside of a kelp forest environment. More specifically, low pH and DO excursions (DO <5, pH <7.7) co-occurred outside the kelp forest environment in the region. I also observed stronger diurnal cycles of oxygen at the top of the water column compared to the benthos, as well as stronger cycles inside the kelp forest in comparison to outside the kelp forest.

Because pH and DO covaried strongly, the kelp environment is therefore dominated by a pattern with low pH stress episodically coinciding with periods of low oxygen stress. These two factors vary at different frequencies inside and outside of the kelp forest environment. Overall, the data in this Chapter suggest that this variation

can have impacts on the biology of the nearshore environment, particularly in the face of global change.

Overview

This work was conducted in collaboration with the Santa Barbara Coastal (SBC) Long-Term Ecological Research (LTER) group from 2014 to 2017. During this time, the SBC LTER provided graduate research support to me, as well as support in boating and diving logistics. Direct funding for this research was provided by California Sea Grant (R/HCME-13), and funds from the UC Santa Barbara Associated Students Coastal Fund. Portions of the data in this chapter have been published (namely, Figure 4) in (Rivest *et al.*, 2016), on which I was a coauthor.

Introduction

Recently, nearshore pH dynamics have been demonstrated to be highly variable in a suite of coastal marine ecosystems (Baumann *et al.*, 2015; Guadayol *et al.*, 2014; Hendriks *et al.*, 2014; Hofmann *et al.*, 2011; Kapsenberg *et al.*, 2017; Kapsenberg and Hofmann, 2016; Kline *et al.*, 2015; Kroeker *et al.*, 2016; Kwiatkowski *et al.*, 2016; Sorte and Bracken, 2015; Unsworth *et al.*, 2012). One emerging aspect of these collective observations is that the importance of macrophytes, or submerged underwater vegetation (SUV), in altering the CO_2 and O_2 of the surrounding water in a daily cycle through photosynthesis during the day and respiration at night (Nielsen *et al.*, 2018).

SUV may also create functionally create refuges from future ocean acidification (OA) on a local scale while also increasing mean levels of dissolved oxygen (DO) and decreasing mean levels of dissolved inorganic carbon (DIC) through net photosynthesis (McLeod *et al.*, 2011; Nielsen *et al.*, 2018). This service may be critical for coastal conservation in California, considering the risk of hypoxia (low O_2) and hypercapnia (high CO_2) for coastal organisms (Gobler and Baumann, 2016), an intensifying future exposure caused by episodic coastal upwelling in the subarctic Pacific (Chan *et al.*, 2017b; Checkley and Barth, 2009; Whitney *et al.*, 2007). This idea that SUVs might create chemical refuges from OA is especially tractable considering that both kelp forests and seagrasses are often seen as critical foundation species and nursery habitats for a variety of ecologically and economically critical fisheries species along the U.S. West Coast (Ruckelshaus *et al.*, 2013). Thus, historically, these 12*Macrocystis*-dominated habitats have been a focus of coastal restoration, protection, and management prior to the emerging discussion of their potential role in climate change mitigation in coastal waters of the U.S (Nielsen *et al.*, 2018; Strong *et al.*, 2014).

Recently, this mitigation capacity of SUVs has been linked to Marine Protected Areas (MPAs) (Nielsen *et al.*, 2018). Kelp and seagrasses in protected areas may be providing a service that was not expected until pH sensors were deployed and these data began to be shared with the science community and managers (Hofmann *et al.*, 2011). In general, the goal of marine protection goes beyond the primary protection of individuals residing in its borders. In particular, the potential for protected populations to seed the areas around them through larval dispersal or migration, often

referred to as the "spillover effect" of marine protection, has been a strong factor in considering the design and effectiveness of marine reserves (Buxton *et al.*, 2014; da Silva *et al.*, 2015). While this may encapsulate the biological benefits of a marine reserve, here I investigate the spillover effect from a different and novel perspective: the extent to which the physiochemical alterations of the water column caused by macrophytes can influence the areas directly surrounding them.

To date, a diurnal variation of physiochemical characteristics has been observed in seagrass beds (Hendriks *et al.*, 2014; Nielsen *et al.*, 2018; Pacella *et al.*, 2018; Unsworth *et al.*, 2012) and kelp forest environments (Britton *et al.*, 2016; Delille *et al.*, 2009; Frieder *et al.*, 2012; Kapsenberg and Hofmann, 2016), but few studies have analyzed this variation over both short timescales and short distances. In this study, I surveyed the kelp forest environment with several deployments of high-frequency oceanographic sensors to understand how these dynamics differ inside and outside of a kelp forest, while analyzing over daily and seasonal time scales.

The carbonate system and DO patterns in kelp forests along the California Current Large Marine Ecosystem (CCLME), observed through sensor deployments (Frieder *et al.*, 2012) and discrete sampling (Koweek *et al.*, 2017), have yielded observations that signal the significance of small distances and and fine temporal variation on this nearshore system (Chan *et al.*, 2017b; Hofmann *et al.*, 2013). Particularly of note is the consistent high-frequency covariation of pH and DO as multiple stressors in these nearshore environments (Frieder *et al.*, 2012) that interact with a mosaic of coastal upwelling along the coastline (Chan *et al.*, 2017b). Variation

can be found regionally and locally, and can occur on a diel basis (Kapsenberg and Hofmann, 2016). Biologically, these patterns all indicate that marine organisms in this nearshore habitat can experience pH and oxygen regimes both higher and lower than those outside of these habitats. In partiular, sessile/benthic adult marine invertebrates in the benthic environment likely experience a range of pH/DO *in situ*, and further, that this gradient varies in strength depending on the proximity to macrophytes. Understanding organismal responses in this complex coastal system will help to elucidate the future of these coastal oceans to complex environmental change (Gunderson *et al.*, 2016).

Given this information, I hypothesize that the kelp forest will alter the local water chemistry by imparting a strong diurnal signal of pH and oxygen. While this will likely be strongest at the top of the water where the kelp biomass is the densest, it will likely reach the bottom of the water column and affect the benthic environment.

To test this hypothesis, I deployed oceanographic sensors to profile the pH and DO of the nearshore kelp forest environment with fine spatiotemporal resolution. The study sites were SBC LTER field sites that have been utilized for long-term ecological and oceanographic data collection. This field project also included a biological outplant experiment co-located with the deployed sensors (Chapter 3) in order to understand the *in situ* effects of this variation on organismal performance.

Methods

Study Site – Mohawk Reef

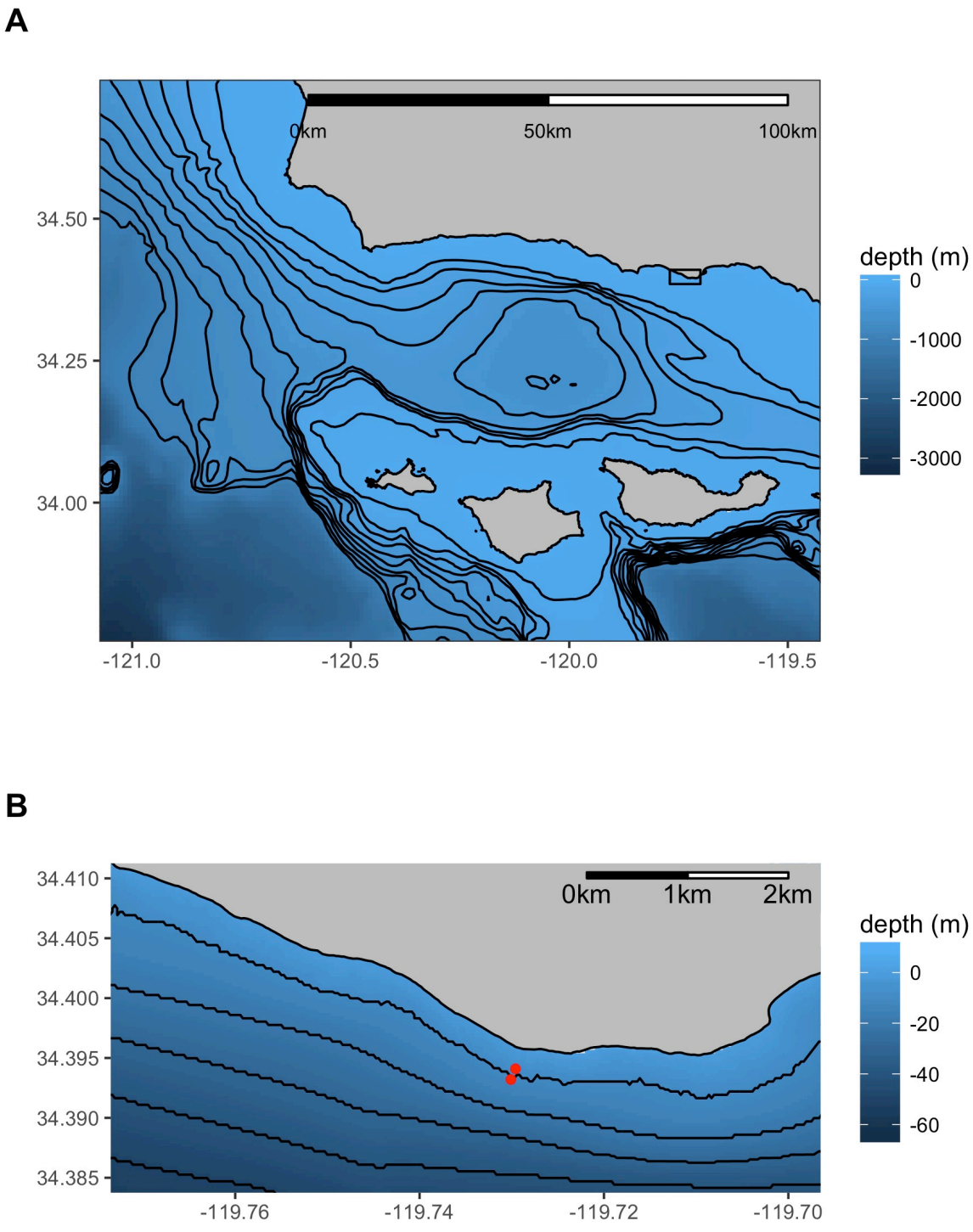
Field research was conducted at Mohawk Reef in the Santa Barbara Channel, CA (Figure 1). Mohawk Reef is a shallow, rocky temperate reef defined by kelp beds of giant kelp (*Macrosystis pyrifera*); although varying with year and season, these beds typically extend 300m along-shore, and from 120 to 200m cross-shore (Gaylord *et al.*, 2007). Mohawk Reef (MKO) is also a permanent long-term research site for the SBC LTER, where the mediation of kelp forest dynamics by abiotic factors such as water attenuation (Gaylord *et al.*, 2007) and wave action (Reed *et al.*, 2011) have been well-characterized.

Sensor types and deployment profiles

The sensor assets (described below) were located at one of two locations at MKO: the inshore site was located inside of the kelp forest at a depth of 7m (34.3941˚N, 119.7296˚W), while the offshore site was located outside of the kelp forest, in an area of sandy substrate at a depth of 11m (34.3932˚N, 119.7301˚W). These two locations were 130m apart, and will hereafter be referred to as the "inside" and "outside" locations, respectively (Figure 1). Each of these sites were marked with a subtidal mooring topped by a spar buoy, and all sensor deployments were conducted on these moorings.

In certain deployments, I arranged sensors vertically along these mooring lines to observe the vertical stratification of seawater chemistry in the kelp forest water

Figure 1. Map of the research site - Mohawk Reef. The inset (B) details the sites relative to the coastline in close proximity (130m apart). The “inside” site is northwest (inshore) of the “outside” site. Isobaths are 100m (A) or 10m (B).



column. Here, I deployed sensors at three depths: (1) just below the mean low tide on the buoy, (2) in the middle of the water column, and (3) 1 m off the bottom. These will be referred to as the "**top**", "**middle**", and "**bottom**" positions, respectively.

The deployed sensor arrays consisted of a combination of SeapHOx® pH-oxygen sensors (assembled by Todd Martz at Scripps Institute of Oceanography) and miniDOT® oxygen sensors (Precision Measurement Engineering). The SeapHOx® as a unit is equipped with a Honeywell DuraFET III pH electrode, an Aanderaa® 3835 oxygen optode, and Seabird® SBE-37 microCAT CTD (Martz *et al.*, 2010; McLaughlin *et al.*, 2017).

Sensors were deployed using established best practices techniques (Bresnahan *et al.*, 2014; Kapsenberg and Hofmann, 2016; Rivest *et al.*, 2016). Briefly, I collected pH samples during the sensor deployments, using SCUBA to trigger Niskin bottles underwater to coincide with a sensor sampling timepoint. These samples were immediately fixed with 0.02% $HgCl_2$ and stored at 15˚C until analyzed with a spectrophotometric pH assay (unpurified *m*-Cresol, Sigma-Aldrich®, assayed in a Shimadzu UV-1800 spectrophotometer at 730nm, 578nm, and 434nm at 25˚C) and an open-cell endpoint titration for total alkalinity (certified 0.1M HCl titrant, titrated using a Mettler-Toledo T50 autotitrator) (Dickson *et al.*, 2007). I calculated alkalinity from raw electrode values using the R package *seacarb* (Gattuso *et al.*, 2017), and used to temperature-correct the measured pH value to match *in situ* conditions. These pH calibration samples were then used to calibrate the data using *seacarb*.

For oxygen measurements, I placed multiple sensors in the same flow-through sea table tanks to ensure precision before and after each deployment, and Winkler titrations were conducted to ensure accuracy after each deployment (Wetzel and Likens, 1991). I calculated oxygen concentration from oxygen saturation using relationships from Weiss (1970) (R package "marelac" v.2.1.6) using salinity values from the long-term mooring time series maintained by the SBC-LTER (Washburn, 2018)

In support of this part of my thesis, I conducted 3 types of deployments from June 2014 to December 2016. The details regarding each of the three deployments are described below:

Sensor Deployment 1: Dense oxygen sensor deployments inside and outside of a kelp forest environment

To understand the differences in daily changes of oxygen content inside and outside of the kelp forest environment, miniDOT® oxygen sensors were placed at all three depths at both inside and outside locations at MKO, for a total of six sensors. Sensors were programmed to log every 10 minutes. I deployed these sensors for 20 days in 2014 (June 2 ~ June 23, 2014). Sensors were retrieved and data were analyzed for patterns of oxygen dynamics as a function of depth and proximity to the kelp forest.

Sensor Deployment 2: Comparing benthic pH-oxygen relationships inside and outside of a kelp forest environment

To compare benthic relationships between pH, oxygen, and *in situ* temperature inside and outside of a kelp forest, SeapHOx® sensors were deployed 1 m off the bottom at both the inside and outside spar buoy locations for four weeks in 2015 (March 10 ~ April 08, 2015). Sensors were programmed to log every 20 minutes. The relationship between pH and oxygen was modeled linearly following Frieder et al. (2012) due to the strong linearity of this theoretically nonlinear relationship over the observed range.

Sensor Deployment 3: Seasonal variation of pH and oxygen in a kelp forest environment

To ascertain the seasonal variation in the pH and oxygen dynamics in the kelp forest environment, an array of miniDOT® and SeapHOx® sensors were deployed at MKO from March 2016 to December 2016. Three miniDOT® sensors were placed inside of the kelp forest at the same three depths as in Deployment 1. One SeapHOx® sensor was placed 1 m off the bottom at the outside moorings, identical to the outside mooring site in Deployment 1. miniDOT® sensors were replaced approximately every three months, and SeapHOx sensors were replaced approximately every 2 months due to battery and biofouling constraints. Sensors were programmed to log every 20 minutes. Due to sensor failure, oxygen and temperature at the top of the water column at the inside mooring was not collected past August 03, 2016. Due to logistic

constraints of deployment, pH, oxygen, and temperature at the bottom on the outside mooring was not collected past October 03, 2016.

Data Analysis

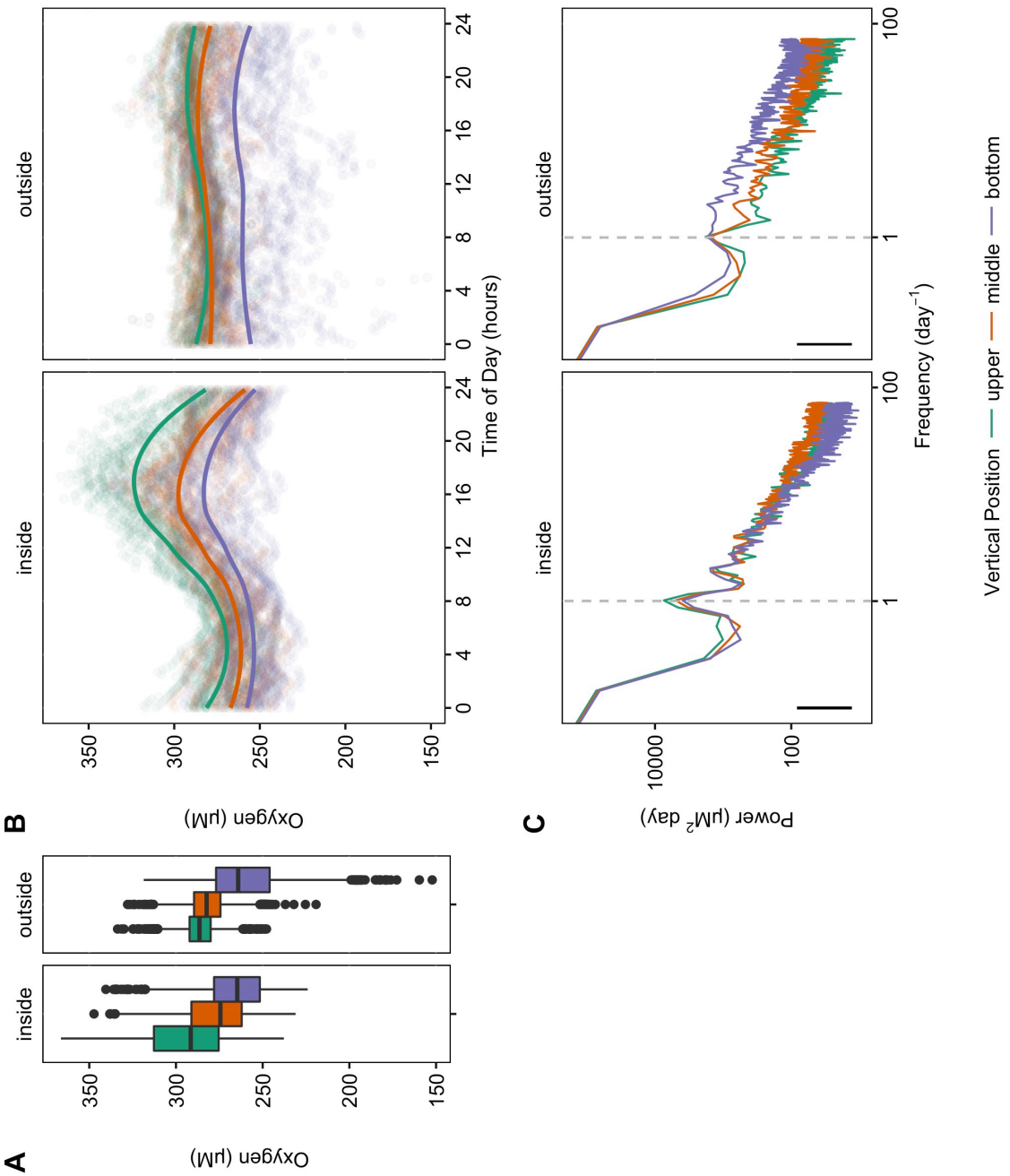
All statistics and analysis were conducted using the R statistical software, as well as the R Studio IDE (RStudio Team, 2017). All power spectral density (PSD) estimates were conducted with 5 overlapping Hanning windows (50%) for 10 degrees of freedom (R package signal, v. 0.7-6) with 95% confidence intervals calculated from an inverse chi-square distribution. Wind data was taken from NOAA NBDC buoy #46054, which was the closest operational buoy for the duration of the study (https://www.ndbc.noaa.gov/), while current data was collected by the SBC-LTER (Washburn, 2018) and averaged across all depths. Current data was rotated into principal axes (u = onshore positive, v = poleward positive), and windstress was calculated from wind velocity (Large and Pond, 1981).

Results

Sensor Deployment 1: Dense oxygen sensor deployments inside and outside of a kelp forest environment

In general, levels of DO were significantly different inside and outside the kelp forest, as well as at each depth (Figure 2). This difference with depth was observed at both sites. In absolute terms, mean values for oxygen were significantly different for each sensor ($F(17868,5) = 1360.6$, $p < 0.0001$, Figure 2a). This was also the case when

Figure 2. Dissolved oxygen regimes at Mohawk Reef for Deployment 1 (June 2 ~ June 23, 2014), where six oxygen sensors were deployed (2 locations, 3 depths) inside and outside of the kelp forest. **A**. boxplots of the oxygen values, taken every 10 min. **B**. Mean daily swings of oxygen. **C**. Power spectra of the deployment for each sensor.



using a repeated-measures ANOVA for time of day ($F(17725, 5) = 1789.5$, $p < .0001$; calculated with Satterthwaite approximation of degrees of freedom). Pairwise comparisons for both a one-way ANOVA and the repeated-measures ANOVA yielded statistical significance for all pairwise comparisons (Tukey post-hoc test, $p<.0001$). I also observed distinct patterns of changes in DO as a diel cycle that was strongly correlated with the proximity to the kelp forest canopy. These daily patterns of oxygen levels were significantly different, both vertically in the water column and in their position inside and outside of the kelp forest (Figure 2b,c). Specifically, the sites inside of the kelp forest exhibited a higher diurnal cycle of DO than those outside, with the top of the water column exhibiting the highest degree of diurnal variation, followed by the middle and bottom of the water column (Figure 2c). All three positions in the water column exhibited a significantly different diurnal pattern (sinusoidal fit, $p<0.0001$ for both vertical position and mooring location), and these differences were also apparent in the PSD (Figure 2c). Overall, the combined significant differences in means and variation of these parameters yields a different oxygen regime for each of these sites and water column depths (Figure 2b).

Sensor Deployment 2: Comparing benthic pH-oxygen relationships inside and outside of a kelp forest environment

Here, I observed significantly different DO and pH at benthic sites inside and outside of the kelp forest environment. Specifically, the benthic site inside of the kelp

forest exhibited stronger diurnal variation of DO as compared to the outside, similarly to what was observed in Sensor Deployment 1.

Examining the relationship between pH and DO yielded a tight correlation (r^2 = 0.9313, Figure 3). The slope of this relationship was not significantly different between the two benthic sites (ANCOVA, $F(4166, 1) = 1.727$, $p = 0.1889$). While the lines fit separately for the sensors exhibited a different intercept ($F(4166, 1) = 145.026$, $p<0.001$), the discrepancy in the intercept was 0.01 pH units – within the margin of error of our spectrophotometric measurements, given an impure *m*-Cresol dye and operator error (Kapsenberg *et al.*, 2015). Thus, data from both sites were used to fit the model correlating oxygen and pH in the nearshore environment, with a resulting relationship of $pH_{total} = 0.001718([O_2]) + 7.519$, with $[O_2]$ expressed in μM.

The power at the diurnal frequency (1 cycle per day) was stronger inside of the kelp forest for DO, pH, and temperature (Figure 4), though not significantly. Notably, the benthic site inside of the kelp forest exhibited a and slightly stronger (Figure 4b) diurnal cycle of both DO and pH, while the outside benthic site exhibited more high-frequency noise throughout this deployment. While oxygen and pH were slightly higher outside of the kelp forest on average, temperature was slightly lower (Figure 4a). There was also a significant increase in diurnal signal of pH and oxygen, as well as a decrease in the absoluve values of pH, oxygen, and temperature, at the end of the deployment on April 6 (Figure 4b). This coincided with a change in wind stress (Figure 4d).

Figure 3. Oxygen and pH inside and outside of a kelp forest environment at Mohawk Reef, 1m off the bottom, for Deployment 2 (March 10 ~ April 08, 2015). Within our detection limits, this relationship is conserved over this distance.

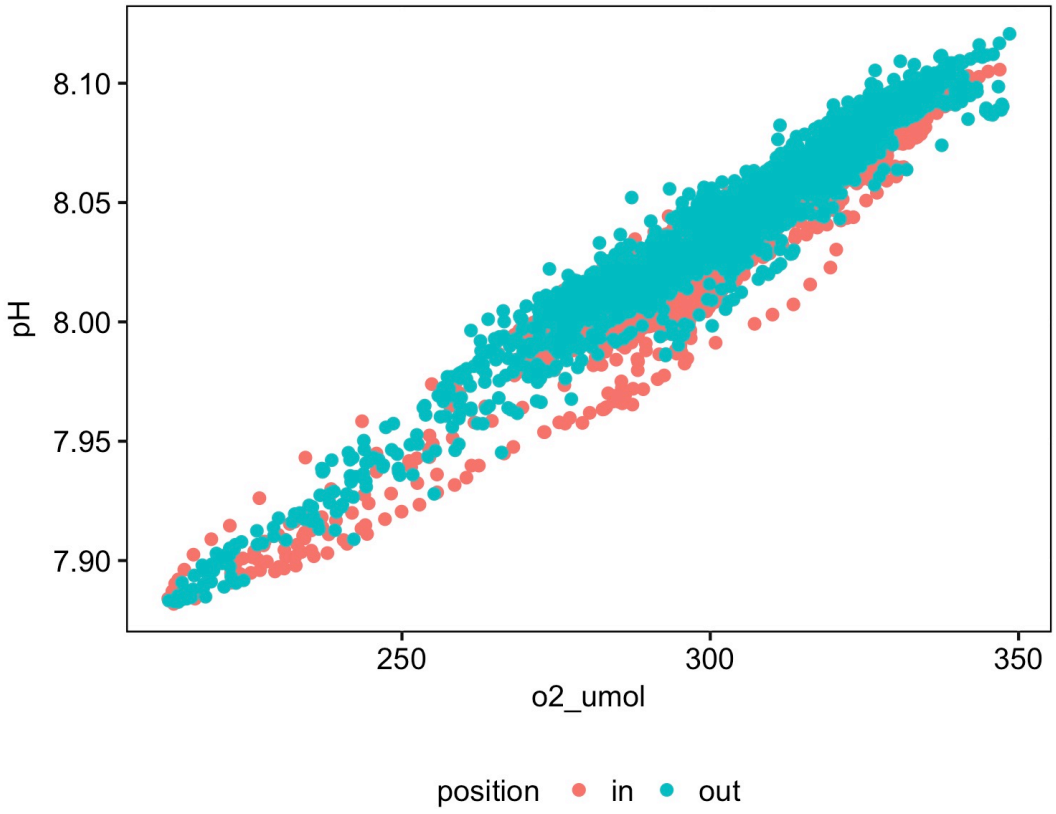


Figure 4. Deployment 2 (March 10 ~ April 08, 2015) at Mohawk Reef: **A.** Boxplot of measured values for each sensor. **B.** a 10-day representative sample of oxygen, temperature, and calculated pH data taken 1m off the bottom, inside and outside of the kelp forest **C.** PSD of the entire deployment, for temperature and oxygen, **D.** measurement of wind stress at the site, taken from NOAA NBDC #46054.

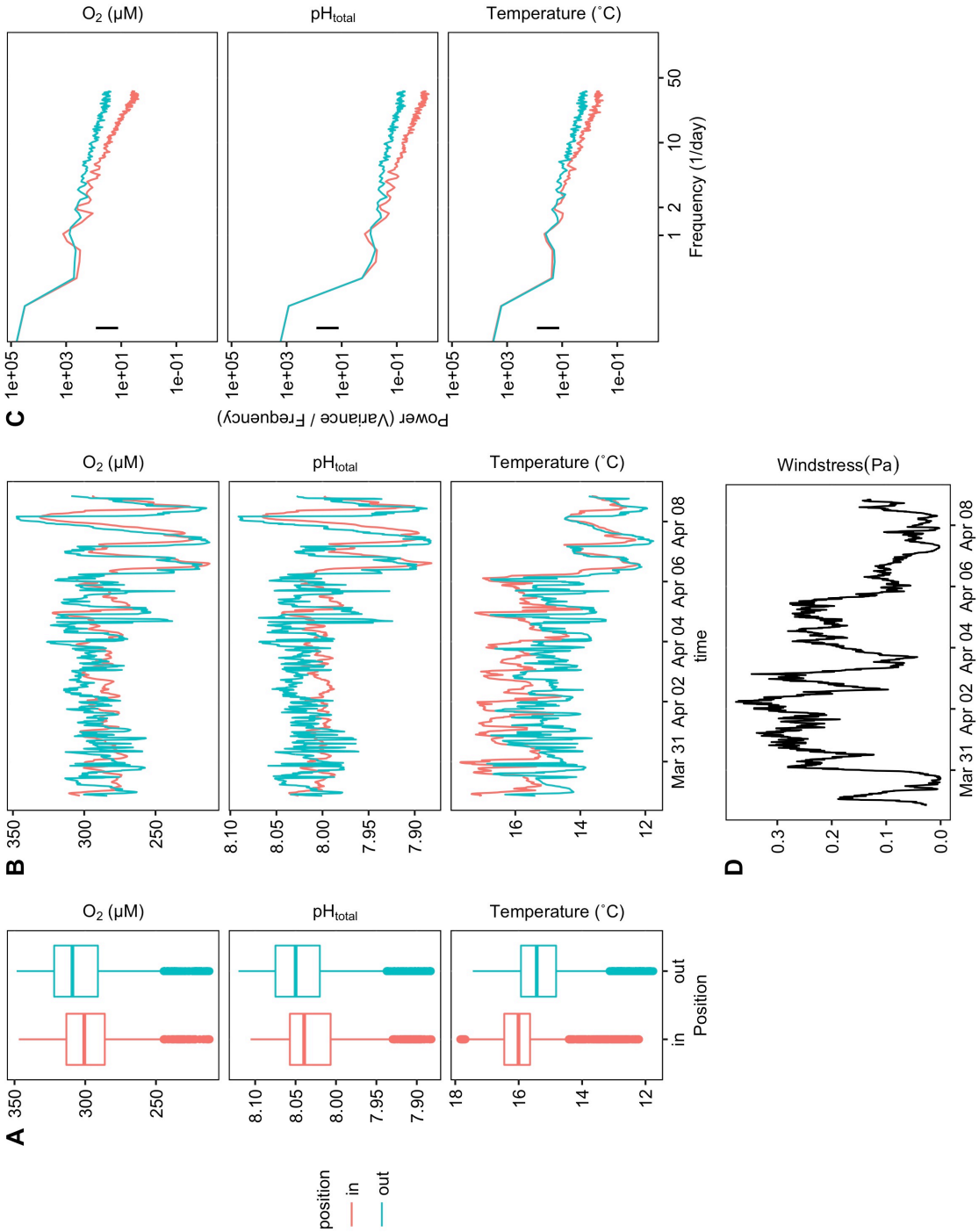


Figure 5. pH, oxygen, and temperature outside of the kelp forest on the benthos at Mohawk Reef during an extreme upwelling event for Deployment 3 (March-May 2016 shown). Also shown is ADCP data from the same site, rotated into principal components, and wind stress calculated from NOAA NBDC #46054.

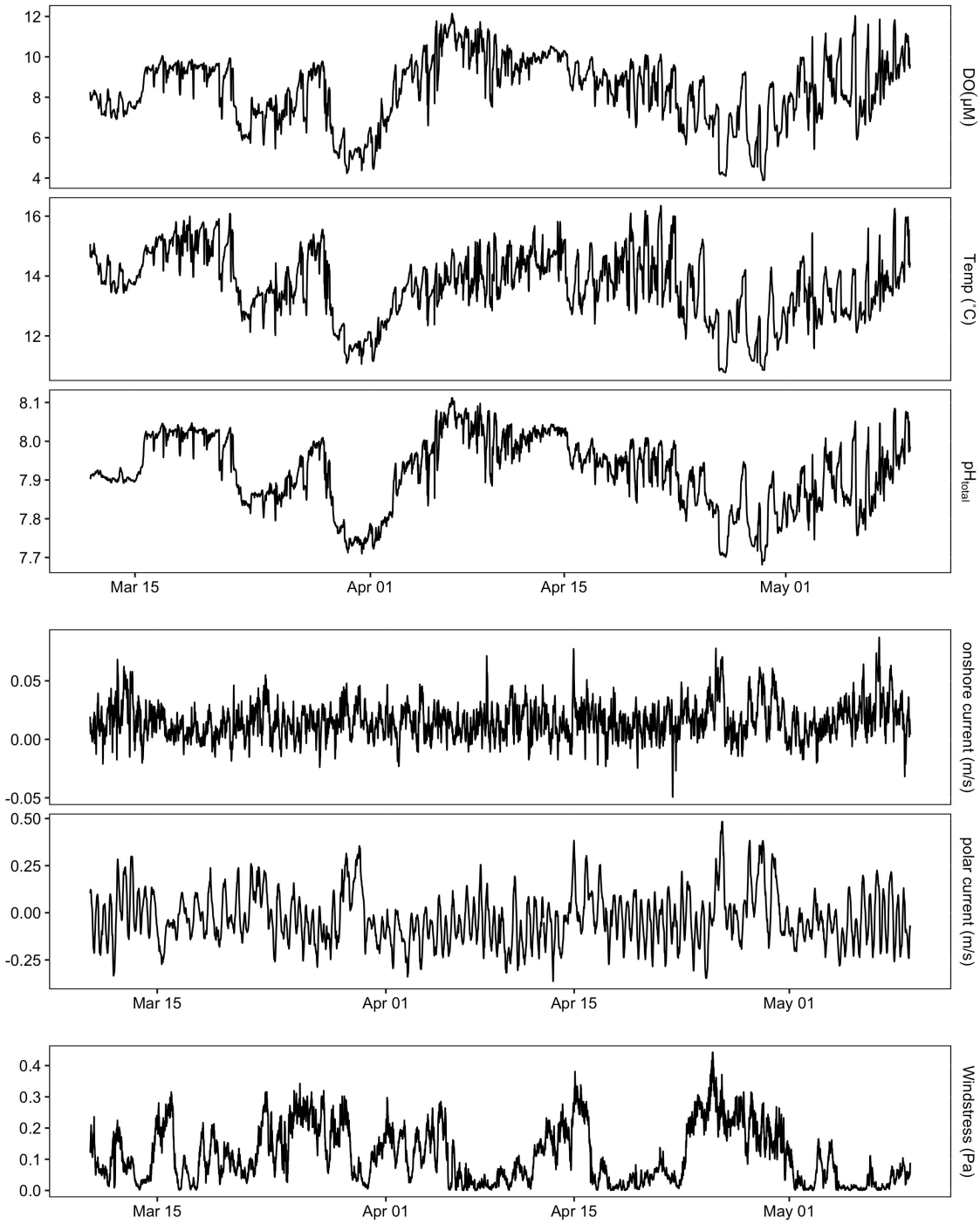
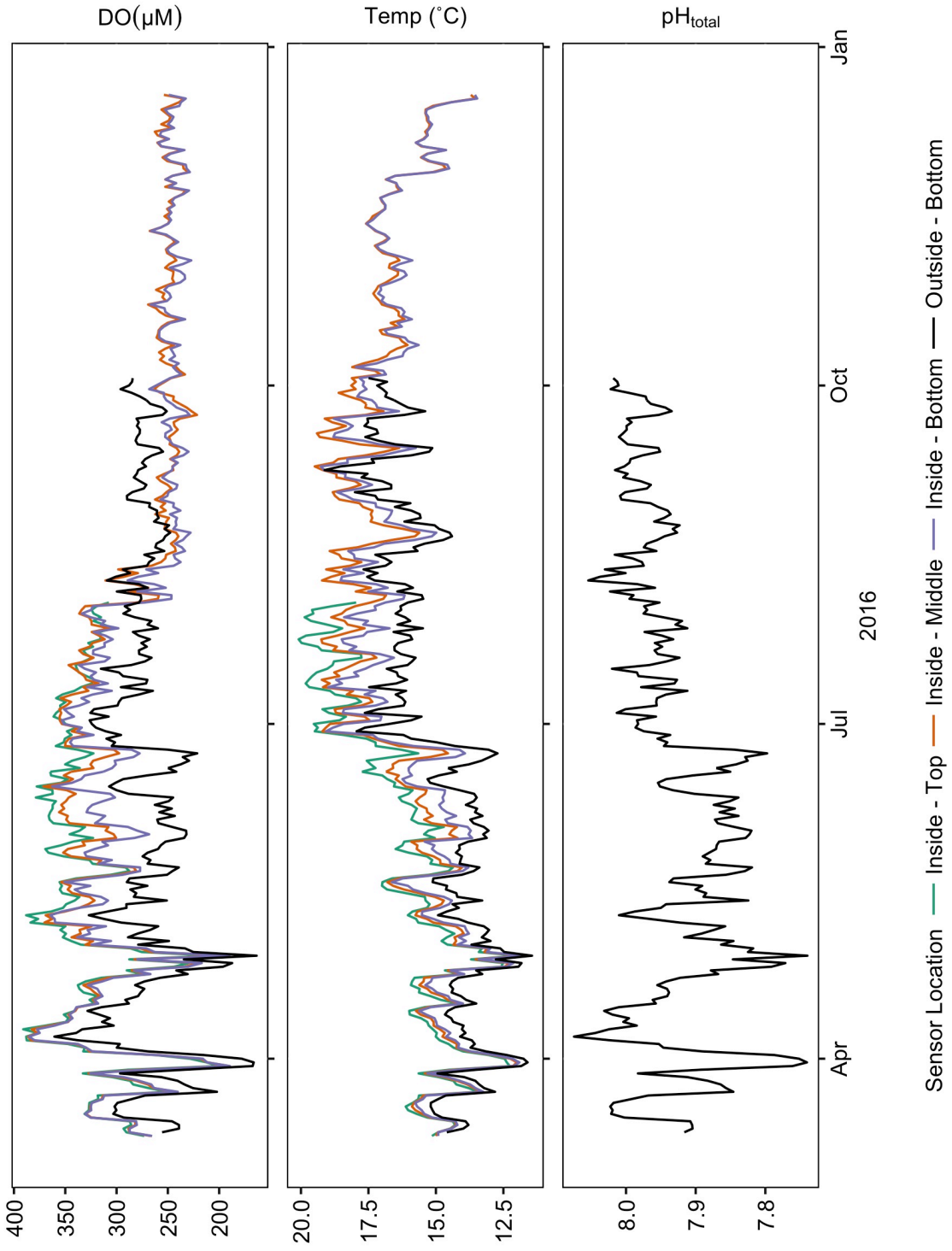


Figure 6. Daily averages of oxygen, pH, and temperature at various sensors for Deployment 3 (March ~ December 2016).



Sensor Deployment 3: Seasonal variation of pH and oxygen in a kelp forest environment

At MKO, oxygen varied greatly with season with a 79µM difference in monthly averages between July and November 2016 at the benthic site inside of the kelp forest. Specifically, DO peaked at 469 µM and declined to 125µM over the course of the six-month deployment (lowest and highest 10 values averaged). Using the linear equation derived from Deployment 2 (Figure 3), this corresponded to a pH range of 8.32 to 7.73. This was observed outside of the kelp forest with a single SeapHOx® sensor (Figure 5), which captured a strong acidic event during March 2016 that was tied to a strong poleward current stemming from a wind relaxation.

Daily averages of oxygen, temperature, and pH (Figure 6) depict a seasonally dynamic kelp forest environment, encapsulating upwelling regimes (e.g. early April) as well as periods of comparatively static conditions (September onwards, Figure 7). This strong seasonal variation was complemented by similar fine-scale variation as observed in Deployments 1 and 2 (Figure 2, Figure 3); that is, a significantly higher absolute DO content inside of the kelp forest, but with a high diurnal variation. This daily cycle varied by depth and season, peaking in the early summer (Figure 7). When examining diel patterns, daily variation in DO peaked on days in early summer, in concert with the maximum daily variation of pH, minimum mean pH, and maximum mean DO, but it was offset from the seasonality of temperature variation, which occurs in late summer to early fall (Figure 7). These data support the fact that the changes in pH and DO were largely <u>not</u> thermodynamically driven due to this temporal decoupling

of summer warming and pH/DO regimes. Comparing the power spectra of two contrasting months (March vs. July) showed a pronounced difference in both the average diurnal peak in the signals, as well as a separation between the signals (Figure 8), with the outside-bottom sensor displaying a significantly lower diurnal signal in the summer when the kelp forest is exhibiting the highest amount of productivity.

Figure 7. Monthly means **(A)** and mean daily swings for each month **(B)** for Deployment 3 (March ~ December 2016).

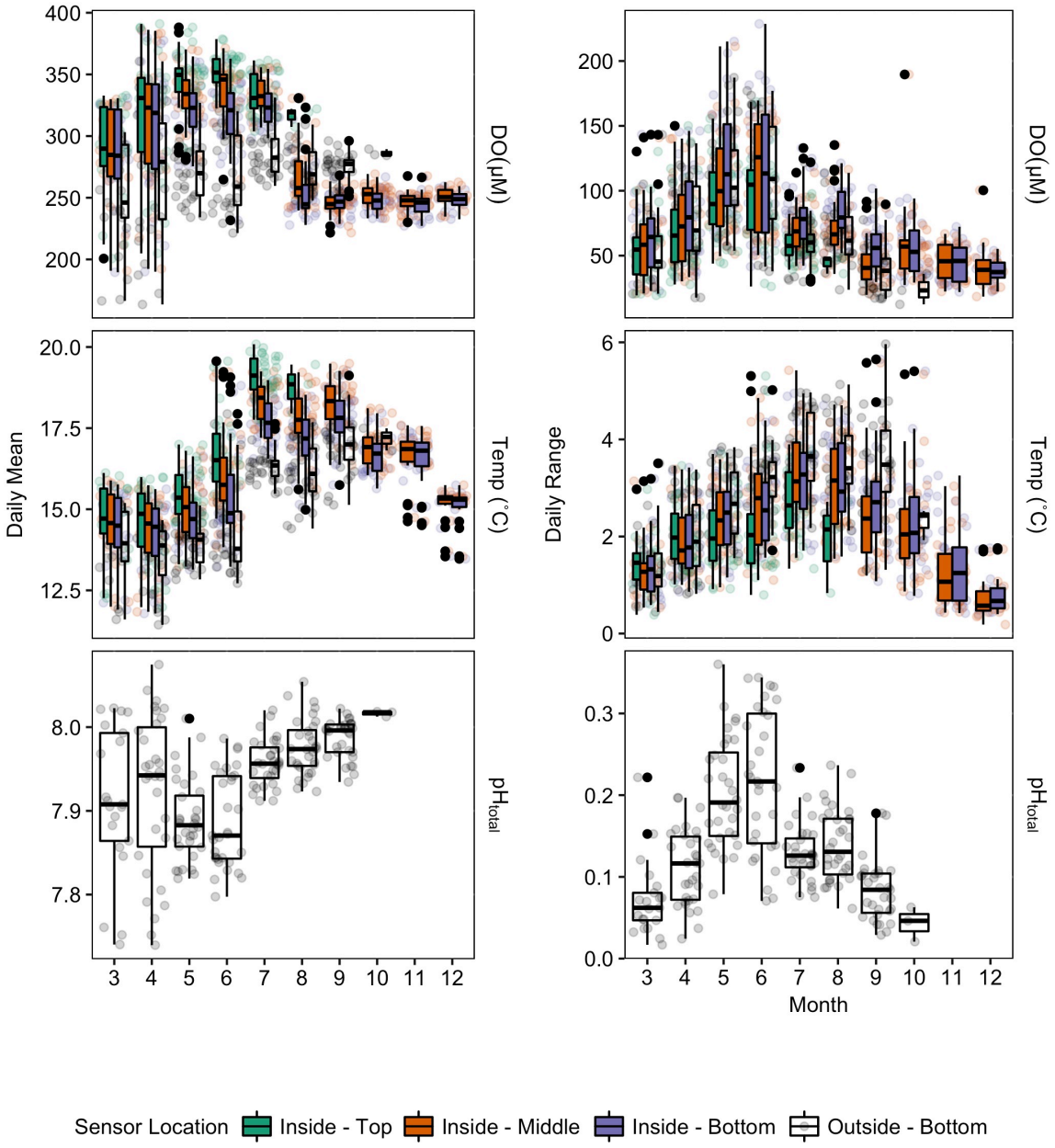
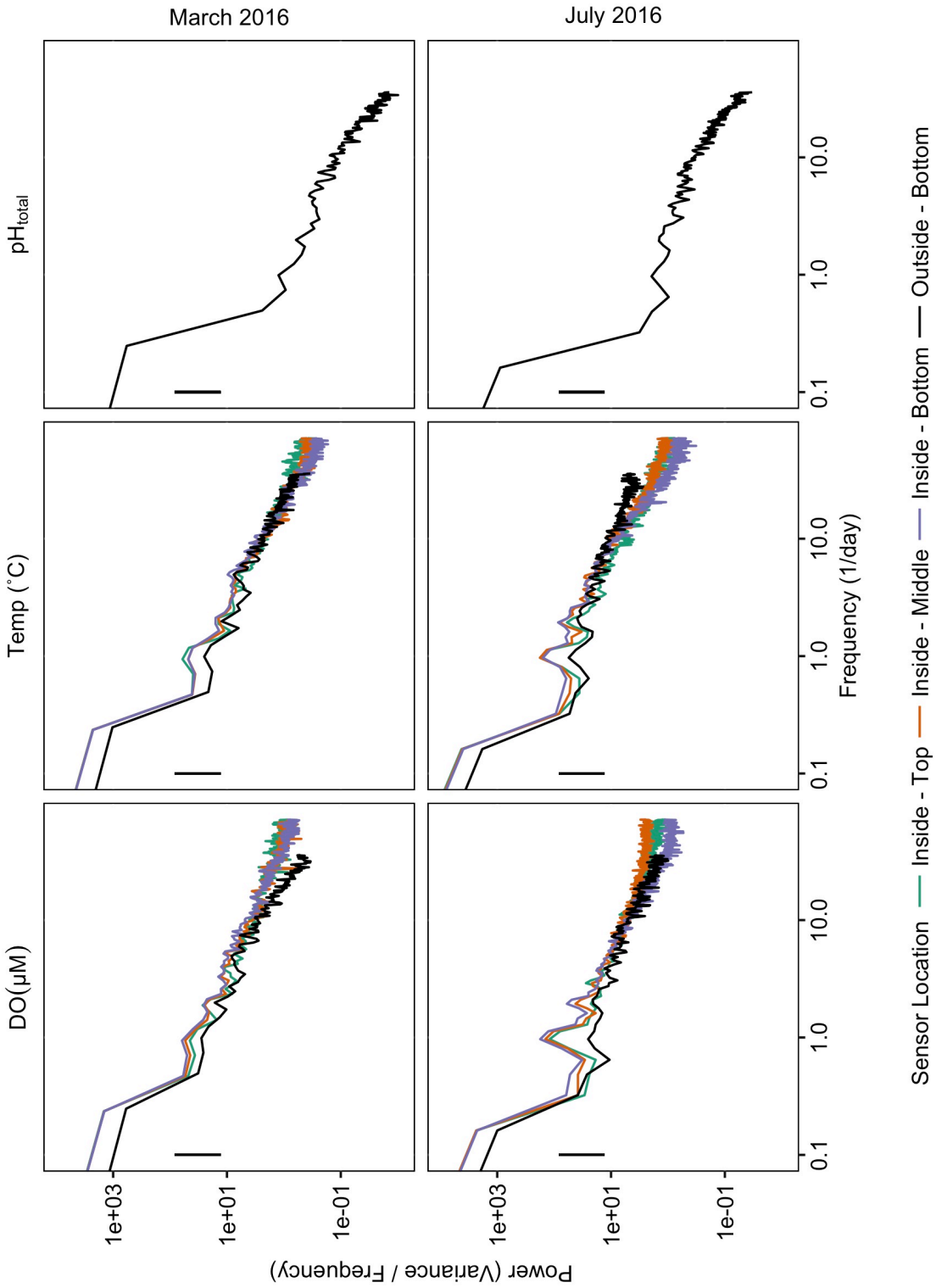


Figure 8. Spectrograms for two contrasting months (March and July) in Deployment 3.



Discussion

The overall goal of this Chapter was to characterize spatiotemporal variability of pH and DO in and around kelp forests at a range of timescales, from daily to seasonal, while comparing sites inside and outside of a kelp forest environment. In general, I found significantly different regimes of oxygen and pH during this series of sensor deployments at MKO. The salient findings of this Chapter are: **1)** DO and pH covaried strongly in the kelp forest environment, and both parameters can decrease to levels that could potentially affect organismal physiology, **2)** pH and DO varied seasonally, both in daily means and in diel variation, and **3)** this diel variation, as well as high-frequency variation, was significantly different inside and outside of the kelp forest. Overall, the patterns of DO and pH captured by this series of sensor deployments may have both biological and ecological consequences for marine biota in this nearshore environment (Breitburg *et al.*, 2018).

The most notable and consistent trend throughout the deployments was the strong covariation of oxygen and pH, which together have the potential to create a physiologically challenging abiotic environment for kelp forest organisms (Figure 3). In absolute terms, the DO values observed in Deployment 3 included values down to 125µM (3.9 mg/L), with 64µM (2 mg/L) being the threshold for hypoxia for many species. These lowest values were observed in March through May, and were not observed in months with the highest daily variation (Figure 6). These episodic, event-scale variations appear to be linked to the shoaling of upwelled water, as they are also coupled with low temperature (Figure 2, Figure 6). The most notable low pH event

occurred in late March (Figure 5), and showed a regime of drastically decreased temperature, pH, and oxygen occurring simultaneously for several days. This was paired with a decrease in wind stress and a subsequent polar flow of water. In contrast, the low, but more variable event on April 30 was accompanied by increased wind stress and a net onshore flow, which more likely indicates an upwelling event.

Contextually, given that global pH is expected to decrease ~0.2 units in the next 100 years (Feely *et al.*, 2009), the potential impacts of these large negative excursions of pH (-0.3) in this time series are likely to have major impacts on coastal marine systems (Gruber *et al.*, 2012).

Although a DO value of 2 mg/L (or 64µM), has long been used as a hypoxic threshold in the context of fisheries, this perspective has been re-visited with researchers noting that this concentration can be a drastic underestimation of sublethal and lethal thresholds of hypoxia when looking across taxa (Vaquer-Sunyer and Duarte, 2008). Here, marine fish and crustaceans have been noted to be the most vulnerable to hypoxia, and 4.6mg/L (or 147µM) is suggested as a precautionary 90-percentile value of survival, especially given the more unpredictable ecosystem recovery after hypoxic events (Steckbauer *et al.*, 2011). In my time series, a value this low is seen most notably for 11 out of 22 hours on April 30, and for over 12 continuous hours on April 26 (Figure 5).

In addition, considering that these decreases in levels of DO are strongly linked to decreases in pH, the multiple stressor scenario of ocean acidification and hypoxia demands a more conservative cutoff for hypoxia (Gobler and Baumann, 2016), barring

antagonistic effects which are generally uncommon for abiotic stressors (Przeslawski *et al.*, 2015). For instance, in studies on red abalone in Monterey Bay (Kim *et al.*, 2013), juveniles showed adverse effects when subjected to low pH (7.5) and low oxygen (5 mg L^{-1}) for 24 hours and attributed the majority of mortality to the hypoxia. This oxygen value was seen for 18 our of 62 hours in early April, then again for 31 out of 80 hours in late April 2016 (Figure 5), though the pH value was not. It is clear that these values recorded in the kelp forest are of concern for local organisms, even taxa that are traditionally thought to be resilient to ocean change (Vaquer-Sunyer and Duarte, 2008)

Lastly, independent of these event-scale changes, DO values decreased with depth in the water column, and also decreased outside of the kelp forest along with pH (Figure 2, Figure 6). In general, pH and DO were very highly correlated in our study (Figure 3). This strong coupling of these two factors has been previously observed, most recently in Frieder et al. (2012) which found a similar relationship except for a slightly different slope and an intercept difference with our margin of spectrophotometric pH error.

Seasonal Variation

The data collected in this study showed a seasonal variation of pH and DO in a kelp forest environment, as has been reported in other studies in California *Macrocystis* forests (Kapsenberg and Hofmann, 2016; Koweek *et al.*, 2017). At MKO, seasonal variation was driven by both event-scale variation, as described above and

displayed in Figure 5, and diurnal variation, as described below and displayed in Figure 7. In particular, the diurnal variation varied strongly with season, peaking in early summer. This was slightly earlier than the peak that was observed previously by Kapsenberg and Hofmann (2016), data that were collected in 2012-2015 at Anacapa Landing Cove, a site in the Santa Barbara Channel positioned on Anacapa Island further out in the Channel. In addition, seasonal variation of diel pH cycles was also generally higher than observed at the Channel Islands site in Kapsenberg and Hofmann (2016). Kapsenberg and Hofmann also observed very little change inter-annually in seasonal pH and oxygen. However, given that the sites on the Northern coast of the Channel Islands sees little wind-driven upwelling, more variable interannual conditions could be expected on the mainland where abiotic conditions have a larger effect. To date, no other kelp forest sites have been instrumented to observe seasonal trends.

Diurnal and high-frequency variation in pH and DO

It is becoming increasingly clear that organismal exposure to pH and hypoxia cannot be completely encapsulated by discussing means and extremes, but that frequency of variation can play a strong factor (Cornwall *et al.*, 2013). As expected, I found that pH and oxygen varied dramatically in this coastal system at MKO. The largest daily ranges of pH and DO were observed at middle and bottom of the kelp forest (Figure 7). One of the large drivers of this change in daily range was at a diel frequency, and corresponded to expected (but not measured) patterns of

photosynthesis and respiration that would track availability of light in the water column. In all power spectra analyzed, the diurnal signal was the dominant periodic function, mirroring results from previous studies in the Southern California Bight (Frieder *et al.*, 2012). Here, both mean DO and diel variation of DO decreased with depth in the water column (i.e. away from the kelp canopy), and decreased outside of the kelp forest (i.e. away from the kelp bed itself).

In addition to differences in diel variation, differences in high-frequency variation were observed both vertically in the water column as well as between sites. High-frequency variation of DO was observed on the benthos outside of the kelp forest (Figure 2) Thus, it appears that kelp forests offer an environment with a strong diel variation of DO and pH, but less high-frequency noise. This pattern is possibly caused by the dampening of water flow around kelp forests, which has been classified at this site in past studies (Gaylord *et al.*, 2007) and contributes to the cleaner and stronger diurnal signal inside of the kelp forest. The deeper, outside site could also be subject to different oceanographic processes, such as internal waves.

Comparison of conditions inside and outside of a kelp forest environment

This study compared a site inside of a kelp forest to one directly offshore with no kelp and sandy substrate. These sites were chosen as Frieder et al. (2012) observed higher gradients cross-shore compared to alongshore, and thus we expected to see a larger variation along this axis. With alongshore currents drastically stronger than

cross-shore currents, it is intuitive that the physiochemical gradients would be stronger heading offshore.

Although Koweek et al. (2017) noted that depth was as great of an indicator of seawater chemistry change as horizontal distance, Frieder et al. (2012) observed that depth was a larger determinant of spatial pH difference than distance. Our study neither refutes or supports these results, as the two sites were at different depths (7m and 11m). Choosing sites that were both different in depth and in kelp presence was intentional, as the main purpose of this study was to investigate the effect of the kelp forest on benthic communities. Regardless, I found a larger difference between the two sensors at the benthos, compared to the sensors at the top and bottom of the water column (Figure 2).

In comparing the three available datasets on California kelp forests, differences in these three systems are likely due to site-by-site differences, as well as sampling methodology. Koweek and colleagues sampled the water column from 9:00-12:00 local time, a time of day when vertical stratification of DO was the highest in my dataset (Figure 2). It is likely that this sampling thus captured the vertical stratification of the kelp forest at its strongest point, in comparison to *in situ* sensor data which allows for around-the-clock data collection. Frieder and colleagues conducted their study in the La Jolla kelp forest in Southern California, with most deployments being conducted midwater at 7m water depth, in an area of the kelp forest 20m deep. This is far beyond the extent of *Macrocystis* beds in the Santa

Barbara Channel, and a larger effect of depth in these La Jolla kelp forests is to be expected.

Biological and Ecological Consequences: What does this mean for coastal communities?

The findings in this Chapter indicate that organisms of the kelp forest encounter a complex mosaic of pH and DO conditions that varied across days and seasons. This pattern has the strong potential to impact ecological and physiological processes for kelp forest organisms. The kelp forest environment provides a generally higher pH, higher DO environment throughout the water column, while also introducing a signature, and possibly predictable, diel signal and diminishing high-frequency variation. Both the absolute change in pH and DO, as well as the frequency of this variation, has the potential to impact biological processes, as diel variations can alter responses of organisms to future ocean change (Britton *et al.*, 2016).

A suite of studies on fluctuating pH often find that changing the variance of pH can affect organism more than altering the mean. Fluctuating scenarios can even affect congeneric species in different ways, even with the congenerics responding similarly to static conditions (Frieder *et al.*, 2014), and as such, the benefits of the kelp forest environment may vary on a species level. This may be particularly important for benthic sessile organisms, that are dependent on the environment on which they settle. In contrast, any difference seen between these sites less than 100m away can be traversed by most mobile organisms. If there is indeed a variation in preference for

pH/DO regimes between species, then a heterogenous environment may provide a large opportunity for organism to move to their regime of preference. individual species must be more extensively surveyed in a multistressor framework to understand the vulnerability of coastal oceans to these ocean conditions (Gobler and Baumann, 2016). Overall, experiments that study the effect of pH variation on organisms generally show disparate results that do not form conclusive trends across taxa, and do not take into account the ability of the organism to move out of a stressful environment (e.g. Alenius and Munguia, 2012; Clark and Gobler, 2016; Davidson *et al.*, 2016; Eriander *et al.*, 2016; Frieder *et al.*, 2014; Keppel *et al.*, 2016; Kim *et al.*, 2013; Roleda *et al.*, 2012), with many of these studies utilizing pH and oxygen variability that generally reflect what I observed here.

Furthermore, research in other systems has shown that parents can alter the performance of their progeny under stress to match seasonal changes in pH (Murray *et al.*, 2014). A more thorough understanding of both species physiology and community interactions will be critical in understanding the true prospect of kelp as a biomediator of global abiotic stressors. Discussion of the biological impacts of this variation, especially in a transgenerational context will be further examined in Chapter 3.

In summary, my data here showed the significance of kelp forests in mediating the pH and DO of the water column through the seasons. While these data help us understand another way that *Macrocystis* acts as a foundation species, one side note of importance is the consideration of the fate of giant kelp itself under future ocean

conditions. To date, few studies have investigated the effect of future ocean conditions on *Macrocystis* physiology, and generally find no effect or a positive effect (Leal *et al.*, 2017; Roleda *et al.*, 2012). Recent studies also challenge the long-held theory that kelp could be a sentinel species for ocean warming (Reed *et al.*, 2016). Regardless, better understanding of the impact of kelp forests on the surrounding water column, as well as its surrounding "sphere of influence", will be a critical piece in predicting the fate of nearshore communities to future change.

III. Transgenerational effects in a kelp forest environment: The influence of in situ adult conditioning on egg quality and the performance of early life history stages of the purple sea urchin, *Strongylocentrotus purpuratus*

Abstract

In the face of rapid global change, transgenerational plasticity (TGP) has been posited as a potential mechanism that can allow survival of organisms through single-generation changes in phenotype via mechanisms such as maternal provisioning and epigenetics (e.g. histone modifications, DNA methylation). While TGP has been observed with climate change stressors in the laboratory environment, there has been little study in how the naturally variable environment can mediate the phenotypic plasticity that confers stress tolerance.

Here, I conduct one of the first studies to investigate transgenerational plasticity *in situ*. To do so, I outplanted adult purple sea urchins on the benthos both inside and outside of a kelp forest environment. These urchins then underwent gametogenesis in the field under the respective abiotic conditions inside and outside the kelp bed. During this 11-week experiment, sea urchins were fed kelp blades every 2 weeks. In addition, oxygen/temperature loggers were placed on the cages to monitor their habitat. I found that adults held outside of the kelp forest produced more numerous eggs, but each egg was less protein-rich. In contrast, adults held inside of a kelp forest produced fewer eggs that were more protein-rich. When these eggs were fertilized and cultured, embryos from mothers held outside of the kelp forest

exhibited different sizes under high pCO_2, while those from mothers held inside of the kelp forest did not. I conclude that not only are there differences in offspring based on maternal condition, but there are also differences in how these offspring respond to stress. These observations have broad implications for the assessment of early developmental stages in a global change context.

Introduction

Variations in the abiotic environment (Chapter 2) may impact resident biota via direct and indirect mechanisms while also altering ecological-evolutionary dynamics (Ghalambor *et al.*, 2007; Lallensack, 2018). In metazoans, the variable and coupled pH/oxygen environment can interact to alter a suite of biological performance metrics including behavioral and physiological processes (Philip W. Boyd *et al.*, 2015; Kroeker *et al.*, 2017). In addition, other processes such as transgenerational platicity (TGP) may play a defining role in shaping the eco-evolutionary trajectory of nearshore organisms to future environmental conditions (Marshall, 2008; Munday *et al.*, 2013; Salinas and Munch, 2012). Here, this transgenerational plasticity (TGP) has the potential to aid in stress response on a timescale more relevant to the rapid rates of global change in the oceans, even potentially facilitating evolutionary rescue through an accelaration in genetic evolution (Price *et al.*, 2003). In this study, I investigated the potential for a predominant form of TGP, maternal effects, to alter the performance of progeny of an ecologically important kelp forest grazer (Pearse, 2006),

the purple sea urchin (*Strongylocentrotus purpuratus*), given the presence or absence of a kelp forest environment.

The purple sea urchin has long been acknowledged as a key species in driving community-level dynamics, notably by grazing down canopy-forming kelp and creating an urchin barren as an alternate stable state (Filbee-Dexter and Scheibling, 2014; Pearse, 2006). As a marine invertebrate with aragonitic spicules and tests potentially vulnerable to ocean conditions with a lower aragonite saturation (Wilt, 2002), the fate of *S. purpuratus* to future ocean change has been heavily studied in an attempt to predict the impact of future nearshore environment along its range (Hammond and Hofmann, 2010; Kelly *et al.*, 2013; Stumpp *et al.*, 2011). Furthermore, previous studies on *S. purpuratus* have shown transgenerational differences in progeny response can arise from adult acclimation to current-day upwelling conditions (Wong *et al.*, 2018). Thus, I anticipate that gametogenesis, when conducted *in situ* in the variable nearshore environment, may mediate TGP of *S. purpuratus* in nature.

In this study, I deployed oceanographic sensors to profile the pH and levels of dissolved oxygen of the nearshore kelp forest environment with fine spatiotemporal resolution, both inside and outside the kelp bed. Co-located with these sensors, I outplanted adult *S. purpuratus* in cages on the kelp forest benthos to acclimatize them to these variable environments during gametogenesis. Finally, I collected and spawned these *S. purpuratus* individuals in order to examine egg quality and to raise embryos in culture to explore TGP *in situ*. After fertilization, the zygotes were raised

to hatched blatulae stage under controlled pCO_2 conditions in the laboratory (400µatm and 1000µatm) to assess the potential for presence or absence of kelp forest envionment to affect the TGP of the offspring under environmental stress.

Given the trends seen in Chapter 2, I predicted that *S. purpuratus* adults held inside and outside of the kelp forest would be exposed to different regimes of temperature, pH, and DO, including different mean values and different frequencies of variation. These differences, while seen vertically in the water column, also will likely persist at the benthos between the cages inside and outside of the kelp forest. Ultimately, I hypothesize these different environmental conditions will elicit a difference in either maternal provisioning of energy or larval response to pH stress.

Methods

The field experiment: Caged adult sea urchins co-located with oxygen sensors

For this part of my thesis, I outplanted adult sea urchins in replicate cages on the benthos in summer of 2017 to have the adults experience different seawater conditions in situ while the adults were undergoing gametogenesis.

For the field experiment, I paired each set of cages with oxygen and temperature sensors to document these conditions. Adult sea urchins were collected from the kelp forest at Mohawk Reef (under California Department of Fish and Wildlife collection permit #SC-1223) near the set of cages inside the kelp forest in August 2017. To ensure that the bulk of sea urchin gametogenesis occurred during the

caged outplants, urchin spawning was attempted prior to the outplants by shock-spawning with a 9-volt battery for three 5-second jolts (Professor Kathy Foltz, pers. comm.). Shock spawning was preferred for this step, as it is a less invasive process than the standard KCl injection used for urchin spawning.

Outplant cages were constructed out of 1-inch PVC-coated welded wire fencing and were shaped as cylinders 50cm in length and 30cm in diameter. When deployed these cages were weighed down with an internal PVC frame filled with sand. A hatch was placed on one side of each cage for urchin access and feeding. For this field experiment, I deployed a total of 6 outplant cages on SCUBA: (1) 3 replicate cages were placed inside the kelp forest on the benthos, anchored to weights in ~7m of water and (2) 3 cages were placed outside of the kelp forest on the benthos, attached to both weights and a 1m sand anchor in ~11m of water. These are the same two sites referred to in Chapter 2.

To begin the acclimatization period, each of the six cages was loaded with 10 adult sea urchins on August 27, 2017. Cages were revisited by SCUBA every two weeks and urchins were fed liberally with giant kelp, *Macrosystis pyrifera*, collected at Mohawk Reef. Urchins were retrieved from the cages on November 14, 2017 after 79 days of acclimatization at the reef field site, and brought back to laboratory seawater facilities at UC Santa Barbara for the embryo culturing experiment.

Each set of cages was paired with a miniDOT® oxygen sensor, which was programmed to collect oxygen data throughout the deployment period at 10-minute intervals. During this period, one of my sensors failed (on the outside mooring). As a

result the data presented here was collected by an identical miniDOT® sensor deployed 1m away (Washburn, 2018), a part of the SBC LTER sensor array at Mohawk Reef. pH sensors were unable to be deployed at these sites due to constraints on time and logistics.

Embryo culturing under controlled pCO_2 conditions

On November 14, adult urchins were collected from their cages, returned to the lab and spawned within 24 hours. Spawning was induced via injection of 1.0M KCl with intermittent shaking. For the adults from both sets of cages I was able to successfully collect gametes from five female urchins for each location. Of these, for the fertilization step, eggs from 5 females from each maternal exposure treatment (inside vs. outside) were crossed with sperm from 1 male urchin. Each male was used to fertilize the pooled eggs from the 5 females of the same treatment, with an identical number of eggs contributed from each female in the pooled egg population. These eggs are designated as "**I**" or "**O**" for "Inside" and "Outside", corresponding to the location of the adult outplant location.

For the culturing step, embryos were raised at 15 °C using pCO_2 conditions chosen to reflect current relaxed and upwelling conditions (400 µatm pCO_2 and 1000 µatm pCO_2, respectively). This gas partial pressure was created using mass-flow controllers (Sierra Manufacturing, SmartTrak 101 and 100L) and equilibrated in seawater using header tanks equipped with 46eteros injectors. This seawater was then pumped to flow-through culturing tanks through irrigation drippers (Fangue *et al.*,

2010). To calculate pCO_2 values, the carbonate system was parameterized by measuring spectrophotometric pH and total alkalinity titration as described in Chapter 2, with the exception that the pH samples were not fixed with $HgCl_2$ and were instead analyzed immediately. These culturing conditions created in the laboratory setting are labeled "**L**" and "**H**" for "Low" and "High", and the crossed maternal conditioning and larval treatments were named with a combination of these two codes. Larvae were spawned from parents conditioned inside of the kelp forest, then raised in a low-pCO_2 environment (**IL)** or raised in a high-pCO_2 envirionment (**IH).** Larvae were also spawned from parents conditioned outside of the kelp forest, then raised in a low-pCO_2 environment (**OL)** or raised in a high-pCO_2 envirionment (**OH).**

These cultures were maintained at 15 °C throughout the experiment using an aquarium chiller and verified via submerged temperature loggers (HOBO Pendant Temperature loggers). These larvae were raised for 18 hours, or through the hatched blastula stage before being sampled.

To assess the impact of maternal conditioning on egg quality, I measured 3 metrics in collected eggs: **1)** average egg length over 3 different axes (n = 30 per mother), **2)** egg area (n = 30 per mother), and **3)** total protein content (n = 3 tubes of 1000 eggs per female). I measured 2 additional metrics for each adult female urchin: **1)** total number of eggs spawned after KCl injection, and **2)** test diameter. To assess the impact of maternal conditioning on embryo traits and development, I measured 3 metrics of hatched blastula embryos: **1)** embryo length over the major axis (n = 30 per

replicate culture vessel), **2)** embryo area (n = 30 per replicate culture vessel), and **3)** embryo total protein content (n = 3 tubes of 1000 embyros per replicate culture vessel).

Egg and embryo morphometrics

Eggs collected from the outplanted *S. purpuratus* adults were preserved in 2% formalin in seawater, and imaged using a trinocular compound light microscope (Olympus BX50) equipped with a digital camera (Lumenera Infinity Lite) and a computer running the associated imaging software (Infinity Capture v. 6.2.0). For morphometric analysis, 30 eggs were measured from each female using ImageJ software (Schneider *et al.*, 2012). In addition, 30 hatched blastula embryos were measured from each replicate larval culturing vessel. All samples were imaged within 30 days of the spawning event which occurred on November 15, 2017.

For eggs, both average diameter and two-dimensional area were measured. As hatched blastula embryos were more ellipsoidal, the major axis (longest diameter that intersects the center) was chosen as a representative diameter. Two-dimensional area was also measured for these embryos (Figure 9).

Biochemical analyses

Prior to fertilization, samples of eggs (n=1000 in 3 cryovials from each female) were flash frozen in liquid nitrogen and stored at -80 °C prior to protein analyses. The tubes were gently centrifuged to pellet the eggs, excess seawater was removed, and the

sample was then frozen in liquid nitrogen prior to storage at -80°C until use in further analysis. Three replicate tubes of 1000 embryos were collected from each replicate larval culturing vessel and preserved as described above for the eggs.

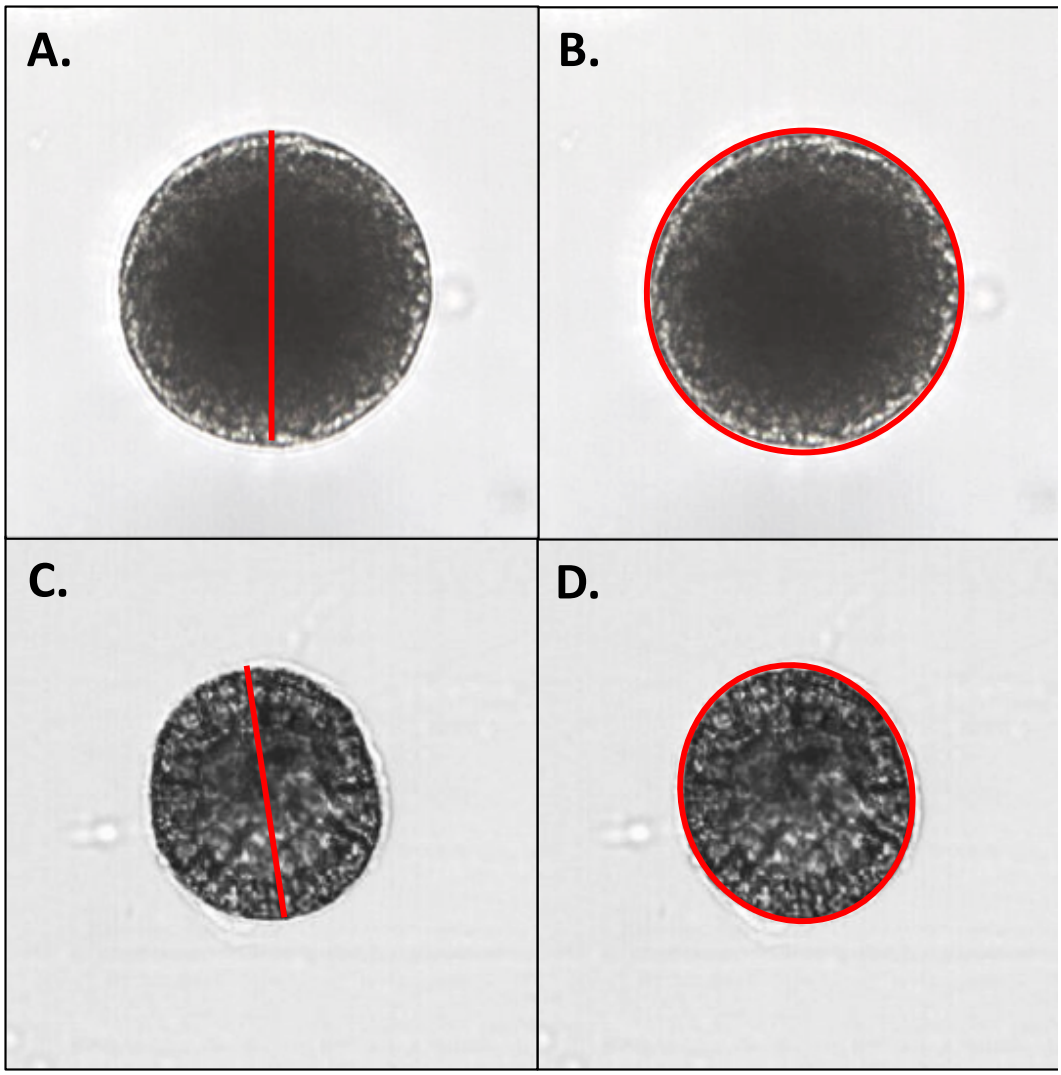
Protein content assays were performed using a microplate BCA protein assay (Catalog number 23225, Pierce Biotechnology) according to manufacturer's instructions.

Embryos and eggs were processed using methods adapted from Byrne et al. (2008) and Prowse et al. (2007); briefly, samples were resuspended and sonicated in a lysis buffer (20mM Tris-HCl, 130mM NaCl, 5mM EDTA, 1% Triton X-100, 1% Sigma-Aldritch Protease Inhibitor Cocktail @ pH 7.6). The resulting homogenates were used in the BCA protein assay within 72 hours of extraction.

Statistical methods

All statistics and analyses were conducted using the R statistical software, as well as the R Studio IDE (RStudio Team, 2017) and the associated packages tidyr, signal, agricolae, and marelac. Morphometric analysis, as well as biochemical analysis, were combined and visualized in tandem using a principal components analysis (PCA) and groupings were tested using a PERMANOVA technique.

Figure 9. Methods used for measuring egg length (**A**), egg area (**B**), hatched blastula length (**C**), hatched blastula area (**D**).



Results

The Environment: Oxygen and temperature regimes at the field site

In general, the oxygen and temperature regimes at these two sites – inside and outside the kelp bed – (Figure 10) mirrored those observed in Chapter 2 in the previous year. Specifically, this pattern was characterized by significantly different DO regimes as a function of the proximity to kelp, particularly in the frequency domain (Figure 10c). In absolute terms, while oxygen content varied significantly between the two sites (repeated-measures ANOVA, $F(10195, 1) = 505.04$, $p<0.0001$), the mean difference in DO between these sites was small, and likely not of biological relevance (245.94 ± 23.7 μM inside of the kelp forest, 250.41 ± 25.38 μM outside of the kelp forest, mean ± s.d.) (Figure 10a). Additionally, DO inside the kelp forest varied more strongly in the diurnal frequency peak, but less so at high frequencies. In contrast, the DO outside of the kelp forest environment was characterized by a lower diurnal variation, as well as more high-frequency variation. This pattern can be visually observed in the time series data (Figure 10b), where the DO inside of the kelp forest exhibited a more consistent sinusoidal pattern, while the DO outside of the kelp forest exhibited a more jagged "saw-tooth" signal indicative of high-frequency variability.

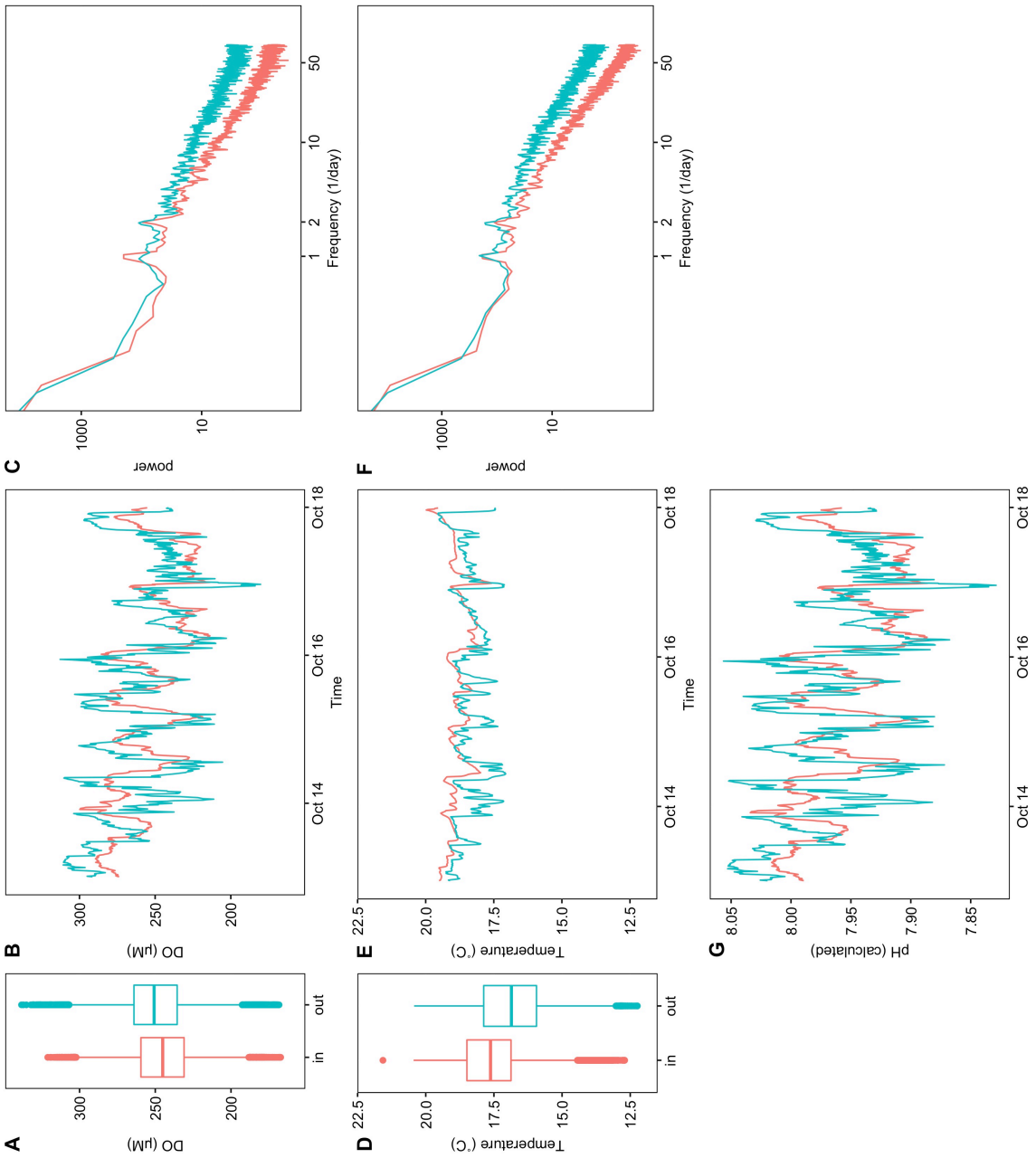
Temperature also varied in both harmonic content and absolute value. In absolute terms, temperature varied significantly between the two sites (repeated-measures ANOVA, $F(10050, 1) = 7032$, $p <.0001$), with the means differing by 0.6°C (17.60 ± 1.28°C inside of the kelp forest, 16.83 ± 1.42°C outside the kelp forest, mean ±

s.d.) (Figure 10d). In the frequency spectrum, similarly to the DO data, temperature exhibited a high- frequency variability outside of the kelp forest in comparison to inside (Figure 10F). This degree of variation can be visualized as high-frequency noise in the representative time series data (Figure 10E). In contrast to DO, however, the diel variation of temperature inside the kelp forest was not notably different than that outside the kelp forest (Figure 10F).

Egg characteristics, morphometrics, and biochemistry

In terms of egg characteristics, the data showed that some characteristics of eggs covaried strongly regardless of maternal exposure, such as egg area and egg diameter (Figure 11, linear regression $p < .0001$, $r^2 = 0.96$). While we expect a square relationship between length and area if the shapes of the eggs were all identical, this relationship was fairly linear over the span of observation. In order to further explore this variation, these three measured metrics (egg diameter, egg area, and egg protein content) were combined and transformed using a PCA, the first two components of which are reported here (Figure 12). With the PCA technique, correlates (e.g. egg diameter and egg area) can be accounted for and majoritavely collapsed onto a primary axis.

Figure 10. Dissolved Oxygen (A,B,C), Temperature (D, E, F), and calculated pH (G) inside (red) and outside (blue) of a kelp forest environment, expressed as boxplots for the entire outplant (A,D), a representative 5 day timeseries (C,F), and a power spectrum (C,F).



Here, eggs from females conditioned inside the kelp forest had 27% higher total protein content, but were 84% fewer in number (PERMANOVA, p = 0.01), while in contrast, eggs from females conditioned outside of the kelp forest were larger in number but less rich in protein (Figure 12).

Embryo characteristics

During the culturing run of 17 hours, pCO_2 levels in the culturing vessels remained consistent to target values for each of the two treatments (target values = 400µatm and 1000µatm, actual values = 413 .59 ± 2.40 µatm pCO_2 and 987.59 ± 31.38 µatm pCO_2 – respectively). For these determinations, carbonate parameters were calculated based on alkalinity and salinity values taken on the second day of culturing (2214.80 µmol kg^{-1}, 33.4 ‰, respectively).

These embryo characteristics measured during the hatched blastula stage were visualized by PCA(Figure 13). Here, because two of the variables (protein content and embryo area) were orthogonal and corresponded well with PC1 and PC2, these original variables were used in analysis in lieu of the principal components.

I did detect some effect of maternal conditioning inside or outside of the kelp forest (Figure 13). Embryos from females that performed gametogenesis outside the kelp, and then were raised under high pCO_2 conditions (abbreviated OH), had a 7% larger two-dimensional body size than the other three treatments (IL and IH, or the offspring of mothers caged inside of the kelp forest and raised at either low or high pCO_2, and OL, the offspring of mothers caged outside the kelp forest and raised under

low pCO_2). This was quantified as a significant effect of embryo area between treatments ($F(3, 26) = 11.156$, $p < 0.001$) and a post-hoc test revealed a significant difference in the area of OH embryos compared to that of other treatments (FDR, = 0.05). In addition, there was a nearly significant effect of embryo protein content ($F(3, 8) = 2.81$, $p = 0.11$). However, no significant pairwise comparisons were found with a post-hoc test for false discovery rate (FDR).

Figure 11. Correlation of Egg area and Average diameter for eggs from mothers conditioned inside and outside of a kelp forest environment.

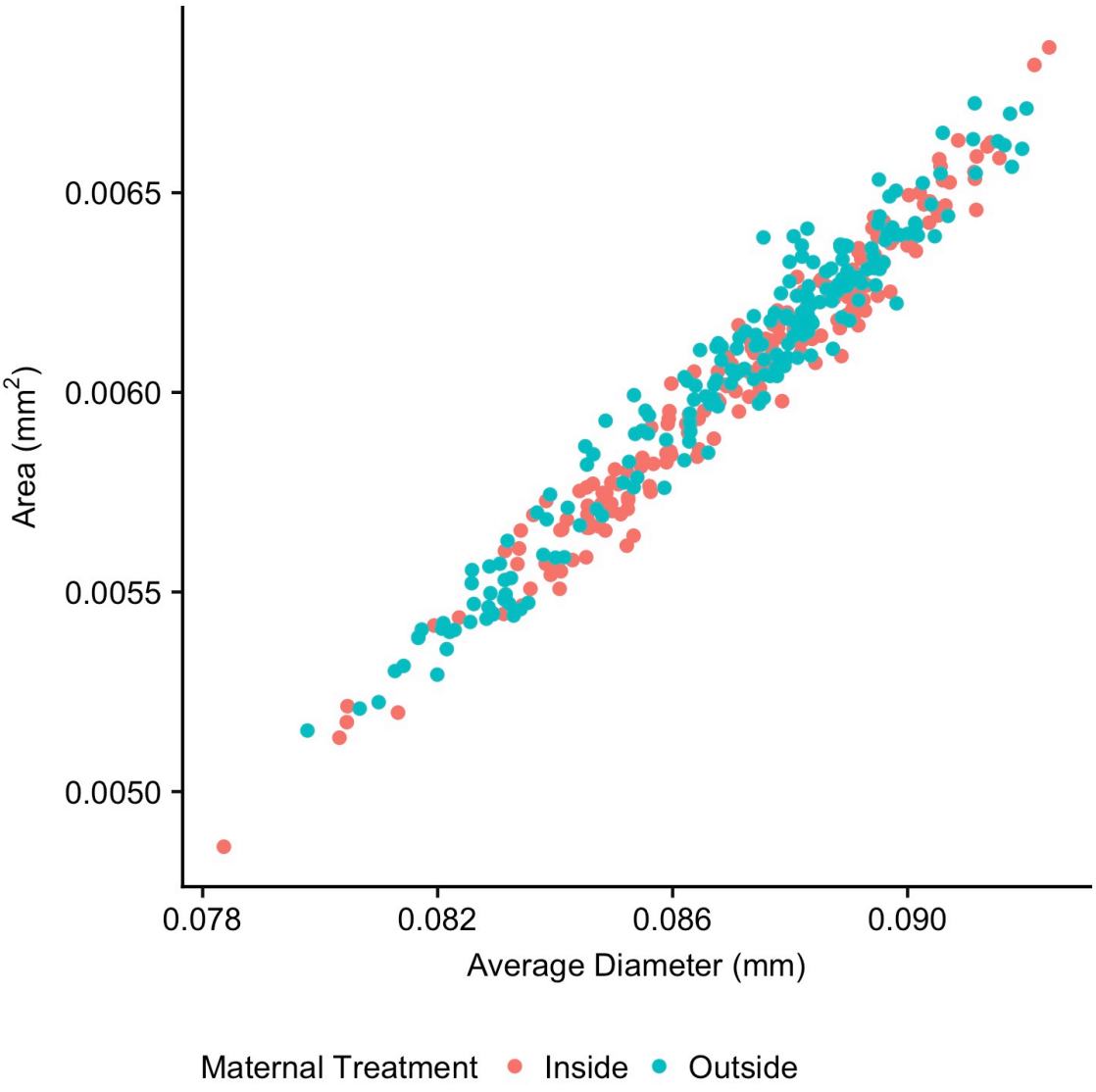


Figure 12. PCA of measurements at the egg level(Egg average protein, Egg aArea, Egg Diameter), averaged to match measurements at the individual female level (Egg count, Test diameter).

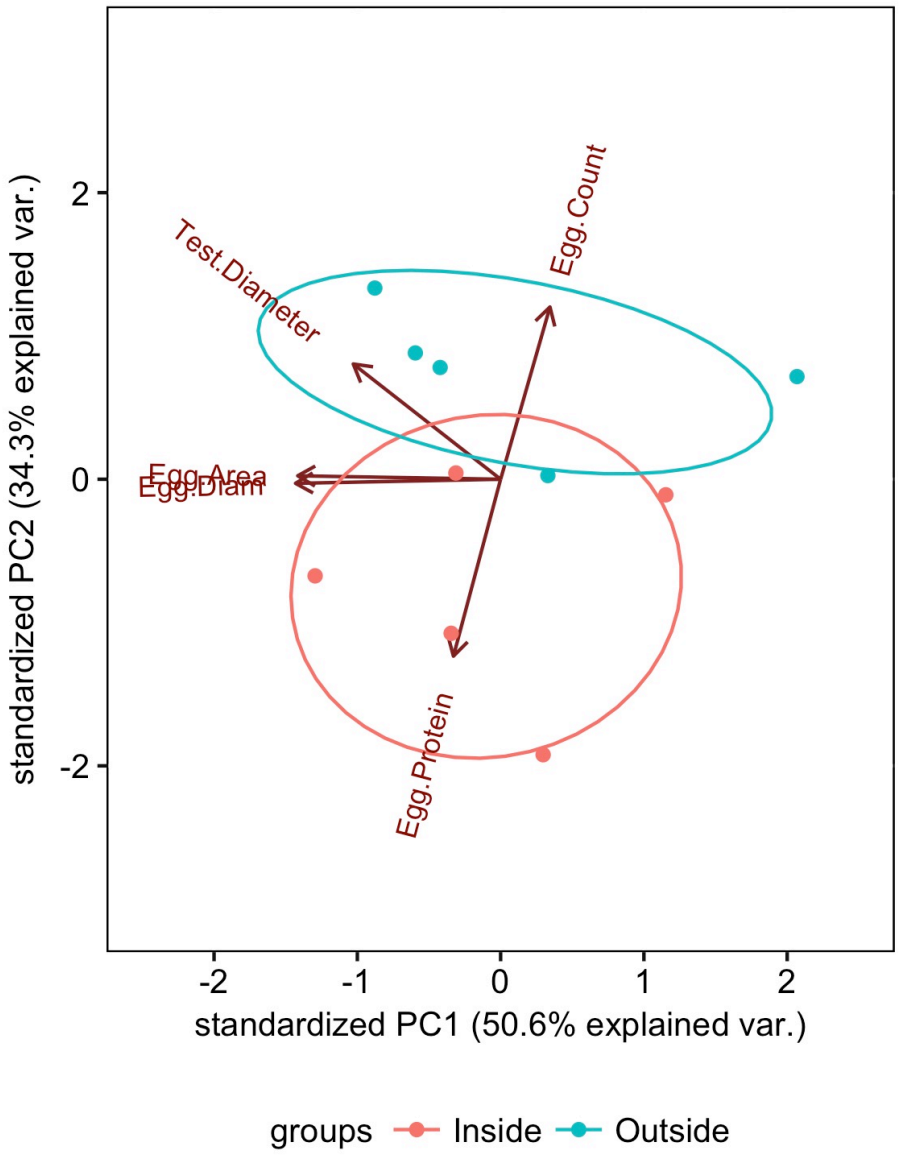
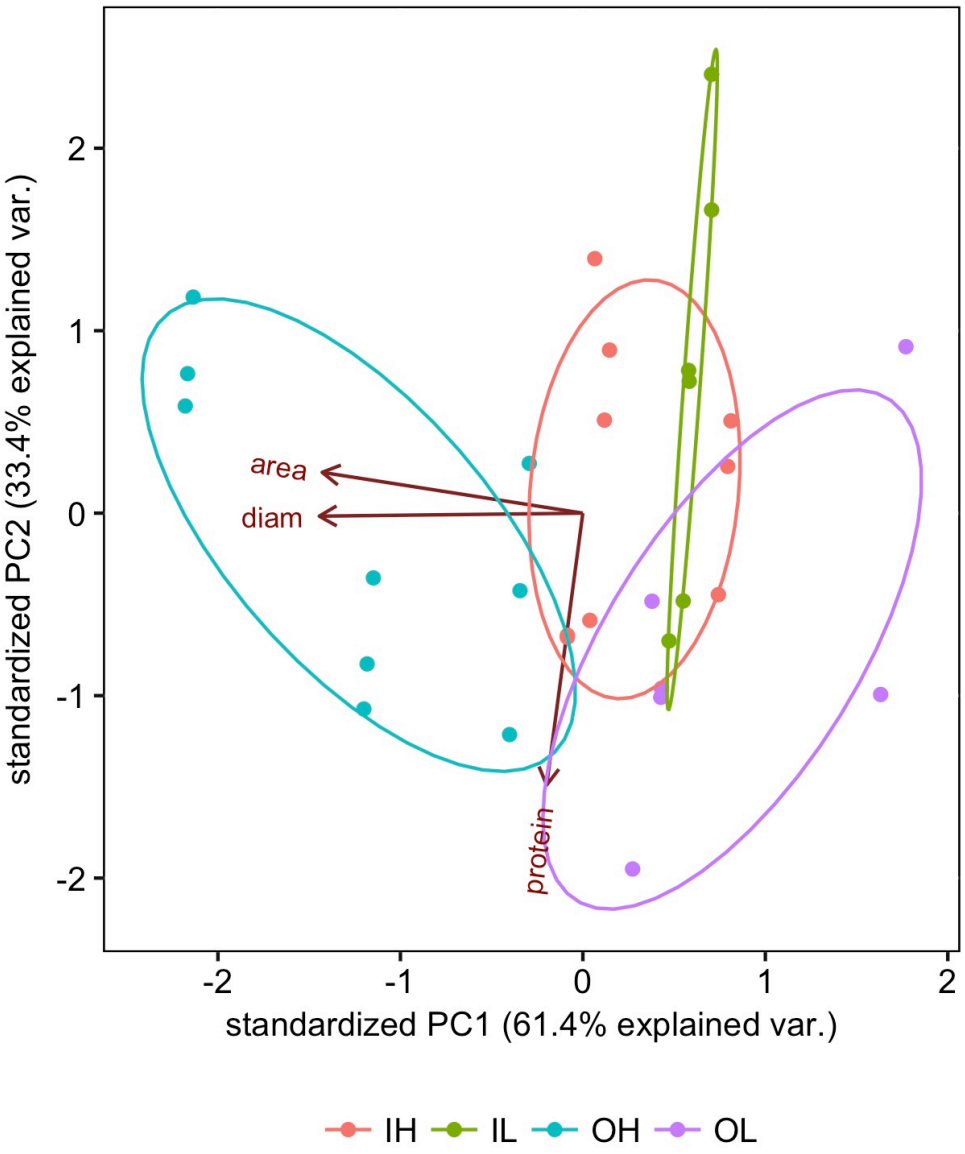


Figure 13. PCA of measurements taken of hatched blastula embryos (17hpf). OH is significantly different from the other treatments (FDR post-hoc test).



Discussion

The overall goal of this Chapter was to investigate the biological impacts of the spatiotemporal variability of physiochemical conditions around kelp forests that was described in Chapter 2. The over-arching question here was: does environmental exposure of the adult females result in a maternal effect that alters the traits, and potentially the performance, of her progeny? Other research in my lab has demonstrated that this process occurs in the laboratory (Wong *et al.*, 2018), and I endeavored to link such a biological process to *in situ* conditions inside and outside a kelp forest.

In general, I found differences in oxygen content and temperature variability at the sites inside and outside of the kelp forest at the Mohawk reef study site, which appeared to impact characteristics of both the sea urchin eggs and early stage embryos. The salient findings of this chapter are: **1)** DO and temperature can vary on very short distances in the nearshore environment, and the presence of macrophytes is likely a large driving factor of this gradient, **2)** sea urchin gametogenesis was affected *in situ* by these seawater conditions, and **3)** this environmental variation also affected traits of the embryos raised using gametes from adult sea urchins conditioned at these sites. Overall, these results suggest that macrophytes may play a key role in the fate of sea urchins during a critical stage in development, potentially leading to ecosystem-level effects in the nearshore environment in the near future.

Sensor conditions during deployment

At the two field sites, adult sea urchins were exposed to different regimes of DO and temperature. This differential experience *in situ* included not only a higher temperature and DO inside of the kelp forest, but also a noticeable difference in the frequency of variability between these two sites (Figure 10). While I did not directly measure pH during these outplants, given the strong coupling between pH and DO I described in this system in the past (Chapter 2), I calculated approximate pH exposure for the deployment (Figure 10).

While there is emerging evidence that the degree of pH fluctuation can modify the outcome of simulated ocean acidification experiments in laboratory-based mesocosms (Frieder *et al.*, 2014; Roleda *et al.*, 2015), it is unclear whether this difference in the frequency of variation will alter organismal response in nature. However, some field studies have begun to explore the ecological and biological consequences of pH variability for natural populations. In a study from Murray and colleagues (Murray *et al.*, 2014), early stage silverside fish (*Menidia menidia*) displayed different tolerances that correlated to the conditions the females experienced in the tidal marsh during the reproductive cycle. In a second study, Britton and colleagues (Britton *et al.*, 2016) found that a species of brown algae, *Ecklonia 6oeteros,* was physiologically adapted to the diurnal pH/DIC conditions of its environment, and performed less optimally at either of the static pH treatments (pH 8.4 and pH 7.8) corresponding to the extremes of this diurnal variation. However, given that the

response to diurnal pH variation was different across different algae (Roleda *et al.*, 2015), or even congeneric invertebrates (Frieder *et al.*, 2014), it is unclear how covarying pH and DO could influence TGP in *S. pupuratus*.

Egg characteristics after parental exposure

It is broadly understood that organisms exhibit thermal tolerance ranges that reflect the variability in their environment, a process that could potentially play a large role in in responding to anthropogenic environmental change (Sunday *et al.*, 2011). However, it is unclear how this response to variation could interact with transgenerational plasticity. In this study, we observed that females outplanted outside of the kelp forest produced more gametes, but each of these had a lower protein content. In comparison, females outplanted inside of the kelp forest spawned fewer gametes that were more protein-rich. This apparent tradeoff in maternal provisioning is often studied in the context of environmental variability and bet-hedging. While some models and systems predict that adverse environments can be optimized for by generating fewer, larger eggs (Olofsson *et al.*, 2009), others empirical studies posit that fitness can be maximized in harsh unpredictable environments by creating many eggs with fewer resources as resource-dependent fitness may not play a large role in more variable, unpredictable environments. These planktrotrophic larvae derive a small amount of their larval nutrient intake through maternal provisioning compared to direct developers and non-feeding larvae (Marshall and Keough, 2007). Because *S pupuratus* early stages do not begin feeding until later in development (as

pluteus at ~72hpf, Strathmann, 1987), this energy storage may play a significant role in the critical early life stages of this organism.

Colder temperatures have been linked to larger optimal egg volume in echnioderms (Levitan, 2000), but we did not see a significant difference in egg size as a function of parental conditioning. It appears that the tradeoff between protein content and egg number may play a larger role in larval success in this scenario compared to egg size.

Embryo response to pCO_2 stress

While there was a negative correlation between egg size and protein content at the egg stage, there were no significant differences in protein between the maternal conditioning and larval treatments at the hatched blastula stage (17hpf). The differences between these treatments were largely driven by embryo size (Figure 13). More specifically, it appears that embryos from females conditioned inside the kelp forest were not significantly different in size, regardless of their exposure to pCO_2 during culturing. However, hatched blastula embryos from females conditioned outside of the kelp forest exhibited a significantly different size in response to pCO_2 conditions, with those raised at higher pCO_2 conditions exhibiting an 8% larger area, on average, in comparison to those raised in the lower pCO_2 treatment.

Previous research has shown that early stage purple urchins are impacted by pH and oxygen change. However the vast majority of this research has been conducted in the laboratory, and usually only examined the effects of high pCO_2 / low

pH using static treatment conditions. Additionally, many studies focus on the impact of pCO_2 treatments on the energetic costs of skeletogenesis, finding that the larvae under high pCO_2 expend more energy (Stumpp *et al.*, 2011) to create shorter spicules (Yu *et al.*, 2011). Here, I review studies that investigated the energetic demands of earlier stages of *S. purpuratus,* specifically in a transgenerational context.

In a transgenerational study on the same species, Wong and colleagues (2018) found that adult female *S. purapuratus* held at static upwelling conditions (1100 µatm pCO_2, 14˚C) during gametogenesis had nonsignificant 3.2% larger eggs than those from mothers raised in ambient conditions for the region (400 µatm, 17˚C), with progeny sizes continuing to reflect this maternal effect. While my outside outplants had both a lower temperature and higher extrapolated pCO_2 (derived from the known relationship between DO and pH at the region), the differences in absolute means of both pH and temperature between my acclimation sites were not as pronounced as those from (Wong *et al.*, 2018). Subsequently, my results did not show a pronounced difference in egg size between maternal conditioning treatments.

In a later study, Wong and colleagues (unpublished data) found that larvae from mothers acclimated to static upwelling conditions (1150 µatm pCO_2, 13˚C) were larger than the larvae from mothers acclimated to non-upwelling static conditions (500 µatm pCO_2, 17˚C). However, there were no significant effects of maternal conditioning on protein content of their eggs. This was the opposite of what was shown in my study, in which maternal conditioning changed protein content and egg

number, but not egg size. However, both studies show a clear effect of the maternal environment on gametogenesis.

In Wong et al (unpublished data), larvae size from non-upwelling-acclimated mothers experienced a significant effect of larval culturing treatment (440and 1050 pCO_2 @ 15˚C), while those from upwelling-acclimated mothers did not. Protein data exhibited similar results, an interaction term in which the effect of the larval pCO_2 treatment is affected by the maternal environment. Even though my maternal treatments are not directly comparable to Wong et al.'s, both show similar broad patterns at both of these stages of development – not only can maternal effect change offspring characteristics, but it can also change the tolerance of offspring to environmental stress in the form of pCO_2.

In other studies, *S. purpuratus* has been shown to not alter its biochemical makeup in the face of experimental ocean acidification (Matson *et al.*, 2012; Pan *et al.*, 2015). However, it is becoming increasingly clear that studies that involve early developmental stages must take into account the genotype or the husbandry conditions of the adults, as both of these factors may play a major role in the biological response.

Summary

Ultimately, this field experiment detected a within-generation plasticity (WGP) effect in response to development under high pCO_2. Note however, that this response was mediated by transgenerational effects – in other words, the differential growth patterns of the embryos was based on the environmental experience of the mother.

The concept that transgenerational plasticity can be a result of climate variability has been posited by Donelson and colleagues (Donelson *et al.*, 2018). Here, the authors noted that TGP may be more advantageous in spatiotemporally stable regions where offspring can utilize parental cues. On the other hand, sea urchins in the in California Current System are exposed to physiochemical conditions that can change over much shorter distances and time scales than larval dispersal. This makes the advantage of TGP less intuitive for this organism and its environment. In this case, the WGP of body size in response to pCO_2 conditions may be explained as the result of the unpredictability of the maternal environment priming the larvae to be able to phenotypically respond to a large suite of conditions. This is supported by observations made by Wong and colleagues where the transcriptome changed in a manner that also suggests that female conditioning can prime the progeny to better respond to extreme conditions during development (Wong *et al.*, 2018). Understanding the role of TGP in nature and the mechanisms that drive such a response are of great interest to the marine science research community at the moment (Donelson *et al.*, 2018, 2018; Ghalambor *et al.*, 2007; Hofmann, 2017), and this is one of very few studies that are designed to investigate this in situ (Murray *et al.*, 2014). Overall, investigating the molecular basis for this plasticity over a multigenerational manipulation may be the key to ultimately understanding this variation in response (Foo and Byrne, 2016). Regardless, any phenotypic plasticity may play a critical component in the survival of marine species to rapid global change (Munday *et al.*, 2013).

Acknowledgements

This part of my thesis was conducted in collaboration with the Santa Barbara Coastal (SBC) LTER in 2017. During this time, the SBC LTER provided GSR support to me, as well as support in boating and diving logistics. While I conceived of, designed, and performed the experiment, I was significantly aided in larval culturing by members of my lab: Juliet Wong, Terence Leach, Logan Kozal, Maddie Housh, Jannine Chamorro, and Xochitl Clare.

IV. A multi-year dataset of ocean pH from McMurdo Sound, Antarctica

Abstract

Polar regions are expected to be highly susceptible to ocean acidification due to cold surface waters that can more rapidly absorb anthropogenic CO_2 from the atmosphere. In the Ross Sea, the largest international marine reserve, the most southern navigable coastline, and one of the most productive ocean areas on earth, there have been very few measurements made of surface water chemistry, and none made during the hostile winters. However, with polar regions experiencing dramatic differences between seasons, previous shipboard cruises have observed large seasonal swings in surface pH in the Ross Sea.

Here, I deployed oceanographic sensors to investigate the seasonal, interannual, and spatial variation of pH over 5 years (2010-2016) at 3 different sites in McMurdo Sound (The Jetty adjacent to McMurdo Station, Cape Evans, and New Harbor). I found an average annual pH decline / pCO_2 increase of -0.189 yr^{-1} and +15µatm yr^{-1}, respectively, which is a far greater change than the expected global averages and thus likely caused by other local factors. Furthermore, I found that the New Harbor site exhibited a delayed seasonal alkalinization event in contrast to the other two sites during the same year, which is consistent with knowledge of the prevailing under-ice currents in the area.

Overview

This work was conducted within the U.S. Antarctic Program during field seasons supported by grants to my advisor, Dr. Gretchen E. Hofmann (U.S. National Science Foundation (NSF) awards ANT-0944201 and PLR-1246202). In association with these projects, I conducted sensor deployments on-site at McMurdo Station, Antarctica in the summers of 2014, 2015, and 2016. I also led diving operations in Summer 2015 and 2016. Previous deployments were conducted by Dr. Paul Matson, Dr. Lydia Kapsenberg, and Professor Amanda Kelley. Parts of two data sets referenced in this chapter were previously published (Kapsenberg *et al.*, 2015), however the remainder of the time series is unpublished and is reported in this Chapter.

Introduction

In coastal oceans, carbonate chemistry can vary drastically over time and space, often at a scale comparable to near-future ocean acidification (OA) (Hofmann *et al.*, 2013; Kapsenberg and Hofmann, 2016; Shaw *et al.*, 2013; Silbiger and Sorte, 2018). Thus, it is critical to evaluate this small-scale spatial variability at study sites, as every region may experience OA in a different context given the local drivers of pH (Shaw *et al.*, 2013).

High latitude seas have been proposed as the 'first in time" of the world oceans to experience signatures of OA, given that cold water can absorb more CO_2 (Steinacher *et al.*, 2009). The Southern Ocean, in particular, acts as a large carbon sink (Arrigo *et al.*, 2008), with the Ross Sea exhibiting a seasonally high saturation of

surface pCO_2 (Sweeney, 2003). This variation has been described to be seasonal, with surface pCO_2 peaking in the winter (McNeil *et al.*, 2010), with a corresponding decrease in pH (Kapsenberg *et al.*, 2015). This is likely driven by the air-surface equilibrium disruption by ice cover (McNeil *et al.*, 2010) as well as the highly seasonal light patterns (Littlepage, 1965).

Organisms in the Southern Ocean, thus, might be impacted by OA in the future (Fabry *et al.*, 2009; Hauri *et al.*, 2016). However, when I began my thesis research, there was little information on the pH and carbonate chemistry in Antarctic waters. McNeil and colleagues had proposed that 450 µatm pCO_2 would be devastating for Antarctic biota (McNeil and Matear, 2008). They predicted that this threshold would be crossed in winter in the year 2030, and no later than 2038. This modeling effort was not matched with *in situ* observations until recently. Some of the first data was reported by my lab for the years 2011-2013 (Kapsenberg *et al.*, 2015). Continuing to understand how these patterns vary over space and time is critical for understanding how the current and future Antarctic environments will shape organismal tolerance to global change.

The overall objective of this chapter was to establish a multi-year timeseries of ocean pH in the Antarctic that also incorporates multiple sites around McMurdo Sound. Here, I contribute to this effort and present seasonal variation in pH we observed over 5 years of autonomous pH sensor deployments at field sites in McMurdo Sound, Antarctica, the most southern navigable body of water in the world. In addition to presenting times series data, I discuss how such a pattern of natural

variation in pH, and as a result, aragonite saturation, might impact the Antarctic marine organisms in this nearshore region of the Ross Sea (further explored in Chapter 5). Lastly, I will discuss the logistical challenges and logistics of sensor deployments at these remote field sites.

Methods

Field Deployments of Oceanographic Sensors

All field work and research diving was conducted under the auspices of the U.S. Antarctic Program (http://www.usap.gov). Sensors were deployed in McMurdo Sound in the southern Ross Sea in the Pacific sector of the Southern Ocean. During the course of this research project, sensors were deployed at three locations: Cape Evans (77° 38.053' S, 166° 24.880' E, 28m depth), the Jetty (77° 51.058' S, 166° 39.865' E, 24m bottom depth), and New Harbor (77° 34.5720' S, 163° 31.7388' E, 24m bottom depth) (Figure 14). Non-commercial, fabricated SeaFET sensors (Martz Lab, Scripps Institute of Oceanography, custom fabrication, Martz *et al.*, 2010) were used in all deployments, and were programmed to record at 4-hour intervals in order to preserve battery life. Using a subsurface float to prevent damage from seasonal fast ice movement, all sensors were installed on subtidal moorings and suspended in midwater at a depth of 18m as previously described in Kapsenberg et al. (2015). All sensor deployments and seawater sampling protocols were conducted according to best practices for the use of pH sensors in ecological field research (Rivest *et al.*, 2016).

Sensors were generally deployed in the Austral Spring, and retrieved the following Austral Spring to be downladed and redeployed.

Calibration samples

Sensors were calibrated to seawater samples taken with Go-Flo® bottles by SCUBA divers, with the collection of the seawater sample timed to coincide with the collection of a data point by the SeaFET. At the surface in a research dive hut, seawater was transferred to 500mL borosilicate bottles (Corning® narrow mouth reagent bottles with a Pyrex® ST stopper), and sealed with greased (Apiezon L) ground-glass stoppers. These bottled samples were brought back to the nearest lab facility (either the Crary Laboratory at McMurdo Station for the Jetty and Cape Evans samples, or the on-site lab facilities at New Harbor field camp for those respective samples) for preservation in 0.02% $HgCl_2$. While this is typically done on-site immediately after collection (Dickson *et al.*, 2007), samples were transported unpreserved due to restrictions on traveling with and working with $HgCl_2$ on sea ice as dictated by the U.S. Antarctic Program. During this transportation period (2 hours maximum), samples were kept cold (<0˚C) and dark to minimize changes in seawater chemistry due to metabolism.

These fixed seawater samples were either analyzed on-site (2013, 2015, 2016, 2017) or shipped at +4˚C back to the University of California, Santa Barbara (2014, 2017) for carbonate system measurements. All measurements were conducted on a Shimadzu spectrophotometer with a 1cm path length (UV-1800 at UC Santa Barbara,

California, USA; UV-2501PC at McMurdo Station, Antarctica) and a Mettler-Toledo T-50 open-cell titrator calibrated using a certified Tris- buffer at pH 7.0 and verified using seawater certified reference materials (Dickson *et al.*, 2007).

Data Analysis

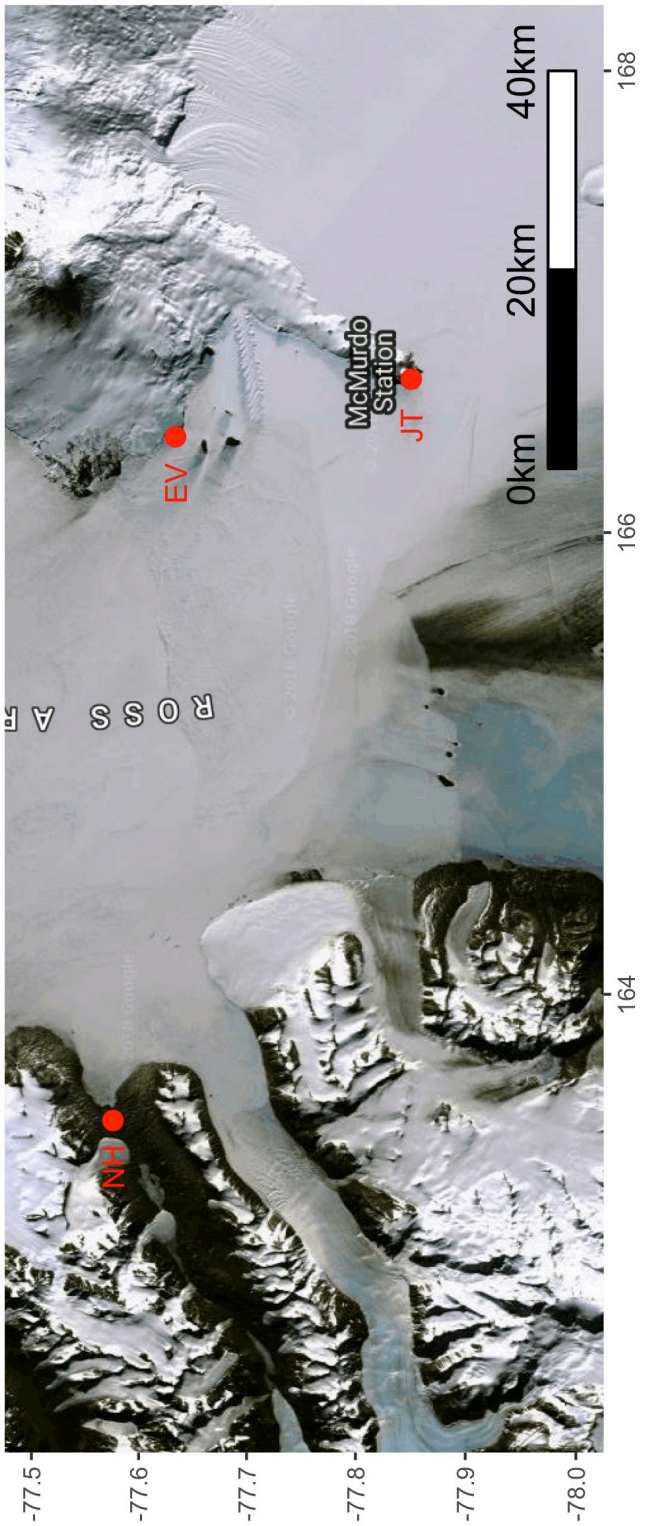
All data analysis was conducted with R v.3.4.3 (R Development Core Team, 2017) and the R Studio IDE (RStudio Team, 2017), with packages seacarb (Gattuso *et al.*, 2017), suncalc (Agafonkin and Thieurmel, 2017), plyr (Wickham, 2011) tidyverse (Hadley Wickham, 2017), lmerTest (Kuznetsova *et al.*, 2017), agricolae (Felipe de Mendiburu, 2017), and cowplot (Wilke, 2017).

First, the measured spectrophotometric pH was converted to *in situ* temperatures using the following equilibration constants K_1 and K_2 (from Roy *et al.*, 1993), K_f (from DOE, 1994), and K_s (from Dickson, 1990). Equilibration constants often recommended in best practices (Dickson *et al.*, 2007) were not used due to their inadequate verified temperature ranges; for example, K_1 and K_2 coefficients (from Lueker *et al.*, 2000) are verified down to +2°C, while K_f (from Perez and Fraga, 1987) is verified down to +9°C. While no published equilibration constants encompass the temperature range of my measurements, I ensured that all equilibration constants used are verified down to 0°C. Pressure was assumed to be 18.199dbar at the sensor site given depth and latitude (Saunders, 1981).

Carbonate chemistry calculations were performed using these same equilibration constants and parameters (Dickson, 1990; DOE, 1994; Roy *et al.*, 1993;

Saunders, 1981). To calculate these values, I used monthly salinity from previous oceanographic observations in the Ross Sea (Littlepage, 1965). Total alkalinity was calculated from salinity using a relationship derived for surface waters in the Southern Ocean (Lee *et al.*, 2006).

Figure 14. Map of the field site. Sites are Cape Evans (EV), Jetty (JT), and New Harbor (NH). EV and JT are on Ross Island, while New Harbor is on the mainland at the mouth of the Dry Valleys, across McMurdo Sound from Ross Island.



Results

Here, I present the data collected over 5 years of deployments of autonomous pH sensors in McMurdo Sound, as well as detailing the challenges of deploying sensors in this environment. In some seasons, battery failures, as well as sensor failures, affected the data set. Overall, sensor deployments depicted a strong seasonal trend, as well as a rate of pH decrease over the 5-year span of the deployments.

Signatures of ocean acidification

Long-term trends of ocean pH were examined using all data. After accounting for site-specific variation, the average pH during this period of time was found to decrease by .0189 +/- 0.00073 pH units a year, or the equivalent of increasing 15.02 +/- 0.67 µatm pCO_2 per year (Repeated measures ANOVA to account for seasonality, $p<0.0001$, Figure 15). This is roughly one magnitude larger than the 1.794 µatm yr^{-1} described by global sampling efforts (Dlugokencky and Tans, n.d.).

Seasonal variation in ocean pH in McMurdo Sound

All sensor deployments (Figure 16) showed the same characteristic pattern: pH was relatively stable and low during winter and early spring, increased rapidly in late December, and slowly decreased through the early winter back to a stable, low pH value. This general trend was observed every year, across all sites. Associated calculated values for aragonite saturation and pCO_2 followed predictable patterns (Figure 17), with pCO_2 decreasing sharply in early summer (December) and then

slowly rising to a stable state in the early winter (June) with aragonite saturation exhibiting an inverse relationship. Monthly pH values peaked in December at 8.3 pH units, with a calculated pCO_2 of 200 µatm and a Ω_{arag} of 2.5. In contrast, monthly pH low values were observed in the months of June through October at 8.05 pH units, with a calculated pCO_2 of 400 µatm and a Ω_{arag} of 1.3.

In addition to these monthly trends, there were differences in monthly high-frequency variation (Figure 18). Here, the pattern of high levels of daily variation peaked in December, similar to the mean monthly pH (Figure 17), and decreased gradually through the winter. These patterns of variation were linked to daylight during transitions in the austral seasons. Specifically, there was a significantly higher diurnal variation during periods of the year in which there is neither 24-hour sunlight or darkness (Figure 19, $p<.0001$).

Differences between the deployment sites

Both the Jetty and Cape Evans sites had similar annual cycles (Figure 16). One distinct difference was New Harbor, which exhibited a later onset of summer alkalinization than the other two sites. This later summer alkalinization was also paired with a distinctly later increase in temperature in the summer (Figure 16).

Figure 15. The pH and temperature values for all years of deployments, for December 1 ~ December 15 of every year to visualize the most stable time of the year.

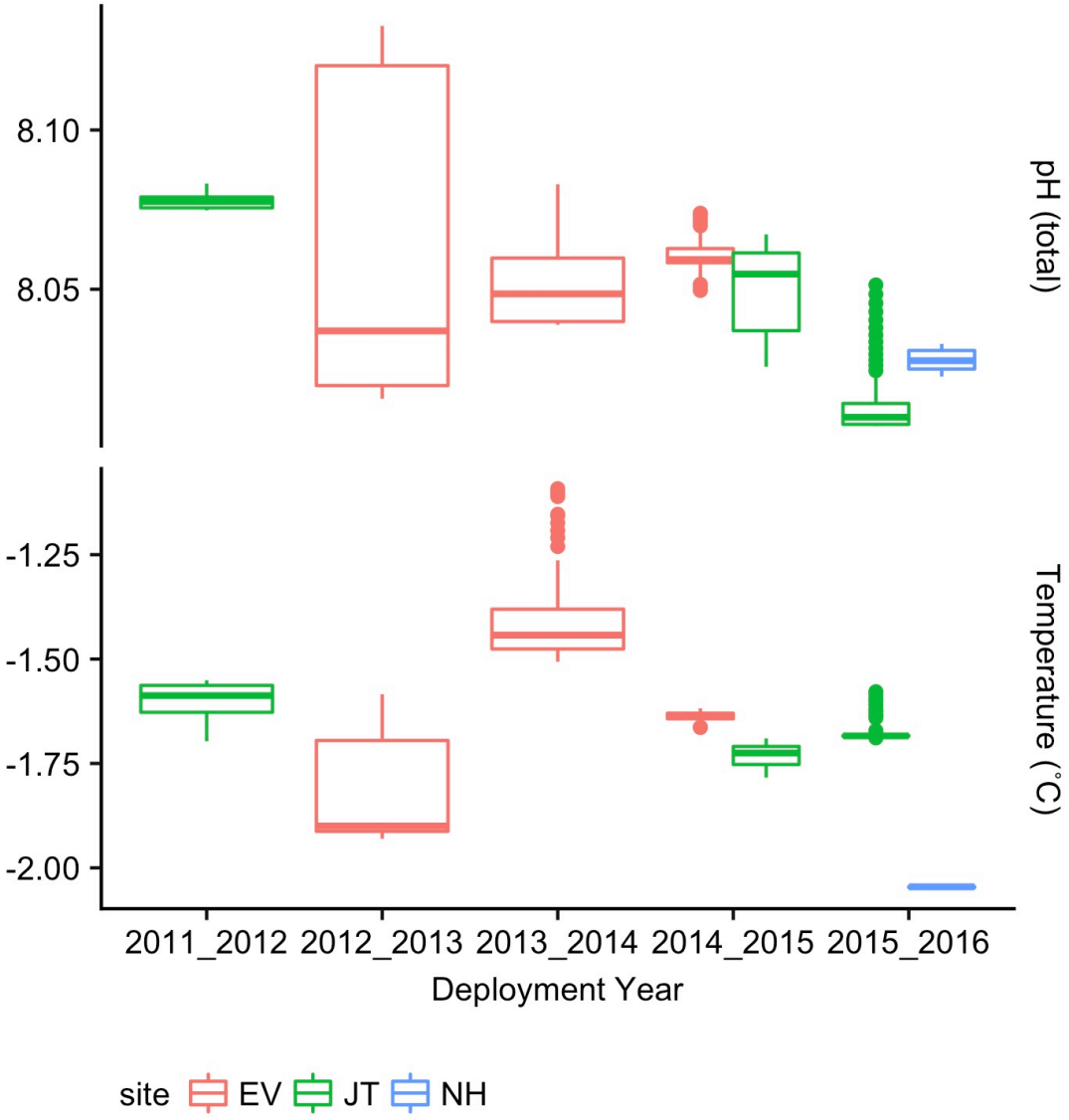


Figure 16. All sensor deployments for the duration of the study, spanning from 2011 to 2016.

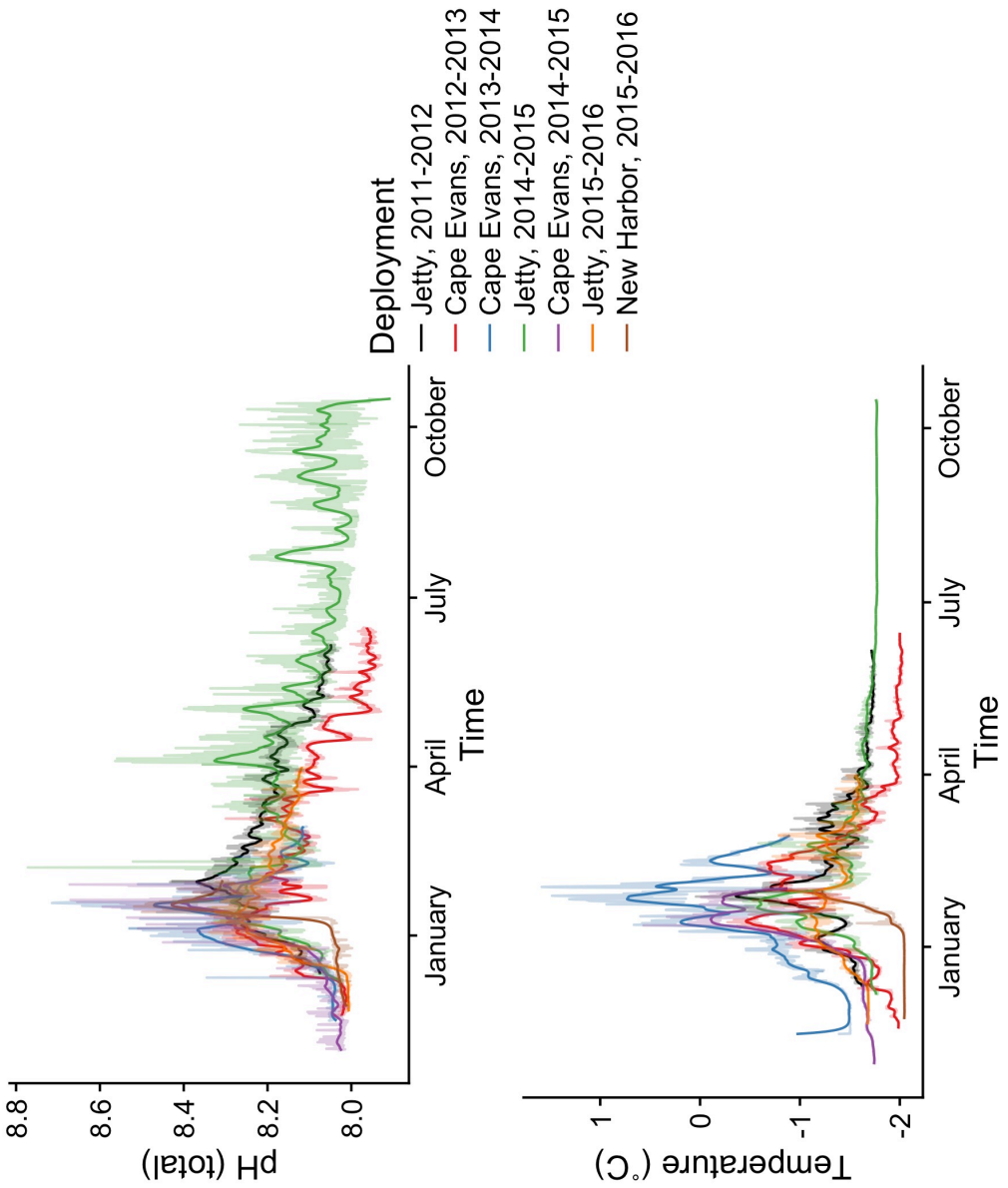


Figure 17. Calculated monthly values of temperature, pH, pCO_2, and Aragonite Saturation state for all deployments.

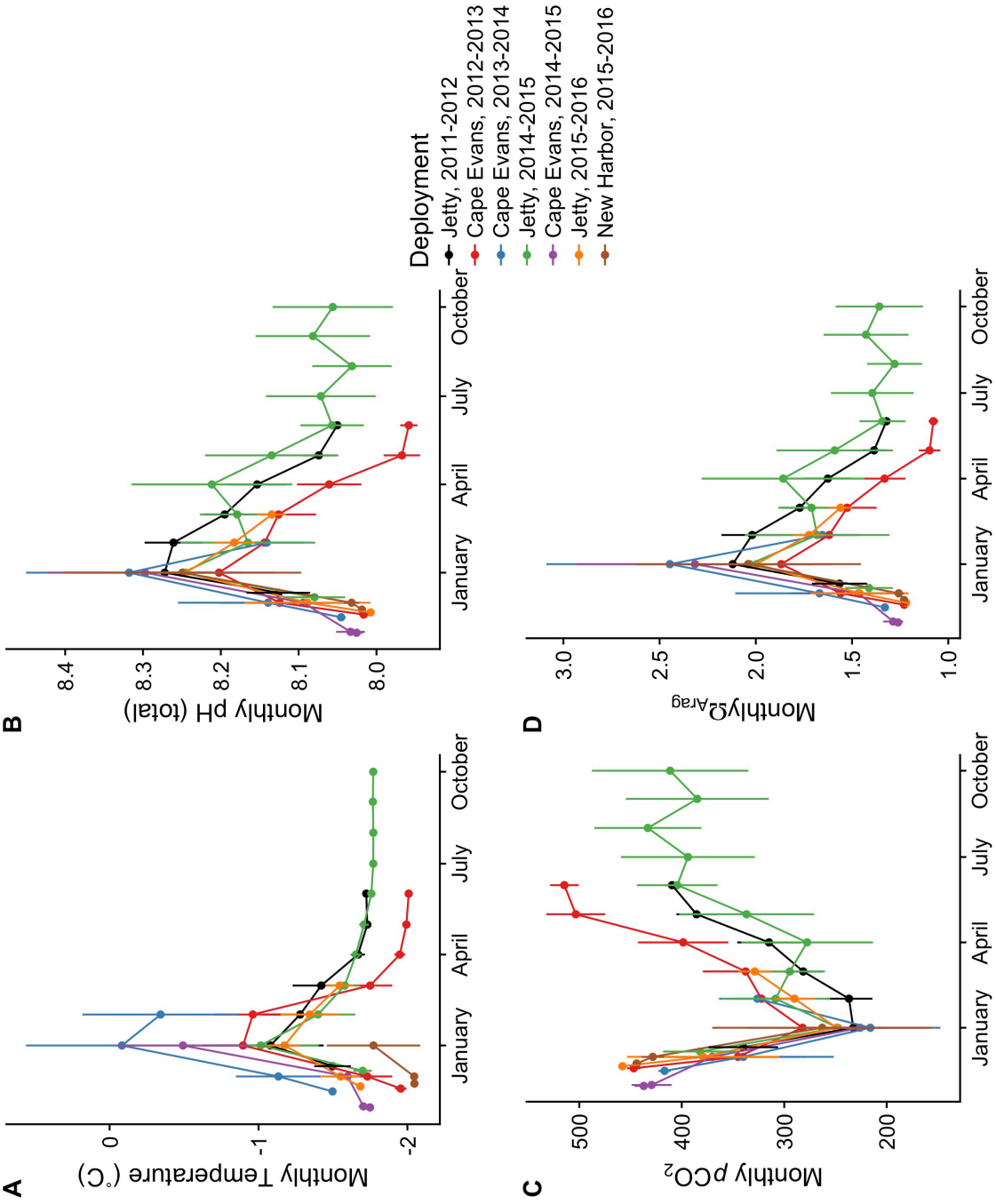


Figure 18. Daily ranges of pH, as a function of site and month.

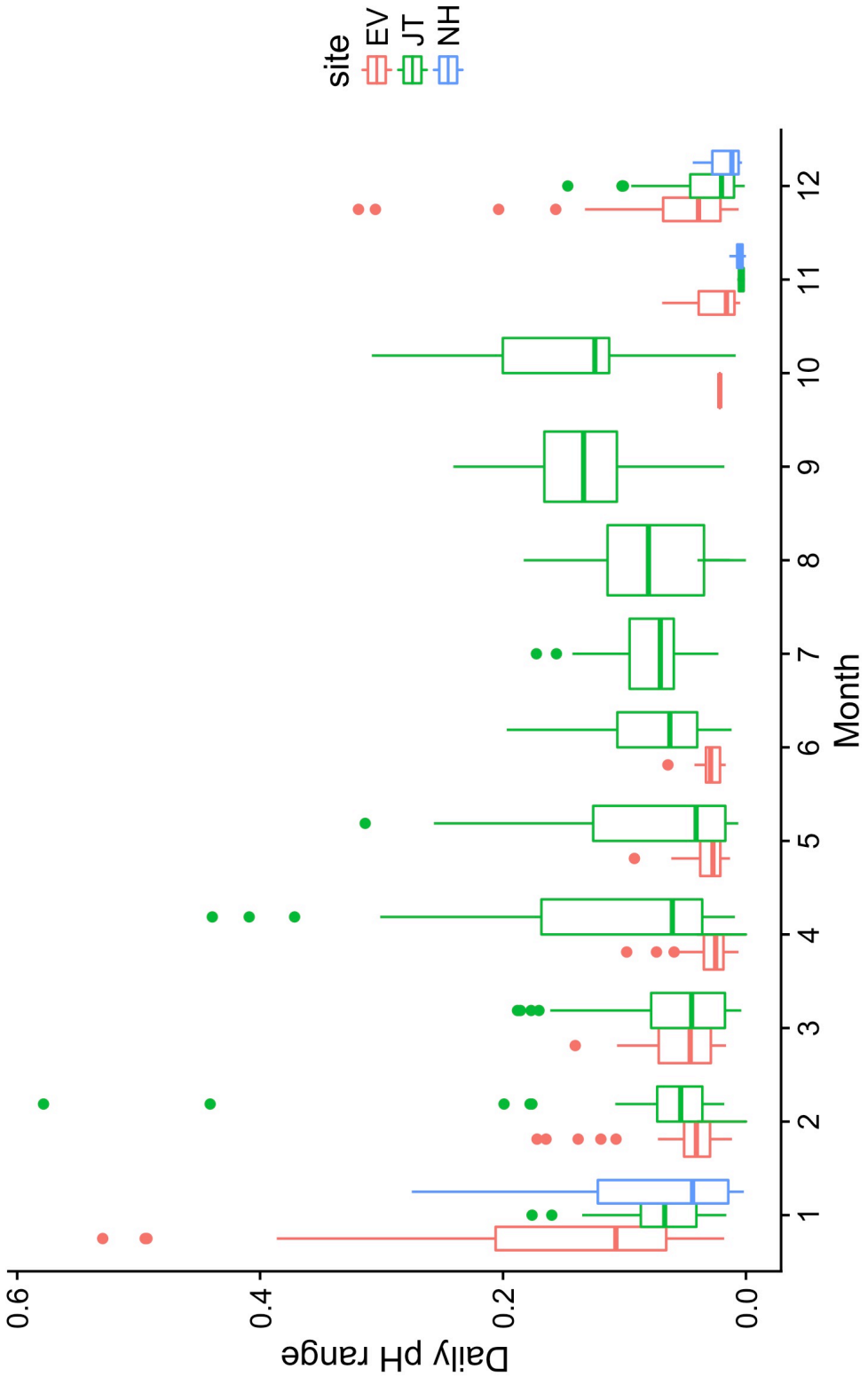
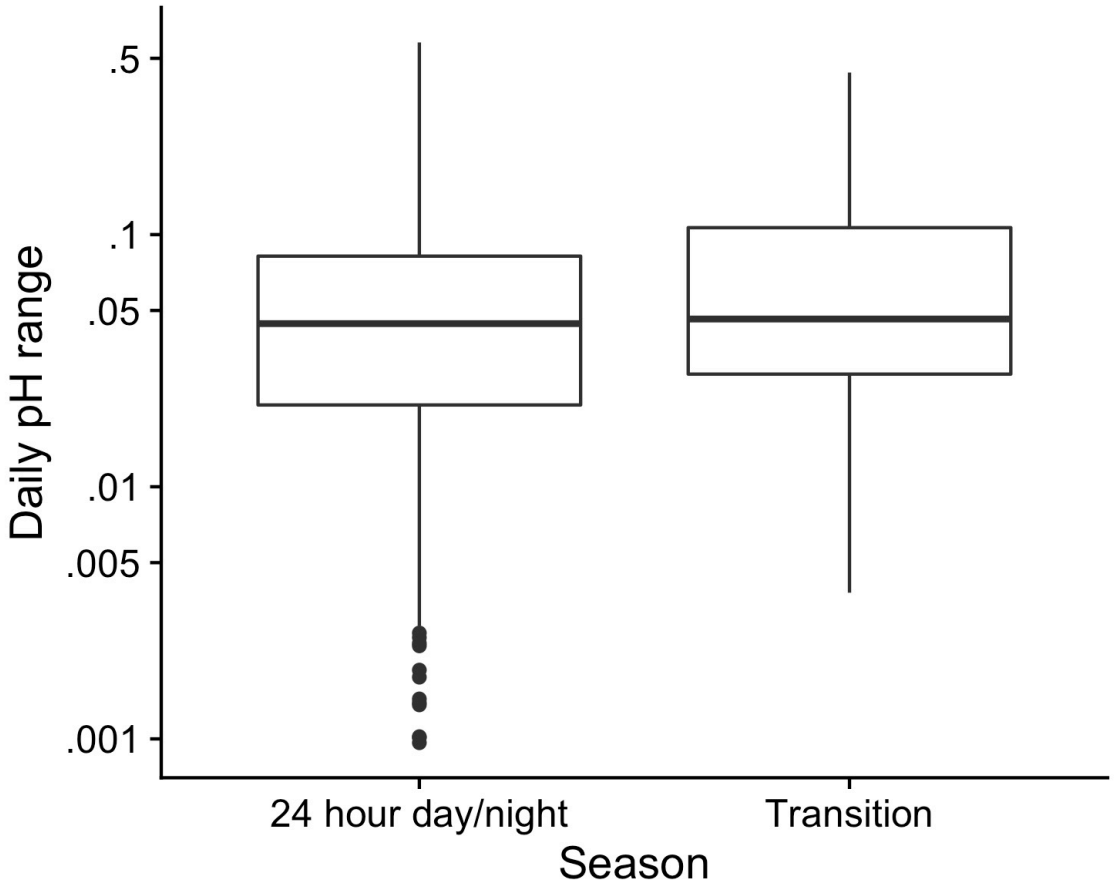


Figure 19. Transition seasons have a significantly higher daily pH range than the rest of the time periods.



Discussion

The goal of this Chapter was to deploy sensors at various sites in McMurdo Sound, Antarctica to generate a multi-year time series of pH in the region, and to assess differences between years and sites. In this analysis, I found **1)** possible evidence of ocean acidification, as denoted by decreases in ocean pH, corresponding to increased global levels of atmospheric pCO_2, **2)** differences in high-frequency variation between seasons, and **3)** differences between seasonal variations between sites within McMurdo Sound.

Evidence of OA & future projections

Over the course of the deployments, pH showed a decline at the Jetty site during the stable period of the year. Given the dynamic nature of pH at the region (average s.d. = 0.111 pH units per deployment), a 5-year time series is realistically insufficient to resolve this large signal of ocean acidification (Keller *et al.*, 2014). This rapid interannual change in pH is thus likely linked to local effects and not purely due to absorption of CO_2 from the atmosphere, given data shown by long-term studies with the Hawaii Ocean Time Series Study and the Bermuda Atlantic Time Series Study, respectively (1.785 µatm per year over 28 years, Bates *et al.*, 2012; 1.92 µatm per year over 22 years, Dore *et al.*, 2009). In these two studies, pH decreased by a relatively modest amount (.001 per year in Hawaii, Bates *et al.*, 2012; .0017 per year in

Bermuda Dore *et al.*, 2009), compared to the large 0.018 per year I observed here in McMurdo Sound (Figure 15).

Seasonal patterns

Despite this large interannual change in ocean pH I observed at this site, the dominant signal throughout the time series was the seasonal pH dynamic driven by photosynthesis via *Phaeocystis* blooms that occur regularly in mid-December in McMurdo Sound (Kapsenberg *et al.*, 2015; McNeil *et al.*, 2010). These photosynthesis-driven dynamics varied significantly through the season. In addition, finer-scale variation was observed at the diurnal scale during the transition seasons between 24-hour darkness and 24-hour sunlight.

Site-specific patterns

Two of the sites – the Jetty site and the Cape Evans site, are on the coastline of Ross Island, in an area seasonally covered by sea ice. In contrast, the third site, New Harbor, is situated across McMurdo Sound in Victoria Land. This site generally exhibits a lower and most stable temperature through the year (Cziko *et al.*, 2014, Cziko pers. comm). While my one deployment at New Harbor (Figure 16) failed shortly after deployment, it is clear that the summer alkalinzation event occurred about three weeks later in that New Harbor deployment than it did in the other deployments at the Jetty and Cape Evans sites in previous years.

While this could be due to differences in local photosynthetic activity, it is more likely due to water mass movements than local effects due to several factors. At the Ross Island sites (Cape Evans, Jetty), the advent of this large alkalinization event seems more linked to a bloom that is generated in the open Ross Sea and carried under the sea ice by ocean currents along Ross Island (DiTullio *et al.*, 2000). Then, this prevailing current heads south along the east edge of McMurdo Sound, past Cape Evans and towards the Jetty site (Figure 14). This current then veers west towards the mainland, then cuts north past New Harbor (Littlepage, 1965). This algal bloom has been observed by divers to occur offshore of Ross Island every summer in mid-December (Peloquin and Smith, 2007; Rob Robbins and Steve Rupp, Antarctic Support Contract, pers. comm), and is also tightly linked to an increase in temperature (Figure 16). Given that both the temperature and pH increase at New Harbor lags behind the seasonal variation witnessed at Cape Evans and Jetty, and that previous work has shown the prevailing currents in the area support this timing, I posit that the alkalinization effects at this site are linked to large water mass movements rather than local productivity.

Comparison with other pH data from the Southern Ocean

While the Antarctic is a location of great concern in the context of future climate change and OA, few data are available for ocean pH in the Southern Ocean. In the Ross Sea, data from oceanographic cruises generally agree that algal primary productivity is the main forcing of the carbonate system in the area (DeJong *et al.*,

2015; McNeil *et al.*, 2010; Rivaro *et al.*, 2017), which is not entirely surprising given that this is considered one of the most productive bodies of water on Earth (DiTullio *et al.*, 2000). Given that this dynamic is so tied to the phytoplankton bloom and the associated hydrography, the fate of this seasonal variation is unclear due to a lack of knowledge about its direct drivers (Mangoni *et al.*, 2017). However, given that current blooms persist in the near future, some have argued the possibility that surface aragonite saturation may be further away than previously expected (2070), or could never happen, when considering algal photosynthesis in a disequilibrium model (DeJong *et al.*, 2015). However, they were unable to measure any carbonate parameters during the austral winter due to seasonal ice cover, which is the season in which aragonite undersaturation would be projected to occur. Thus, further monitoring of Southern Ocean pH through sensors, during times of year when oceanographic cruises cannot travel to these sites, will be critical to attaining a full picture of the annual cycles in carbonate parameters that will set the stage for future ocean change.

A similar seasonal cycle is observed on the West Antarctic Peninsula, with productivity increasing pH during the summer months, but with a larger change in seasonal alkalinity due to melting and runoff which leads to slight carbonate ion dilution (Gibson and Trull, 1999; Legge *et al.*, 2017). This meltwater also stratifies the water column, facilitating these plankton blooms (Legge *et al.*, 2017) – a process which is likely less important in the cold surface waters of the Ross Sea. Thus, while the Western Antarctic Peninsula also shows a strong seasonal signature, it is mediated by

melting ice, which may change drastically in the near future with sea ice in rapid decline (Comiso *et al.*, 2008)

In East Antarctica, researchers with the Australian Antarctic program have recently launched an underwater free-ocean CO_2 enrichment experiment (FOCE, Gattuso *et al.*, 2014) aimed to monitor ambient ocean pH while manipulating pCO_2 in benthic chambers (Stark *et al.*, 2018). This study found very stable pH conditions at two locations in O'Brien Bay, Casey Research Station, Antarctica, from December to February, a period of time when I observed maximum variability in pH in McMurdo Sound. This disparity between my data and that of the FOCE experiment exemplifies that the seasonal effects that I observed at several sites in McMurdo Sound, and that have been observed in the greater Ross Sea region (McNeil *et al.*, 2010) cannot be extrapolated to other Antarctic coastlines. Such regional variation must be accounted for when considering the response of Antarctic marine organisms to future ocean change (Hofmann *et al.*, 2013).

Biological consequences

The purpose of this study was to frame oceanographic variation at this field site in a biological perspective – how do these large seasonal changes, high-frequency variations, and seasonal dynamics impact biological processes both currently and in the future?

In the case of interannual change, it is clear that the Southern Ocean in general is reaching aragonite undersaturation rapidly due to the cold surface waters (Hauri *et*

al., 2016; McNeil *et al.*, 2010). If rapid change of this magnitude continues locally in this region, it has the potential to more rapidly reach aragonite undersaturation in the future. This undersaturation would likely occur first during the winter months, a time of year with no light and sparse food. This limitation of resources may challenge taxa that might otherwise tolerate the energetic costs of stressful pH conditions in other biomes (Johnson *et al.*, 2016). Like in many systems, these multistressors (low food availability and acidification) are hard to decouple and should be considered together when designing lab experiments, especially those that consider the energy budget of organisms in response to global change (Sokolova, 2013).

These seasonal implications may also affect organisms' phenology. Some organisms, such as the Antarctic sea urchin, are gravid in the austral spring just before the large alkalinization event (Chiantore *et al.*, 2002). This may mean that sea urchin larvae are developing during the time of year most amenable for growing their early skeletal rods. However, acidification may stress adult urchins over the preceding winter, changing variables such as larval energy allocation in times of stress (McAlister and Moran, 2012).

Sensor Deployment Challenges

Given the logistical constraints of deploying sensors subtidally in a dynamic environment, sensors deployments were generally successful. However, this particular 5-year dataset was marked by the complexities of deploying relatively new instruments in a fast ice, subzero seawater environment, combined with the

inaccessibility of the field sites in the winter, and the evolution of battery technology for these sensors.

The seawater temperatures around McMurdo Sound are known to cause spontaneous ice formation (i.e. brash ice) on any subtidal substrate (Dayton *et al.*, 1969). This also occurred on deployed sensors and the associated mooring equipment. This caused several challenges; most notably in 2014, one sensor accrued so much brash ice that it achieved the ~50kg of buoyancy necessary to overcome the lead weight anchoring it to the bottom. This sensor, deployed at the Jetty site, was not recovered, and led to subsequent deployments utilizing heavier weights to anchor the sensor at the bottom, which were more challenging to both deploy and retrieve.

Brash ice cover also affected the sensors in more unexpected ways. Specifically, the pH probes on the sensor would often become enveloped in ice, potentially creating an enclosed chamber of seawater around the probe that was not representative of local oceanographic conditions. This may have also increased the corrosion of nearby metal on the sensor through redox reactions (Professor Todd Martz, Scripps Institute of Oceanography – pers. comm), leading to failure of certain external parts of the sensor.

In addition to ice cover, one challenge of these sensor deployments was the absolute temperature of their environment. Delrin was chosen for custom sensor deployments due to its tolerance to colder temperatures (DuPont, n.d.). In addition, it was found that alkaline batteries had a significantly lower energy capacity these deployments due to their inefficiency at low temperatures. As a solution, custom

lithium batteries were constructed in order to prolong the life of the sensor under Antarctic sea ice.

While adopting these lithium batteries lifted a prime limiting factor in my deployment times, they were accompanied by their own set of challenges. Unlike alkaline batteries, which have a no-load voltage which can reflect the remaining battery capacity, lithium batteries do not decrease in voltage over use until near-empty, making their assessment difficult. Thus, for later deployments using lithium batteries, care was taken to record the length of deployments conducted on each battery to ensure that a used battery was not being swapped into a sensor.

Summary

Given the implications for ecologically important Antarctic organisms such as pteropods, I explored the variability of Antarctic pH at a variety of temporal scales. Overall, I detected seasonal variation in pH as well as a decline in average ocean pH over the 5-year time series. This evidence of ocean acidification may not represent a pure signal of actual anthropogenic CO_2, but rather may be related local drivers in McMurdo Sound. However, ecologically, the data do imply that the waters of the Ross Sea, despite being comparably invariant in temperature and pH as compared to temperate waters (Hofmann *et al.*, 2011), do present potential abiotic challenges to marine organisms and can fluctuate on an interannual scale. Furthermore, the data indicate that the typically low Ω_{arag} of the Southern Ocean will be exacerbated in the winter months in the Ross Sea.

Notably, in my biological studies (Chapter 5, I examined how low pH in winter might impact the physiology of juvenile pteropods, a shelled pelagic mollusc, that over-winters under the sea ice. For that study, the pH data reported here were used to frame the pCO_2 mesocosm experiments described in Chapter 5, where I applied these seasonal pH extremes in an experiment designed to test the physiological tolerances of *Limacina helicina antarctica*, an important member of the macrozooplankton in the Southern Ocean.

V. Additive effects of pCO_2 and temperature on respiration rates of the Antarctic pteropod *Limacina helicina antarctica*

Abstract

The Antarctic pteropod, *Limacina helicina antarctica*, is a dominant component of the zooplankton in the Southern Ocean and serves a key role in the short Antarctic food web, connecting key trophic levels in a harsh and seasonally variable climate. With a shell made from aragonite, a crystalline form of calicium carbonate ($CaCO_3$) with a relatively high solubility in seawater, *L.h. antarctica* has the potential to be adversely affected by future conditions that may hinder the growth of its structurally critical shell. This structure also play a key role as ballast, as these organisms are vertical migrators that depend on their ability to change buoyancy in order to feed on phytoplankton and defend themselves against predation. Finally, as *Limacina spp.* and other thecosome pteropods are cosmopolitan species that form a critical role in food chains globally, understanding their physiology under future change in hostile environments may shed light on the fate of pteropods globally.

Here, we collected Antarctic pteropods using plankton hand-tows at Cape Royds, Antarctica. These pteropods were maintained in a flow-through experimental system held at two temperatures (-0.8˚C, 4˚C), and three pCO_2 conditions that reflected current and future extremes. These small organisms were then individually subjected to respirometry trials as a proxy for their metabolic response to these conditions.

To date, this is the second study analyzing the metabolic rates of Antarctic pteropods in the Ross Sea, and the only one that studied juveniles in the Austral Spring. This is a critical season for *L. h. antarctica*, as they are coming out of winter starvation and soon facing a growth spurt brought on by a local algae bloom. This study is a snapshot of pteropod physiology in the context of this strongly coupled phenology and seasonality in a harsh environment.

Overview

In this chapter, I conducted respirometry trials at various temperature and pH acclimations to assess the fate of the Antarctic pteropod to near-future ocean change. This biological data set pairs with Chapter 4, and examines the impact of current and future extremes of pH on the respiration rate of these pteropods. There data were collected during the 2015-2016 Summer Field Season as part of research associated with a project in the U.S. Antarctic Program (NSF award to Dr. Gretchen Hofmann, NSF award PLR-1246202). In addition, the research presented in this Chapter was conducted in collaboration with my advisor, Dr. Gretchen Hofmann, and Ms. Juliet Wong, an EEMB Doctoral Candidate in the Hofmann Lab. This Chapter has also been published in *Conservation Physiology* (Hoshijima *et al.*, 2017).

Introduction

Organismal responses to singular abiotic stressors have long been utilized to investigate the role of physiological tolerance in shaping ecosystems and communities (Somero, 2002). However, assessing physiological conditions in response to multiple environmental stressors has recently become recognized as a critical step towards predicting how populations will persist in the future in response to anthropogenic environmental change (Philip W. Boyd *et al.*, 2015; Gunderson *et al.*, 2016; Todgham and Stillman, 2013). As climate change will involve complex physical factors changing together (Gunderson *et al.*, 2016), experiments investigating environmentally relevant

multistressor exposures are quickly becoming a critical component of global change biology.

In line with the process of global climate change (GCC), there is an emergent need to find species which can be used to assess ecosystem health. These organisms, often referred to as indicator species, can allow for a better understanding of global ocean conditions, especially in remote locations, as long as the species is well-studied in the context of these global stressors (Landres *et al.*, 1988). Certain animals may also act as valuable sentinel organisms, helping to indicate the first signs of GCC impacts on ecosystems(Bednaršek *et al.*, 2014; Johnson and Hofmann, 2017; Manno *et al.*, 2017). For instance, thecosome pteropods possess a very fragile shell composed primarily of aragonite (Comeau *et al.*, 2010; Orr *et al.*, 2005), a more soluble form of $CaCO_3$ that is particularly vulnerable to ocean acidification (OA) (Comeau *et al.*, 2010; Mucci, 1983). Previous studies have shown evidence of shell dissolution and malformations under future pCO_2 levels (Lischka *et al.*, 2010; Orr *et al.*, 2005), which may adversely impact the shell's protective capacity against predators or infection, or the shell's ballast ability, which aids in vertical migration (Comeau *et al.*, 2010). Through this lens, the overarching goal of this study was to examine the response of an important member of the zooplankton in the Southern Ocean, the pteropod *Limacina helicina antarctica*, to temperature changes and simulated OA.

Although it is well understood that GCC and OA are drastically increasing global sea surface temperatures and reducing the mean surface ocean pH levels at an accelerated rate (0.6-2.0°C increase and a 0.3 – 0.32 pH units decrease by the year

2100) (IPCC, 2013) , changes in ocean conditions are predicted to be particularly dramatic in polar seas (Steinacher *et al.*, 2009) with marine communities in the Arctic and Southern Ocean expected to experience significant changes in physiochemical conditions in the next decade (Hauri *et al.*, 2016). In particular, the Southern Ocean has already warmed at a rate almost double that of the global trend in the upper 1000m of ocean waters (0.17˚C in the Southern Ocean between the 1950's and the 1980's) (Fyfe, 2006; Gille, 2002; Levitus *et al.*, 2000). Additionally, the surface waters of the Southern Ocean are inherently susceptible to OA due to the high solubility of gases in cold waters, with surface ocean undersaturation of aragonite ($\Omega_{arag}<1$) expected to develop by the year 2030 (Hauri *et al.*, 2016; McNeil and Matear, 2008). Considering these observed trends, understanding how Antarctic marine organisms respond to the combined impacts of warming and acidification is of paramount importance for predicting how these factors will impact the unique ecosystem that persists in the Southern Ocean (P. W. Boyd *et al.*, 2015).

In the context of future ocean change, organisms that inhabit the Southern Ocean may be limited in their ability to adapt or migrate. Polar ectotherms often possess slow growth rates and long generation times, which generally result in a lower capacity for adaptation (Peck, 2005; Pörtner *et al.*, 2007). Furthermore, unlike some temperate and tropical species that have been observed to migrate poleward with increasing oceanic temperatures (Poloczanska *et al.*, 2013; Sorte *et al.*, 2010; Sunday *et al.*, 2012), polar species are already restricted to high latitudes and will more likely undergo range contractions with continuing GCC (Whiteley, 2011). In addition to

being stenothermal, possessing a narrow range of thermal tolerance from adapting under very stable conditions for millions of years (Peck, 2005; Somero, 2012; Sorte *et al.*, 2010), these organisms may potentially be living at or near their thermal tolerance maxima (Whiteley, 2011).

In contrast, seasonal pH variation in the Ross Sea (Kapsenberg *et al.*, 2015) indicates that Antarctic species may have more physiological tolerance and potential for adaptation to OA stress than expected. Indeed, larvae of the sea urchin, *Sterechinus neumayeri*, were found to be remarkably tolerant to OA and warming (Kapsenberg and Hofmann, 2014), although other studies have shown sensitivity of calcification processes in early stages (Byrne *et al.*, 2013). Stress responses, however, can vary greatly by species (Breitburg *et al.*, 2015; Byrne and Przeslawski, 2013; Przeslawski *et al.*, 2015; Stillman and Armstrong, 2015). Additionally, although variability in pH has been characterized across a variety of spatiotemporal scales (Hofmann *et al.*, 2011), the high seasonality of pH in Antarctic surface waters indicates the potential for spawning phenology of polar organisms to be a critical factor in assessing the exposure of early life stages to near-future OA (Kapsenberg *et al.*, 2015).

In the Southern Ocean, *L. h. antarctica* is a dominant member of the zooplankton (Hunt *et al.*, 2008). Thecosome pteropods are a key study organism in polar regions, holding an essential role in food web dynamics and energy transfer (Bednaršek *et al.*, 2012b; Hunt *et al.*, 2008; Willette *et al.*, 2001) by acting as major consumers of phytoplankton (Bernard and Froneman, 2009; Perissinotto, 1991) while serving as prey for a variety of organisms, including gymnosome pteropods,

euphausids, salps, and a multitude of pelagic and demersal fish species (Hunt *et al.*, 2008; Lancraft *et al.*, 1991). As such, *L. h. antarctica* is a key player in the simple, yet fragile Antarctic food web and directly impacts the food sources of higher trophic levels, including predatory birds and mammals such as penguins, whales, and seals (Davis *et al.*, 1999; McClintock *et al.*, 2008; Seibel and Dierssen, 2003). Thecosome pteropods also play a sizeable role in geochemical cycling of both organic and inorganic carbon, producing fecal pellets and calcium carbonate ($CaCO_3$) shells that sink to deeper waters (Berner and Honjo, 1981; Collier *et al.*, 2000; Fabry, 1990; Gilmer and Harbison, 1991; Manno *et al.*, 2009; Tsurumi *et al.*, 2005). Manno *et al.* (2009) estimated that *L. h. antarctica* could contribute as much as 72% of the total organic carbon export in the Ross Sea, and Honjo (2004) found that south of the Polar Front, pteropods were the main contributor to an inorganic $CaCO_3$ flux of 110 mmol C $m^{-2}yr^{-1}$. Overall, *L. h. antarctica* holds a key role in Southern Ocean ecosystems, but unfortunately, changing ocean temperatures and pH levels may adversely impact this species. Due to this considerable abundance and ecological importance of *L. h. antarctica* in the Southern Ocean, as well as its potential vulnerabilities to elevated temperatures and pCO_2 levels, it is critical to investigate how this species copes with multiple abiotic stressors and consequently, the capacity of these populations to withstand dynamic environmental change.

To complicate matters, the interaction effect between pCO_2 and temperature stress can vary drastically, and can have key physiological consequences (Todgham and Stillman, 2013). An additive interactive effect may occur if each individual stressor

does not interact with one another, or if one or more of the stressors has no significant effect – as pCO_2 and temperature have been observed in certain mollusk species (Talmage and Gobler, 2011). However, other organisms, such as collector urchin larvae, has been shown to respond to these same two stressors antagonistically when the negative effects of one stressor are offset by the other (Sheppard Brennand *et al.*, 2010). Lastly, the two stressors may exacerbate one another, resulting in a synergistic interaction effect between stressors as has been observed in the Arctic pteropod (Lischka and Riebesell, 2012). Understanding the interaction of environmentally co-occurring stressors in sentinel species, especially in areas the world's oceans potentially most vulnerable to global change, is thus a key research priority in global change biology.

Here, we examined the nature of two key 98eteroscedast, pCO_2 and temperature, on the respiration rates of the Antarctic pteropod *L. h. antarctica*. Interrogating metabolic rate as a proxy for organismal energy dynamics is a useful strategy in global change studies and can provide valuable insight into its physiological state (Sokolova, 2013). Hypothetically, under OA stress alone, pteropods may increase their metabolism to support shell repair, or to maintain calcification under elevated pCO_2 conditions. In addition to shell effects, elevated pCO_2 levels can also alter physiology by interacting with internal and external body fluids, causing acid-base imbalances and reduced capacity for oxygen transport (Fabry *et al.*, 2008; Fangue *et al.*, 2010; Melzner *et al.*, 2009). Metabolic suppression has been identified as an adaptive mechanism by which some organisms cope with hypercapnia, but

prolonged metabolic suppression will have consequences on growth and survival (Dymowska *et al.*, 2012; Fabry *et al.*, 2008). In contrast, some species appear to respond to OA by increasing their metabolic rates, although this comes at an energetic cost (Stumpp *et al.*, 2011; Wood *et al.*, 2008). Increased temperatures will also increase metabolic rates due to enzyme kinetics based on the Boltzmann-Arrhenius model (Dell *et al.*, 2011), which may interact with the consequences of a high pCO_2 environment (Gunderson *et al.*, 2016).

In nature, individuals of *L. h. antarctica* may modify their metabolic rate as a result of seasonal and developmental changes. *L. h. antarctica* have a lifespan of one to three or more years (Bednaršek *et al.*, 2012a; Hunt *et al.*, 2008) in which juveniles overwinter before maturing into adults in the early summer (Gannefors *et al.*, 2005; Hunt *et al.*, 2008). Overwintering juveniles may be especially susceptible to elevated pCO_2 levels and temperatures because, during the wintertime when algal food supplies are limited, polar organisms are known to lower their metabolism as a means of conserving energy (Hagen and Auel, 2001; Hirche, 1996), possibly decreasing their resistance to abiotic stressors (Lischka *et al.*, 2010). Maas *et al.* (2011) demonstrated that *L. h. antarctica* lowered its metabolic rate, measured by a decrease in oxygen consumption, under conditions of reduced food availability. Over the winter, the Arctic pteropod subspecies, *L. helicina helicina*, temporarily stops growing, perhaps lowering its activity and calcification processes while relying on internal lipid stores (Lischka and Riebesell, 2012). Metabolic suppression may decrease an organism's ability to cope with OA and/or warming, or else metabolism must be increased in

order to respond, effectively forcing the use of energy reserves at a time when conserving energy is a necessity (Lischka and Riebesell, 2012). Thus, research on juvenile *L. h. antarctica* provides valuable information on what is potentially its most vulnerable life history stage (Manno *et al.*, 2017).

Here, we examined the response of juvenile Antarctic pteropods, *L. h. antarctica*, to abiotic stressors related to both temperature and pH by measuring their metabolic rate under laboratory treatments reflective of current and future Southern Ocean abiotic conditions. We also describe an experimental setup designed to maintain pteropods under controlled pCO_2 and temperature conditions. Using a microplate respirometry system, oxygen consumption measurements were recorded during two-week exposures to a combination of three pCO_2 levels and two temperatures chosen to reflect current and predicted future ocean conditions during both winter and summer seasons in this region. Additionally, oxygen consumption rates were measured along a range of elevated temperatures to determine the critical thermal maxima (CT_{max}). Due to their reliance on their relatively vulnerable aragonitic shell, our goal was to examine the impacts of differential pCO_2 and temperature conditions on juvenile *L. h. antarctica* by assessing degrees of metabolic change.

Materials and Methods

Field collection of pteropods

Pteropods were collected ~1 km from shore near McMurdo Station, Antarctica at a research hut site on first-year fast ice (77°50'54" S, 166°35'55" E) with a maximum water depth of 170 m. Collections were made using a fixed-frame bongo net (two 50 cm diameter x 150 cm length nets with 333 µm mesh and cod ends with 200 and 333 µm mesh) deployed to a depth of 50 m through a 1.3 m diameter hole drilled in the sea ice. Floats attached to each cod end minimized pinching of the net, while a swiveling double shackle allowed the net to rotate and tilt along with the water currents. The duration of each bongo net deployment was approximately 24 hours. Collections were performed throughout the 2015 austral spring and summer, and selected tows supported three experiments described in this study: (1) an experiment run at ambient temperature, (2) an experiment run at an elevated temperature, +4°C, and (3) an experiment to determine the thermal tolerance of *L. h. antarctica* (referred to as E1, E2, and E3, respectively). E1 pteropods were collected on the 21st and 22nd of October, E2 pteropods on the 11th of November, and E3 pteropods on the 30th of November. Following collection, pteropods were immediately transported to the Crary Lab aquarium facilities at McMurdo Station, where they were held in 500 mL polycarbonate Nalgene containers filled with seawater at ~ -0.8°C for no longer than 1 day until the start of each experiment.

Experimental design

For CO_2 exposure experiments (E1 and E2), pteropods were held in a flow-through zooplankton culturing system with controlled levels of pCO_2 while maintaining sufficient flow for long-term experiments. The reservoir mixing system that generated the target experimental pCO_2 levels was constructed following Fangue *et al.* (2010). Briefly, filtered, CO_2-scrubbed (Sodasorb, Smith's Medical, St. Paul, MN, USA), dried (W.A. Hammond Drierite Co, Stock no. 23001, Xenia, OH, USA) air was mixed with pure CO_2 using SmartTrak™ 100L Series Mass Flow Controllers and MicroTrak™ 101 Series Mass Flow Controllers (Sierra Instruments, Monterey, CA, USA), respectively. These reservoirs were held in a sea table that kept the treated seawater at the desired experimental temperature (~ -0.8°C for E1 and ~ 4°C for E2) to represent the current average temperature and an elevated temperature based on the Representative Concentration Pathway (RCP) 8.5, the scenario that represents the highest greenhouse gas emissions (IPCC, 2013; Moss *et al.*, 2010; Riahi *et al.*, 2011). The pCO_2 treated seawater was then pumped into the zooplankton flow-through culturing system.

Acidification treatments were chosen to represent the range of current monthly means of pH in McMurdo Sound (measured 2 km away from the pteropod collection site), as well as modeled end-of-century pH based on an equilibrium model (Kapsenberg *et al.*, 2015). Target pH values for E1 were 8.2 (a low pCO_2 treatment of ~264 µatm, representing the average pH of the current highest-pH month), 7.95 (a mid pCO_2 treatment of ~500 µatm, representing both the average pH of the current

lowest-pH month, as well as the average pH of the future highest-pH month), and 7.7 (a high pCO_2 treatment of ~920 µatm, representing the average pH of the future lowest-pH month) (Table 1). Target pH values for E2 were equivalent to those of E1 for the mid and high pCO_2 treatments; however, the low treatment was set at 8.11 pH units (~337 µatm) because the 8.2 pH value from E1 at 4°C resulted in an environmentally improbable pCO_2 value (~265 µatm).

The zooplankton culture system used for E1 and E2 consisted of 3 culture vessels per treatment (9 culture vessels total) submerged in seawater held at the experimental temperature (either -0.8°C or 4°C). Culture vessels were created by modifying clear 1-liter polycarbonate plastic tanks, accompanying lids, and 400 µm mesh baffles that are often used in zebrafish research (Model AHLT3, Pentair Aquatic Ecosystems, Cary, NC, USA). To ensure that the animals were exposed to a continuous flow of pCO_2 treated seawater, water input was regulated using irrigation button drippers (W221B, DIG Irrigation Products, Vista, CA, USA), at a flow rate of 2 liters per hour. The volume of one vessel was replaced on a 20-minute basis. As a result, the pCO_2 levels in the replicate culture vessels were highly similar within a treatment and tracked the reservoir pCO_2 concentrations closely (see Results, Figure 20). For each experiment, approximately 400 pteropods were randomly selected and added to each of the culture vessels. Only actively swimming individuals were taken for experiments. Individuals were not fed during these experiments.

Table 1. Average seawater chemistry conditions during the 2-week experiments of E1 (-0.8˚C) and E2 (4˚C). Values are expressed at mean ± s.d. The rationale for pH conditions is based on environmental data and local modeling based on Kapsenberg *et al.* (2015)

Treatment	*p*CO2 (µatm)	pH	Ω_{arag}	Rationale for pH conditions
E1 (ambient temperature)				
low	315 ± 14	8.13 ± 0.02	1.71 ± 0.06	Present-Day Summer
mid	427 ± 11	8.00 ± 0.01	1.33 ± 0.02	Present-Day Winter / Future Summer
high	901 ± 34	7.71 ± 0.01	0.70 ± 0.02	Future Winter
E2 (elevated temperature)				
low	379 ± 16	8.07 ± 0.02	1.77 ± 0.06	Present-Day Summer
mid	513 ± 10	7.95 ± 0.01	1.39 ± 0.02	Future Summer
high	961 ± 14	7.69 ± 0.06	0.81 ± 0.01	Future Winter

Carbonate chemistry analysis

Seawater chemistry was analyzed for the culturing tanks as previously described (Johnson *et al.*, 2016) . Water samples were fixed with 0.02% mercuric chloride and stored at 4°C until analyzed (Dickson *et al.*, 2007). Total alkalinity measurements were performed using an open-cell titration method (Mettler Toledo T50, Columbus, OH, USA) and pH readings were conducted using a *m*-Cresol spectrophotometric assay (Clayton and Byrne, 1993) conducted on a Shimadzu spectrometer (Model UV2501PC, Kyoto, Japan). Carbonate chemistry parameters were calculated using CO2calc (Robbins *et al.*, 2010) using constants from (Lueker *et al.*, 2000).

Sampling

For E1 and E2, respirometry measurements were made at the start of each experiment, and following mesocosm exposures of 24 hrs (T_1), 48 hrs (T_2), 4 days (T_4), 7 days (T_7) and 14 days (T_{14}). At each timepoint, 18 individuals were randomly selected from culture vessels of each treatment and pooled for respirometry measurements. Individuals were not returned to the culturing tanks after respirometry was conducted, and thus each respirometry trial was conducted with different remaining individuals.

Respirometry

Oxygen consumption rates were measured using a microplate system (Model SY1000, Loligo Systems, Viborg, Denmark). Each respirometry plate contained 24 closed-cell glass chambers with a 200 µL capacity, with a PreSens oxygen spot (PreSens, Regensburg, Germany) at the base of each chamber. Each plate was calibrated at two oxygen concentrations: 100% (aerated seawater) and 0% (1% Na_2SO_3 in milli-Q) at 2˚C. All measured oxygen values were temperature-corrected to this calibration. At each timepoint, each plate was submerged in seawater (pCO_2-treated water for E1 and E2, ambient seawater for E3), and a plastic transfer pipet was used to remove any visible air bubbles from the plate chambers. A single, visibly swimming pteropod was carefully added to a chamber using a transfer pipet, and the chamber was quickly sealed with a screw-on plastic cap lined with a PTFE seal. Care was taken to eliminate air bubbles from the plastic caps during the capping process. Six chambers per plate, spread across multiple rows and columns, were filled with only treatment seawater and were used as blanks to account for any background respiration due to microbial activity. In E1 and E2, the three plates were randomly assigned to a treatment in each timepoint to avoid any potential differences between plate calibrations (i.e., plate effects). Once every respirometry chamber was filled and sealed, each plate was then submerged in a flow-through acrylic water bath (Model CH10505, Loligo Systems) connected to a larger, temperature-controlled tank via a pump (Model MD7, Danner Manufacturing Inc, Islandia, NY, USA) that was held at the desired experiment temperature with a heat pump (IsoTemp 730, Fisher,

Waltham, MA, USA). Although the temperatures were held as accurately as possible, the heat in the laboratory limited the lowest temperature of the respirometry system to -0.6˚C for E1. Thus, for E1, respirometry was conducted 0.2˚C higher than the experimental exposure temperature.

Each water bath was nested above an array of 24 optodes corresponding to the oxygen-sensitive spots in the respirometry chambers, which recorded optode phase values for each chamber every minute for approximately 6 hours, or until oxygen concentrations fell below 60% saturation. Optode phase values were converted into oxygen concentrations using spreadsheets provided by PreSens. Following the oxygen measurements, pteropods were carefully removed and stored at -1˚C until they were weighed. All individuals were visually inspected for mortality after the respirometry measurements were complete. Wet weight values were obtained by gently blotting individuals dry on a KimWipe (Sigma-Aldrich, St Louis, MO, USA) before transferring them to a microbalance (Cahn C-31; Thermo-Fisher, Waltham, MA, USA). Pteropods were then placed in a freeze dryer overnight (LabConco FreeZone, Kansas City, MO, USA) and reweighed to obtain dry weight values.

Thermal tolerance

For E3, pteropods were kept at -0.8˚C until the start of each respirometry run. Oxygen measurements were collected using the microplate respirometry system (described above) at several different temperatures: -0.8˚C (approximate ambient temperature), 2˚C, 4˚C, 6˚C, 8˚C, 10˚C, 12˚C, and 14˚C. For each temperature, one

plate was filled with pteropods (n = 18) and run for 6 hours, or until oxygen concentrations fell below 60% saturation. Pteropods were then visually assessed for mortality and wet and dry weights were obtained (similar methods to E1 and E2).

Data analysis

All statistical analyses were run in R (v.3.2.4). For each chamber, the rate of oxygen consumption over time was quantified by linear regression. For each microplate, the oxygen consumption rate of the six blank chambers were averaged and subtracted from the oxygen consumption rate of the sample chambers to account for any microbial respiration. There was a significantly different scaling coefficient between timepoints (ANCOVA, $p = 0.003$), but not between treatments (ANCOVA, $p = 0.321$), likely due to the change in dry weight over time. Thus, different scaling coefficients were derived for each timepoint in each experiment (E1 and E2), and used to correct pteropod respiration rates separately for each respective timepoint and treatment. Pteropod respiration rates were mass-corrected using the mean mass in each experiment (145.76 mg in E1, 142.21 mg in E2) using mass-correction equations from (Steffensen *et al.*, 1994). As the mass of organisms remained constant over E3, they were mass-corrected using a common scaling coefficient ($\alpha = 0.585$), to a mean common mass (152.78 mg).

Oxygen consumption values were compared between treatments using two-way ANOVAs after testing for significance against a mixed-effect model (R package lmerTest v.2.0-32) which included individual glass chambers as a random effect to

account for any sensor variation between each oxygen-sensitive spot. Post-hoc Tukey HSD tests were conducted to resolve pairwise differences between treatments and timepoints.

Q_{10} values were also calculated to compare metabolic rates (R_1, R_2) measured at different temperatures (T_1, T_2) as:

$$Q_{10} = (R_2/R_1)^{10^\circ/(T2-T1)}$$

Results

At a survivorship level, time in the culturing system tanks did not affect the juvenile pteropods during the experiment. We observed a very low percentage of mortality throughout the duration of E1. During E2 <1% total mortality was observed during the first 8 days of the experiment, after which there was a ~4.4% daily mortality rate observed for all treatments until the termination of the experiment (Day 14). This E2 mortality rate was not significantly different between treatments (Cox proportional hazards regression model, $p = 0.57$).

Throughout the exposures of E1 and E2, carbonate chemistry remained highly stable (Figure 20, Table 1). Seawater temperature also remained stable, with an average temperature of -0.97 ± 0.07˚C during E1 and 3.97 ± 0.08˚C during E2 (mean ± s.d.). Salinity was also maintained at stable levels: 35.9‰ for E1 and 35.5‰ for E2.

Oxygen consumption (E1 and E2)

Oxygen depletion in the respirometry chambers occurred at very consistent rates, with samples being eliminated from the analysis *a priori* only if they exhibited

an $r^2 < 0.8$ when plotting oxygen consumption over time. Here, only 7 out of 611, or 1.1%, combined for E1 and E2 were eliminated from the analysis. As expected from the results of elevated temperature, average respiration rates for E2 were higher than those of E1 (Q_{10}, averaging across all treatments and timepoints = 1.7; Q_{10} values will be further explored in E3 results).

Oxygen consumption was mass-corrected using different scaling coefficients for each timepoint and experiment (Figure 21). These scaling coefficients decreased significantly over time in E2 (-0.04 day $^{-1}$, p = 0.031), but did not show a significant directional trend over time in E1 (p = 0.417). All further respirometry results were conducted on mass-corrected values where these coefficients were used to correct individuals from each timepoint to that experiment's mean body mass (145.76 mg for E1, 142.21 mg for E2). Analyses replicated using a common mean scaling coefficient for each experiment yielded similarly significant end results.

In E1, timepoint ($F(4, 250) = 4.577$, p = 0.001), treatment ($F(2, 250) = 7.863$, p <0.001) and their interaction effect ($F(8, 250) = 2.413$, p = 0.016) were statistically significant. For the effect of treatment, a post-hoc Tukey test concluded that respiration rates in the high pCO_2 treatment were significantly higher than the other two treatments. Respiration rates generally increased over time (Figure 21). The interaction term likely refers to the emerging effect of treatment over time (Table 2).

Similarly, in E2, timepoint ($F(4, 251) = 4.636$, p = 0.001), treatment ($F(2, 251) = 4.782$, p = 0.009), and their interaction effect ($F(8, 251) = 4.059$, p < 0.001) were significant. For the effect of treatment, a post-hoc Tukey test concluded that the

Figure 20. Seawater chemistry conditions throughout the 2-week experiments of E1 (left, -0.8℃) and E2 (right, 4℃).

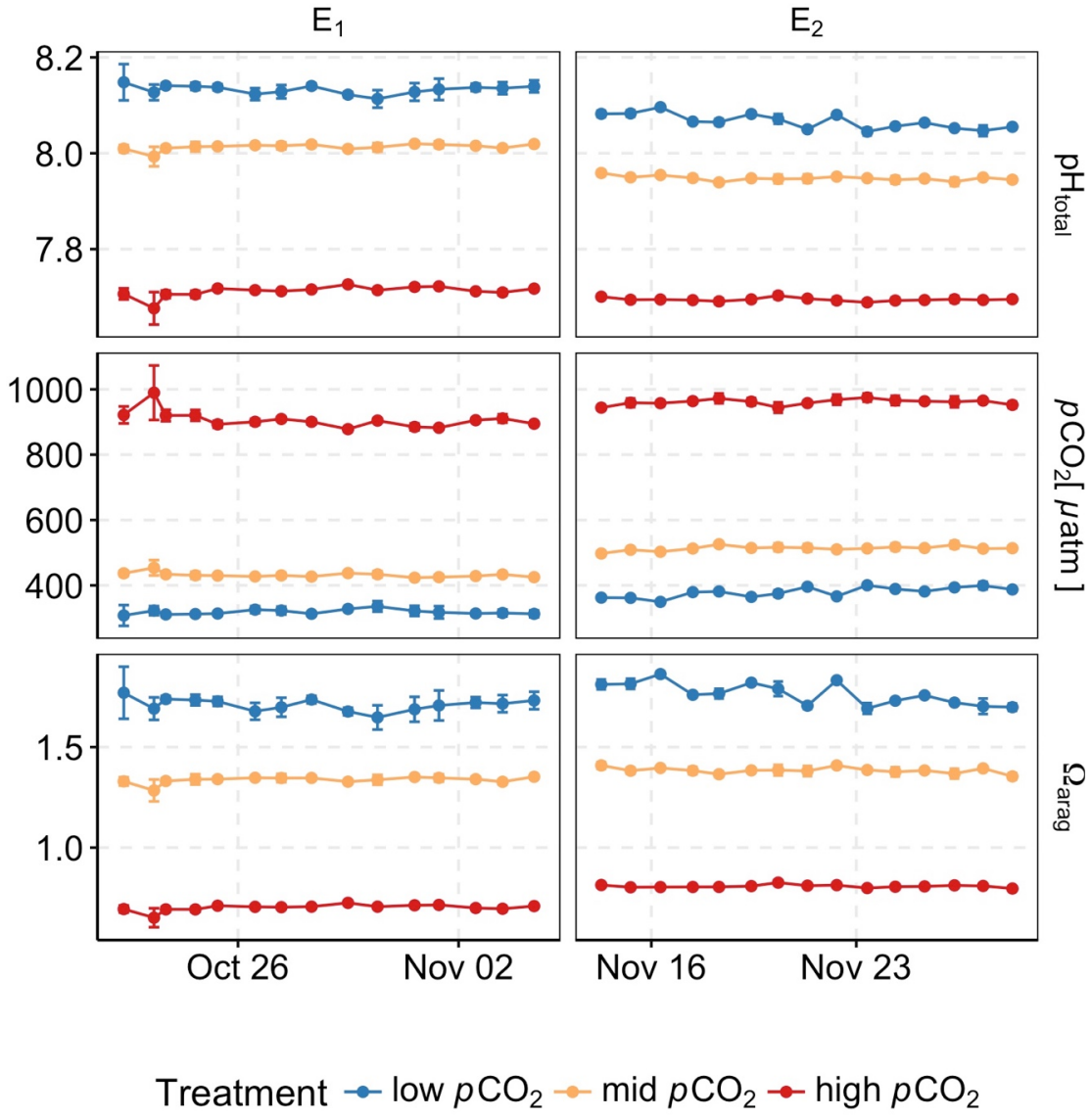


Figure 21. Oxygen consumption over time for each pCO_2 treatment (low, mid, high), and temperature (E1, acclimated to -0.8˚C and measured at -0.6˚C; E2, 4˚C).

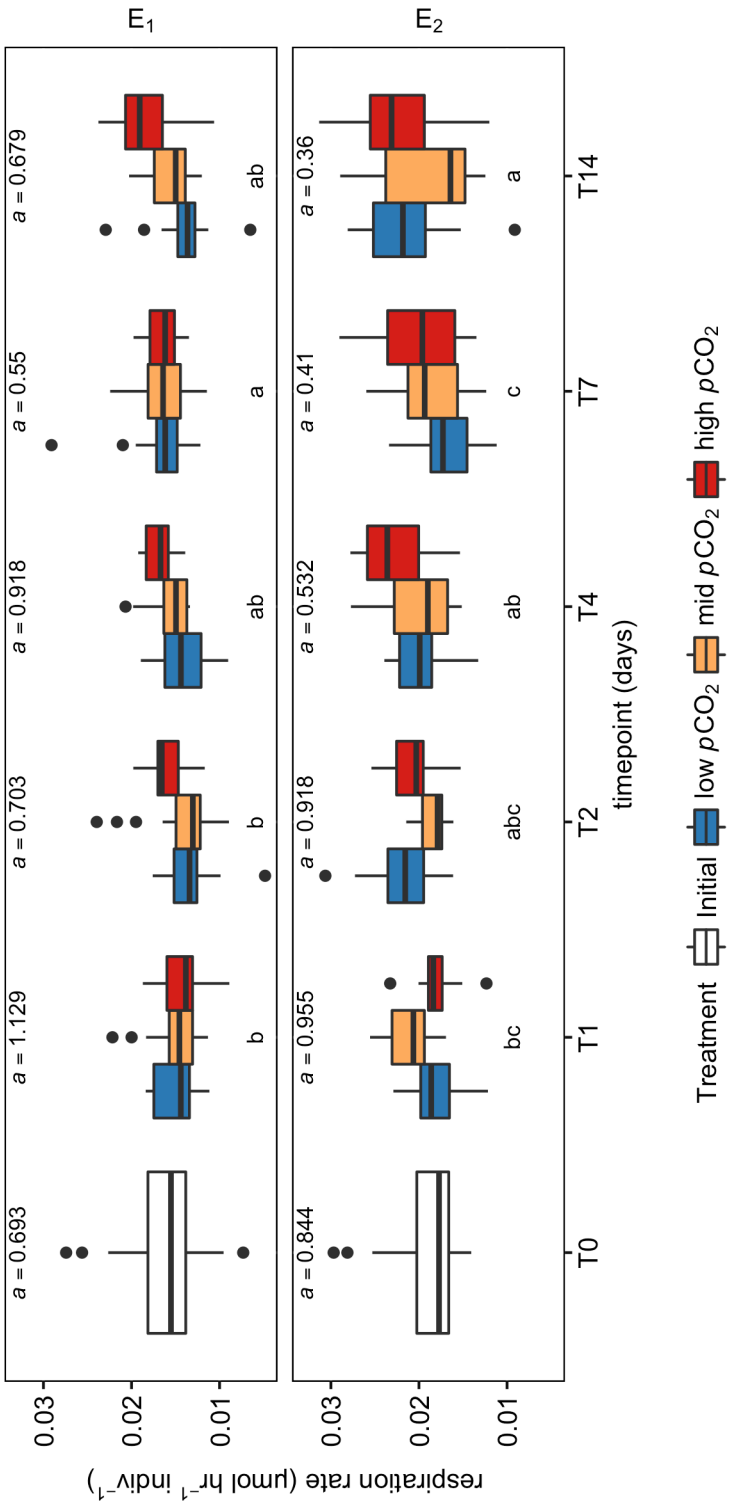


Table 2. Post-hoc Tukey groupings of treatments and timepoints for E1 and E2.

Factor	mean respiration rate (μmol hr^{-1} $indiv^{-1}$)	Tukey grouping
E1 - timepoint		
T7	0.0168	a
T14	0.0159	ab
T4	0.0159	ab
T1	0.0150	b
T2	0.0146	b
E1 - treatment		
high pCO_2	0.0166	a
mid pCO_2	0.0155	b
low pCO_2	0.0149	b
E1 - timepoint : treatment		
high pCO_2:T14	0.0183	a
high pCO_2:T4	0.0173	ab
low pCO_2:T7	0.0171	ab
high pCO_2:T7	0.0167	abc
mid pCO_2:T7	0.0166	abc
high pCO2:T2	0.0161	abc
mid pCO2:T4	0.0159	abc
mid pCO2:T14	0.0156	abc
low pCO2:T1	0.015	abc
mid pCO2:T1	0.0150	abc
mid pCO2:T1	0.0150	abc
high pCO2:T1	0.0148	bc
low pCO2:T4	0.0145	bc
mid pCO2:T2	0.0144	bc
low pCO2:T14	0.0141	bc
low pCO2:T2	0.0135	c

E2 - timepoint		
T14	0.0222	a
T4	0.0210	ab
T2	0.0202	ab
T1	0.0196	b
T7	0.0193	b
E2 - treatment		
high pCO_2	0.0217	a
low pCO_2	0.0200	b
mid pCO_2	0.0196	b
E2 – timepoint : treatment		
high pCO_2:T14	0.0244	a
high pCO_2:T4	0.0234	ab
low pCO_2:T14	0.0227	abc
low pCO_2:T2	0.0219	abc
high pCO_2:T7	0.0213	abcd
mid pCO_2:T1	0.0213	abcd
high pCO_2:T2	0.0205	abcd
low pCO_2:T4	0.0198	bcd
mid pCO_2:T4	0.0197	bcd
mid pCO_2:T14	0.0196	bcd
mid pCO_2:T7	0.0194	bcd
high pCO_2:T1	0.0190	bcd
low pCO_2:T1	0.0185	cd
mid pCO_2:T2	0.0182	cd
low pCO_2:T7	0.0171	d

respiration rates in the high pCO_2 treatment were significantly higher than both that of the mid and low pCO_2 treatment. The pattern of increasing respiration rates over time observed in E1 were not as apparent as in E2 (Figure 21), and appeared to become more heterogeneous between timepoints over time. Similarly, the interaction term likely refers to the emerging effect of treatment over time (Table 2).

Oxygen consumption during acute high temperature exposures (E3)

Mortality was not observed during the six-hour acute temperature exposures and respirometry measurements conducted in E3. Across the observed temperature range (-0.8˚C to 14˚C), oxygen consumption increased predictably with temperature (Figure 22) and was heterogeneous between tested temperatures ($F(7, 135) = 35.47$, $p < 0.001$). Although the mean respiration rate measured at 14˚C was slightly lower than the mean respiration rate measured at 12˚C, this difference was not significant (Tukey's HSD, $p_{adj} = 0.779$), and thus there was no discernible critical temperature threshold. Similarly, a segmented linear regression conducted on an Arrhenius plot of the data (inverse of temperature (Kelvin) plotted against $\log_{10}$(respiration rate)) could not discern a breakpoint (R package "segmented", v. 0.5-1.4). The Q_{10} value calculated over the temperature extremes (-0.8 to 14˚C) was 3.04 (Table 3).

Weight

Generally, dry weights of pteropods agreed well with their respective wet weights for E1 and E2 (Figure 23)

, $r^2 = 0.67$). In E1, although there was no significant change in pteropod wet weight, dry weight of pteropods was heterogenous over time (Figure 24) ($F(5, 287) = 9.254$, $p < 0.001$).

In E2, although there was a small significant effect of treatment on dry weight ($F(2,284) = 3.49$, $p = 0.03$), there was a more significant effect of treatment on wet weight ($F(2,284) = 13.488$, $p < 0.001$). For both dry and wet weight in E2, pteropods from the high pCO_2 treatment had a lower average mass than the other treatments. At the same time,

the interaction term between treatment and timepoint was only significant for wet weight ($F(8,284) = 2.69$, $p = 0.007$), which fluctuated to a further degree during the experiment.

Mean dry and wet weights of pteropods in E3 were 0.152 mg and 0.452 mg, respectively, and there were no significant changes to either of these factors between temperature runs in E3 (ANOVAs, $p = 0.39$ and $p = 0.46$, respectively). The entirety of E3 used the same cohort of pteropods and all temperature exposures were completed

within three days of collection from the field.

Table 3. Calculated Q_{10} values determined for several ranges of temperatures.

Temp	-0.8°C ~ 2°C	-0.8°C ~ 4°C	-0.8°C ~ 6°C	-0.8°C ~ 8°C	-0.8°C ~ 10°C	-0.8°C ~ 12°C	-0.8°C ~ 14°C
Q_{10}	2.5	3.5	2.0	3.3	3.1	3.9	3.0

Figure 22. Respiration rate as a function of temperature (E3), plotted as an Arrehnius plot (inverse Kelvin temperature on the x-axis, log of respiration rate on the y-axis) with error bars indicating standard error. Respiration rate is expressed as nM hr^{-1} $indiv^{-1}$, and is mass-corrected to the average mass of the pteropods in E3.

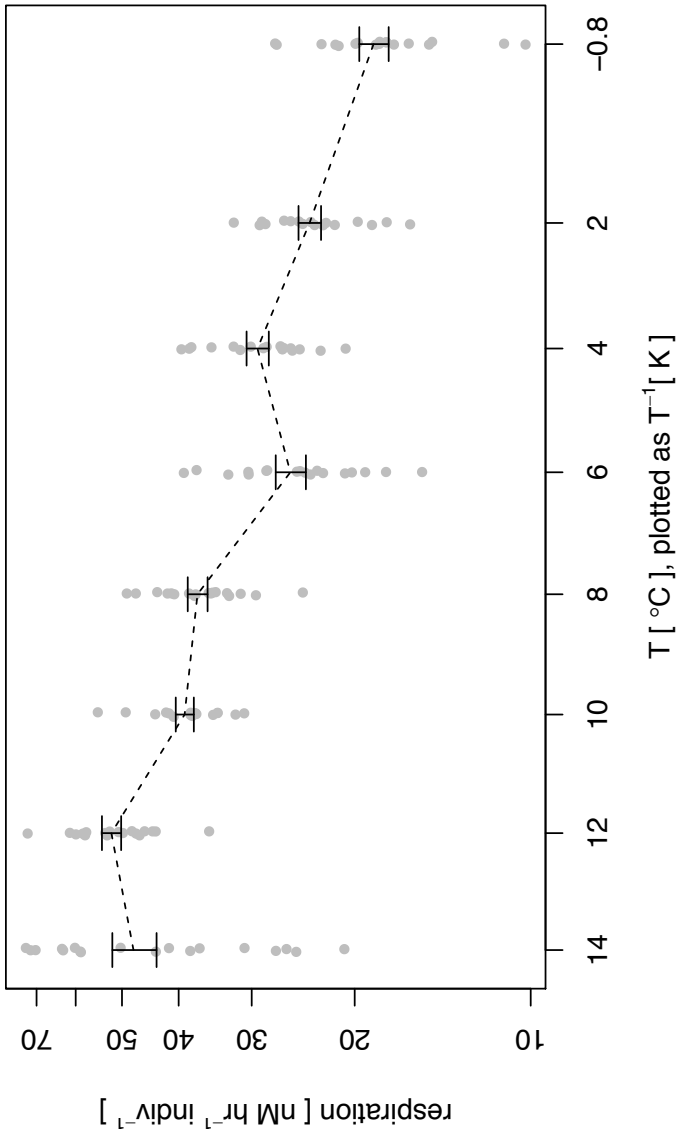


Figure 23. Correlation between pteropod wet weight and dry weight (each expressed in milligrams), with histograms along each axis. This correlation is linear ($r^2 = 0.69$, $p < 0.001$).

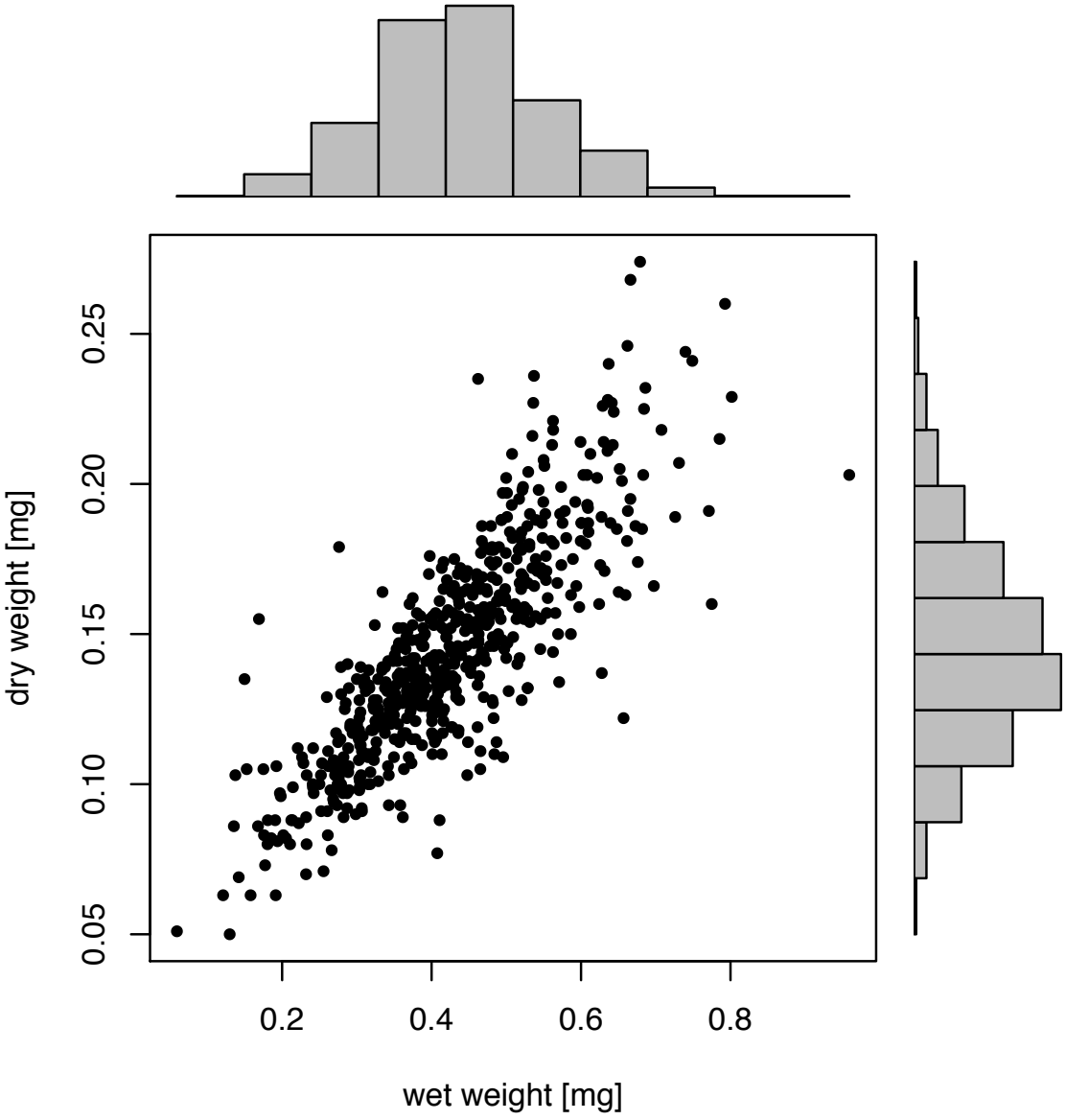
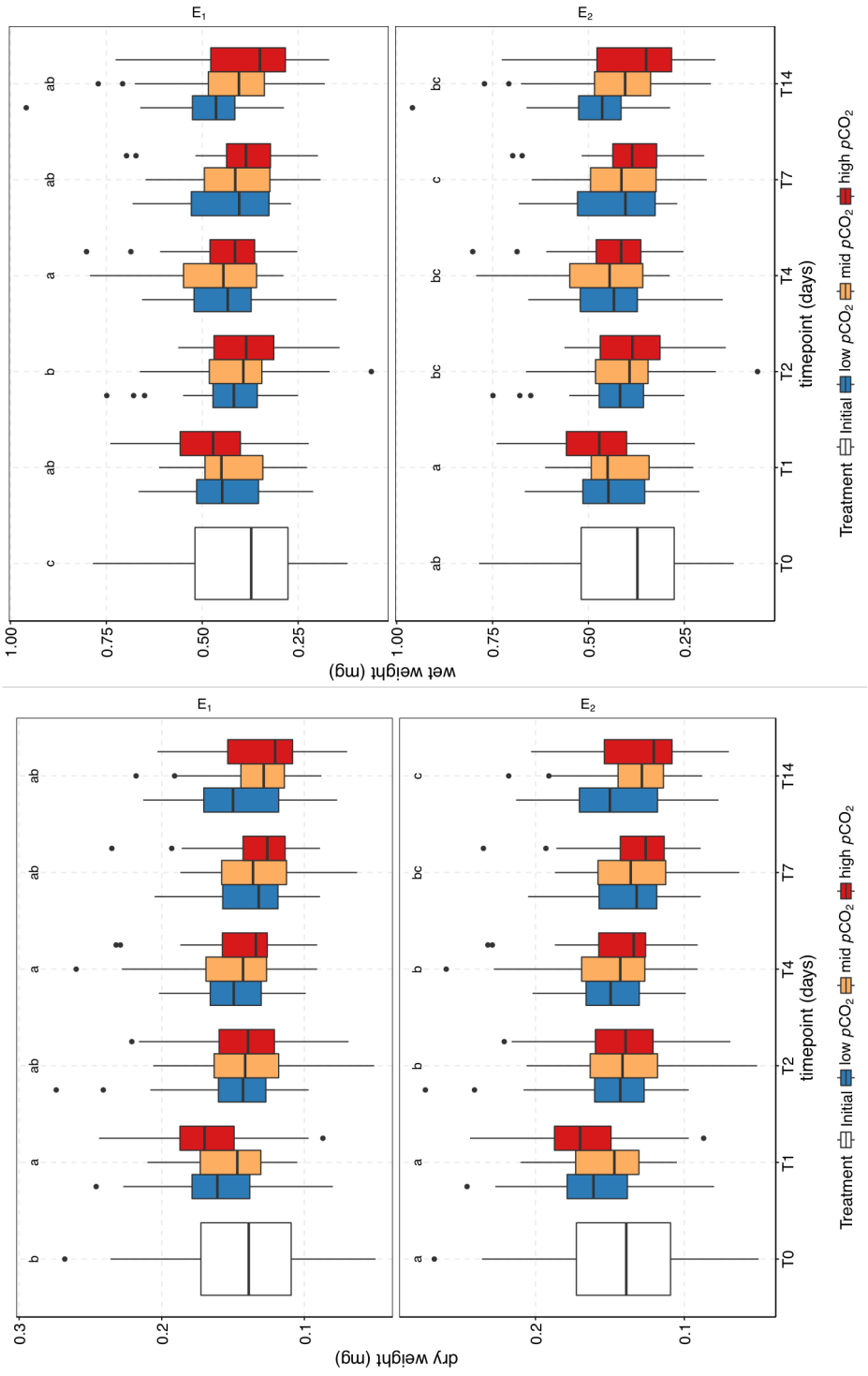


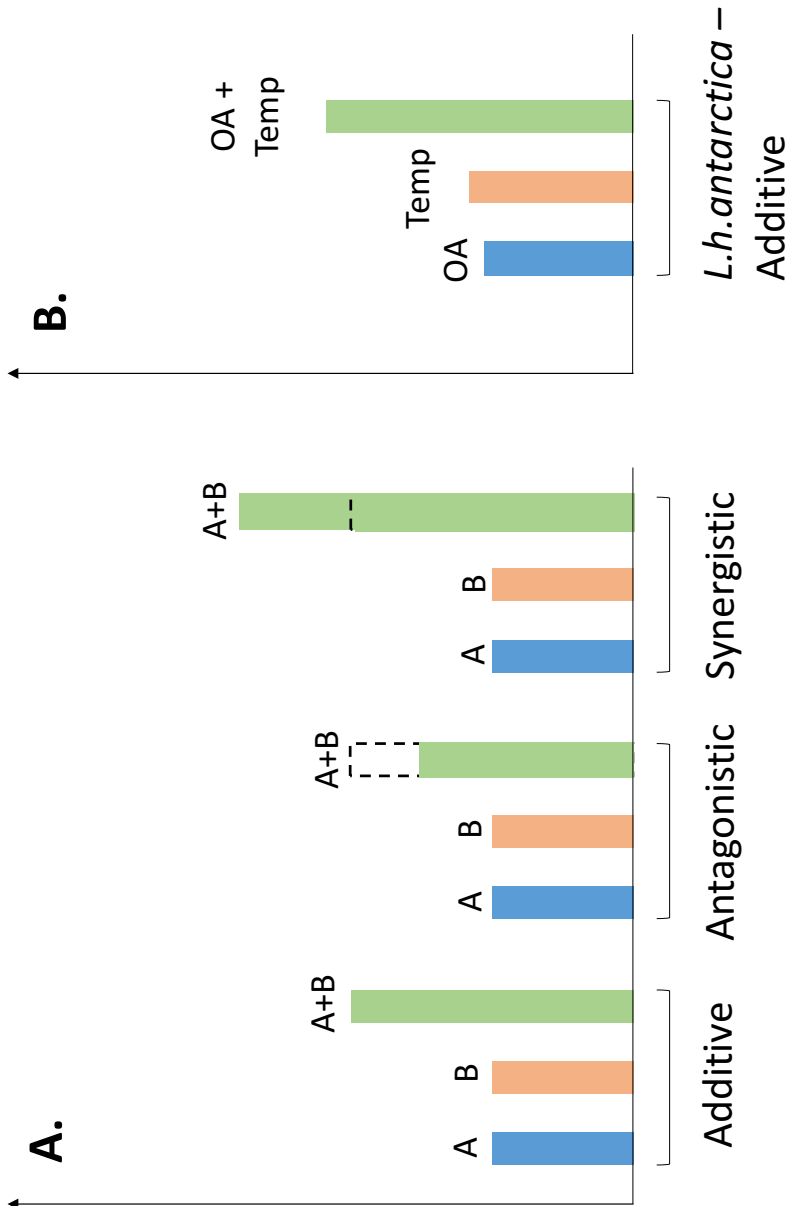
Figure 24. Pteropod wet and dry weights (mg) over time, for each pCO_2 treatment (low, mid, high), and temperature (E1, -0.8℃; E2, 4℃). Letters represent post-hoc Tukey test groupings ($p < 0.05$) conducted separately for each weight type and experiment.



Discussion

Multiple stressors can have complex interacting effects on physiological processes in ectothermic animals (Gunderson *et al.*, 2016). Characterizing these organismal responses is critical to estimating the potential vulnerability of species to global change (Boyd, 2011; Gunderson *et al.*, 2016; Todgham and Stillman, 2013). In this study, we examined the metabolic rate of juvenile-stage pteropods in response to the interacting effects of pCO_2 and temperature. We found that metabolic rate was increased by elevated pCO_2 at both experimental temperatures. Our results show that the interaction of temperature and pCO_2 elicited an additive response in juvenile pteropods (Figure 25).

Figure 25. A conceptual diagram of multistressor interactions (A) in comparison with multistressor results for *L. h. antarctica*'s metabolic response to simulated OA and temperature stress (B). Dotted bars indicate the additive response. Antagonistic responses are lower than the additive response, while synergistic responses are higher than the additive response. An additive response could also be indicated by a lack of a significant interaction term between the effect of the two stressors. Conceptual portion of the figure was adapted from Todgham and Stillman (2013).



Specifically, at ambient temperature (-0.8°C, E1), pteropods exposed to the high pCO_2 treatment exhibited elevated respiration rates relative to those exposed to low pCO_2 conditions. This increase in metabolic rate is consistent with a 124eteroscedast outcome with increased costs of maintenance (Sokolova, 2013), possibly due to *L. h. antarctica's* ability to self-repair its shell from the inside by secreting aragonite in response to physical scarring caused by undersaturation (Peck *et al.*, 2016). As the shell's function is to protect the pteropods, as well as to help regulate their buoyancy, its repair is a critical component of their energy budget under future undersaturated conditions of OA, and evidence of dissolution on shells has been documented on Antarctic pteropods collected from the wild (Johnson and Hofmann, 2016). The increase in metabolic rate as a response to high pCO_2 may also be due to increased energetic demand for acid-base balance (Sokolova, 2013), which may be critical in providing an internal environment for this critical aragonite shell repair.

An effect of pCO_2 exposure on metabolic rate was also observed in E2, where pteropods exposed to the high pCO_2 treatment exhibited elevated respiration rates relative to those exposed to low and mid pCO_2 conditions. The effect of temperature alone resulted in 1.29-fold higher respiration rates in E2 than E1 (over a 4.6°C temperature change, or Q_{10} = 1.752). Although E1 and E2 cannot be directly compared because the animals used were from different collection days, incorporating respirometry results into a model shows that there is no significant interaction term between pCO_2 treatment and temperature ($p = 0.309$). The effect of elevated CO_2 on

respiration rate did not change drastically between temperature treatments (high pCO_2 treated organisms had 1.112-fold higher respiration rates in E1, and 1.081-fold higher respiration rates in E2 when compared to low pCO_2-treated organisms). These trends designate the observed interaction as additive – where the effect of the two stressors additively affect metabolic response, without a positive or negative interaction term. In contrast, antagonistic effects yield an effect less than the sum of the individual stressors, while synergistic effects yield an effect greater than the sum of the individual stressors (Figure 25). In this case, neither pCO_2 or temperature are the key driver – they act on similar scales and do not exhibit an interaction term.

We also observed that the variance in metabolic rate increased over time in every treatment in E2 (Figure 21b). This does introduce some moderate 125eteroscedasticity (differential variance between groups) into the analysis (Breush Pagan test, R package lmTest v.0.9-35, $p < 0.0001$) which cannot fully be corrected by a Box-Cox transformation and yielded similarly significant results when run through the same model. Thus, we present the data as uncorrected. This increase in variation could be due to a variation in response to high pCO_2 emerging through time, or to different degrees of starvation over the experimental period that interacted with the pCO_2 response and manifested itself as an increase in variance.

Due to the large range of body sizes in E1 and E2, these analyses were conducted after accounting for the contribution of mass to metabolic rate. This was done by normalizing the respiration rate to pteropod mass following a calculated scaling coefficient (Steffensen *et al.*, 1994). However, pteropod mass decreased

throughout the experiments, and especially began to deviate between treatments in E2 (Figure 24). The general weight decrease is most likely due to the starvation of the pteropods during our two-week long incubations, as the tanks in our culturing system were fed from a filtered seawater intake. It was not logistically feasible to account for a feeding regimen in our limited season length in the Antarctic. The difference in mass between treatments by the end of the experiment mirrors our expectations based on our measurement of respiration rate; that is, the individuals for the high pCO_2 treatment had a decreased body mass over time, possibly due to their increased metabolic demand in a low-pH environment causing them to burn through energy reserves. Although dry weights of sampled pteropods were significantly different between treatments by the end of E2, as there was no difference in the scaling coefficient (ANCOVA, $p = 0.321$) between treatments of each timepoint. Thus, scaling coefficients were calculated separately for each timepoint of each experiment (Figure 21), then applied to correct the respiration rate to an average body mass for each experiment.

Logistically, it was necessary to conduct sequential plankton tows to collect animals for the respirometry trials. Thus, it is important to note that pteropods from E1 and E2 were collected 23 days apart and represent different cohorts of field-collected individuals. Although water chemistry at the collection site remained fairly stable during this time (Johnson *et al.*, 2016) and initial body mass was similar between collections, austral spring bridges winter dormancy and summer time growth, and pteropods could undergo drastic transcriptomic changes during this

season (Johnson, 2017). Overall, conducting E1 and E2 concurrently with the same cohort of collected individuals was not logistically feasible due to field collection and laboratory tank space constraints in a remote field location.

In general, studies that have examined the temperature/pCO_2 multistressor scenario in marine metazoans have found that antagonistic effects, along with additive effects, are far more common than synergistic effects in adult marine invertebrates (Byrne and Przeslawski, 2013) and are also relatively common in early life history stages (Przeslawski *et al.*, 2015). Indeed, it is becoming increasingly clear that there is no unifying theory regarding multistressor effects on a physiological level, and that species-to-species variation can affect these interactions significantly (Lefevre, 2016).

In this additive interaction, we found that temperature and pCO_2 stress operated on a similar magnitude to affect metabolic rates– a result which contrasts research on other Antarctic ectothermic species. In studies on early stage Antarctic dragonfish, *Gymnodraco acuticeps,* increased temperature significantly increased development rates and embryonic metabolic rate (Flynn *et al.*, 2015). However, although pCO_2 stress alone did not negatively affect embryo physiology, there was a synergistic interaction when embryos were exposed to both pCO_2 and high temperature. Similarly, a study rearing larvae of the sea urchin, *Sterechinus neumayeri,* in pCO_2 and temperature treatments found that there was no effect of either individual stressor on the ability to withstand acute larval heat stress (Kapsenberg and Hofmann, 2014). However, rearing urchin larvae at both high pCO_2 and high

temperature resulted in a decreased ability to tolerate acute heat stress at certain early developmental stages. Overall, studies seem to indicate that multistressor relationships in the Antarctic can vary between species, and that this interaction can occasionally make certain species less vulnerable to experimental conditions of ocean change than previous studies have suggested (Peck, 2005).

Although metabolic rates of polar pteropod species are highly understudied, there have been three other studies that have observed the effects of lab-manipulated pCO_2 on *Limacina spp*, only one of which was conducted in the Antarctic. In the northern hemisphere, studies collecting pteropods from Kongsfjord, Spitsbergen have found varying effects of pCO_2 and temperature based on collection time, species, and acclimation duration. Specifically, Lischka and Reibesell (2017) generally found an increase in metabolic rate with increasing temperature and pCO_2 in acute exposures of L. h. helicina collected in January and February, with the exception of the highest-temperature (6.8˚C), where an intermediate pCO_2 (650, out of a range of 180-880) caused the highest increase in metabolism (Comeau *et al.*, 2010). This "hormesis-type" effect, where an intermediate treatment yielded the highest metabolic increase, was prominent at all temperatures during the paired 9-day acclimation experiment. In contrast, *L. h. helicina* collected from the same site in May-June showed no metabolic response to pCO_2 manipulation at ambient temperature (0˚C), but there was an observed increase in respiration rate when increasing pCO_2 at high temperature (4˚C) after a 24-hour incubation. The hormesis-type effect described in Lischka and Riebesell (2017) was not observed in our study in either experiment. However, Lischka

and Riebesell (2017) also observed different multistressor effects in the congener *L. retroversa* collected at the same site, which did not exhibit any interaction between temperature and pCO_2 effects. Altogether, these studies in the Arctic show marked differences in pteropod response to these multistressor conditions at different acclimation durations and collection times – even in closely related species. Specifically, the seasonal differences are likely a situation that, while not unique to polar ecosystems, may be strengthened there due to the strong seasonality experienced at high latitudes compounded by the collection of different life history stages at different times of the year.

In contrast, Seibel *et al.* (2012) resolved a metabolic suppression under high CO_2 at concentrations analogous to the "low" and "high" treatments utilized in E1. While Seibel and colleagues collected *L. h. antarctica* near our collection site (the three sites are 25 km, 35 km, and 75 km north), they were collected far later in the austral summer. This is reflected in the pteropod mass – the pteropods collected for E1 and E2 in October and November 2015 (0.05-0.95 mg) for our study were less than 10% of the mass of those collected by Seibel *et al.* in January and February (0.8-15 mg). Although seasonal development of pteropods in the Antarctic is poorly documented, this growth spurt coincides with phytoplankton blooms in the Ross Sea region (Goffart *et al.*, 1999). This corroborates previous studies that suggest *L. h. antarctica* potentially exhibit a one-year life cycle, spawning and senescing in late summer (Hunt *et al.*, 2008). The fact that this phytoplankton bloom plays a large part in pteropod phenology, growth and development is supported by Seibel *et al.*'s disparate results

(2012) between replicate experiments conducted in consecutive years, which is hypothesized to be a function of low ocean chlorophyll abundance already suppressing metabolism in the second year of trials. Given that phenology and interactions with seasonality have been seen as key factors in governing the Arctic pteropod's response to pCO_2 and temperature, we posit that these factors drove the differences between the results in this study and that of Seibel and colleagues.

In the case of seasonality, observations of pH in McMurdo Sound show austral summer (January and February) as the least acidic period, with a strong seasonal shift towards most acidic conditions in the austral winter (Kapsenberg *et al.*, 2015). This is most likely caused by the stark seasonal day/night cycles at high latitudes, which result in 24-hour darkness during the winter and 24-hour daylight during the summer. Considering the high pH seasonality in the Ross Sea region, it is probable that pteropods at different life stages during a different time of year will exhibit a disparate response to pCO_2 stress. Furthermore, different life stages will experience different magnitudes and duration of pCO_2 stress, both of which have been seen as critical factors in determining calcification capacity in pteropods collected from the wild in the U.S. Pacific Northwest (Bednaršek *et al.*, 2017a). This highlights the importance of considering life stage, phenology, and current-day seasonal variation of abiotic stressors when studying global change biology, as impacts during juvenile stages may also be difficult to recover from (Hettinger *et al.*, 2012; Manno *et al.*, 2017). If hypercapnia elicits a different response at different life stages, the seasonal trend of

pH in McMurdo Sound may play an integral part in this organism's future viability under environmental change.

To contextualize our high-temperature experiment (E2) in the scope of *L. h. antarctica*'s thermal tolerance, we measured respiration rates at ambient pCO_2 levels at increased temperatures (E3) to find that individuals tolerate temperatures far higher than their current, natural environment. The McMurdo Sound region does not see surface temperatures above 0°C (Cziko *et al.*, 2014), yet respiration rates of *L. h. antarctica* increased predictably with temperature following the Arrhenius equation (Alcaraz *et al.*, 2013) up through 14°C. However, it is the case that these acute temperature exposures do not account for every aspect of animal physiology, such as acclimation, and it is possible that growth, energetics, and reproduction cannot keep pace at these higher temperatures. Although the experimental temperature exposures of 4° in E1 and E2 are well within the acute thermal tolerance of *L. helicina antarctica*, it is possible that, especially in conjunction with the additive stressor of OA, pteropod populations in the Southern Ocean may be negatively impacted in the near future.

Changes in resting metabolic rate can play a critical role in an organism's energy budget, potentially drawing resources away from other energetically costly processes such as growth and reproduction. In this light, any changes in respiration rate in response to multiple environmental stressors may be critical for the fate of marine organisms in the near future. Additive responses, such as those seen here (Figure 25), reflect the results of individual stressor experiments in a multistressor scenario. Other species may exhibit a more or less extreme response. While it is

clearly unfeasible, in many regions or taxa, to design experiments that will fully simulate all parameters of future oceans, focusing on a multitude of potentially harmful or co-occurring stressors may be necessary to fully tease apart the fate of future oceans.

Globally, marine ecosystems are projected to face multiple, concurrently changing variables in the future, with abiotic factors that are unique to a particular habitat type and region, of which OA is just one stressor (Breitburg *et al.*, 2015). Emergent examples of this are scenarios of OA and thermal stress in coral reef ecosystems (Hoegh-Guldberg *et al.*, 2007) or persistently covarying stressors like OA and hypoxia in temperate kelp forest ecosystem (Frieder *et al.*, 2012). Further study of the energetics of marine organisms in response to potentially interacting abiotic factors may lend additional insight into understanding the vulnerability of species to future ocean change. Eventually, investigating the root cause and patterns that lead to certain species exhibiting additive effects may not only allow for a better understanding of stress physiology, but may also allow for a critical, more accurate glimpse of future community dynamics in a changing world.

VI. Conclusions

There are a suite of stressors projected to impact coastal organisms in the near future. Understanding the magnitude, variation, and covariation of these stressors in the environment, along with organismal performance under current and future conditions, is critical to understanding the fate of marine communities in the near future. Overall, I have found that the coastal waters can vary strongly in oxygen and pH across short scales of time and space. These traits can also covary, raising the need for experiments that focus on this multiple stressor scenario. Furthermore, upon exposing organisms to different physiochemical conditions, both in the lab and in the field, I detected differences in metabolism, transgenerational plasticity, and within generational plasticity.

The novel findings for each chapter are described below:

***Chapter II**: Spatiotemporal variability of seawater chemistry and dissolved oxygen in a kelp forest environment*

In this chapter, I found that not only does both low-pH and low-DO stresss co-occur in the kelp forest, but it also varies with depth and with the presence or absence of kelp. The top of the water column at Mohawk Reef was dominated by a strong diurnal cycle, especially within the kelp forest. Absolute levels of DO, diurnal range of DO, and stratification of DO all change throughout the seasons. This is one of the few studies that documents DO an pH in a kelp forest environment, and the first to compare nearby sites inside and outside of a kelp forest.

Chapter III: *Transgenerational effects in a kelp forest environment: The influence of in situ adult conditioning on egg quality and the performance of early life history stages of the purple sea urchin,* Strongylocentrotus purpuratus

In this chapter I found that offspring perform differently under stress depending on the environmental exposure of their parents to a kelp forest environment. This is tied to an apparent maternal tradeoff between egg protein content and egg number. This is the first experiment showing transgenerational plasticity in an outplant experiment through the lens of evolutionary rescue in global change biology.

Chapter IV*: A multi-year dataset of ocean pH from McMurdo Sound, Antarctica*

In this chapter, I found that pH changes seasonally in the McMurdo Sound region. This pH cycle is consistent across multiple years, but appears to differ at one of the sites located on the mainland – consistent with knowledge of the prevailing under-ice currents in the McMurdo Sound region. . Looking for a trend of ocean acidification in this region yielded a -0.189 yr^{-1} decrease, which is likely driven by factors shorter than that of global change.

Chapter V: *Additive effects of pCO_2 and temperature on respiration rates of the Antarctic pteropod Limacina helicina antarctica*

In this chapter, I found that juveniles of the Antarctic pteropod increased its respiration rate additively under a multiple stressor scenario of low pH and high temperature. Furthermore, I found that *L. h. antarctica* can tolerate acute temperature exposure far beyond the environmental range at the collection site, which indicates that they may be more resilient to thermal stress than their presence in Antarctica may indicate.

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