AN ABSTRACT OF THE THESIS OF

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Title: Quantifying Sensitivity and Adaptive Capacity of Shellfish in the Northern California Current Ecosystem to Increasing Prevalence of Ocean Acidification and Hypoxia

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The severity of carbonate chemistry changes from ocean acidification is predicted to increase greatly in the coming decades, with serious consequences for marine species- especially those reliant on calcium carbonate for structure and function (Fabry et al. 2008). The Northern California Current Ecosystem off the coast of US West Coast experiences seasonal variations in upwelling and downwelling patterns creating natural episodes of hypoxia and calcite/aragonite undersaturation, exacerbating global trends of increasing ocean acidification and hypoxia (OAH) (Chan et al. 2008) (Gruber et al. 2012). The goal of these experiments was to identify thresholds of tolerance and attempt to quantify a point at which variance in responses to stress collapses. This study focuses on two species: *Cancer magister* (Dungeness crab) and *Haliotis rufescens* (red abalone). These species were selected for this study based on their economic and ecological value, as well as their taxonomic differences. Respirometry was used as a proxy for metabolic activity at four different scenarios mimicking preindustrial, upwelling, contemporary upwelling, and distant future conditions by manipulating dissolved oxygen and inorganic carbon (DIC) concentrations. Both species showed a decrease in mean respiration rate as OAH stressors increase, including an effect in contemporary upwelling conditions. These results suggest that current exposure to ocean acidification (OA) and hypoxia do not confer resilience to these stressors for either taxa. In teasing apart the effects of OAH as multiple stressors, it was found that Dungeness crab response was more strongly driven by concentration of dissolved oxygen, while red abalone data suggested a strong interactive effect

between OA and hypoxia. Not only did these two different taxa exhibit different responses to a multiple stressors, but the fact that the Dungeness crab were secondarily impacted by acidification could suggest that current management concerns may need to be focus more strongly on deoxygenation.

Quantifying Sensitivity and Adaptive Capacity of Shellfish in the Northern California Current Ecosystem to Increasing Prevalence of Ocean Acidification and Hypoxia

by
Hannah Margaret Gossner

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Hannah Margaret Gossner, Author

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1.1 Ocean Acidification and Carbonate Chemistry Dynamics

Since the industrial revolution, the atmospheric concentration of carbon dioxide has increased by more than 43% from 280 ppm to today's value of approximately 408 ppm (IPCC 2014). Currently, carbon dioxide and other greenhouse gasses are at levels that have not been reached in over 800,000 years (IPCC 2014). While some of these gasses are necessary to maintain the habitability of Earth, the rapid concentration increase is being shown to have a myriad of negative effects on the planet (IPCC 2014). Current global conditions would be much worse, however, without the oceans' function as a net carbon sink (Sabine et al. 2004, Orr et al. 2005). Thus far, the ocean has absorbed approximately 30-48% of post-industrial anthropogenic carbon dioxide emissions due to a sequence of carbonate chemistry chain reactions (Sabine et al. 2004, Devries et al. 2017) (*Equation 1*).

$$CO_{2(g)} \Leftrightarrow CO_{2(aq)} + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow HCO_3^- + H^+ \Leftrightarrow CO_3^{2-} + 2H^+ \qquad [Eq.\ 1]$$

Once dissolved into seawater, CO_2 undergoes hydrolysis to form carbonic acid (H_2CO_3). Carbonic acid dissociates first into hydrogen (H^+) and bicarbonate (HCO_3^-). The free hydrogen ion is then available to bond with carbonate (CO_3^{2-}) to become more bicarbonate (Zeebe and Wolf-Gladrow 2001). The favorability for aqueous carbon dioxide to move down this reaction causes a reduction in the concentration of carbon dioxide in surface waters, allowing further diffusion across the air-sea boundary (Zeebe and Wolf-Gladrow 2001, Sabine et al. 2004). As atmospheric carbon dioxide increases, so too does the amount of carbon absorbed by the ocean due to thermodynamic equilibration.

While essential to partial remediation of anthropogenic carbon dioxide emission, continuous uptake is not without consequence. Increased concentration of hydrogen ions from the above reaction directly results in a decrease in pH of the currently mildly basic (pH ~8.1) ocean (Orr et al. 2005, Doney et al. 2009). This phenomenon has become known as ocean acidification (OA), and widespread evidence already exists to document these changes in global ocean chemistry. Surface waters are especially effected due to the air-sea interface, with an average drop closer to 0.1 units (Brewer 1997). By the end of the 21st century it has been estimated that surface ocean pH may drop by 0.3-0.4 units (100-150% increase in hydrogen ions), with a maximum, potential distant future drop of 0.7 units (Brewer 1997, Caldeira and Wickett 2005, Orr et al. 2005).

Changes in pH are not the only chemical repercussions of this process. Shifts in carbonate chemistry also include changes in concentrations of carbonate (CO_3^{2-}) and bicarbonate (HCO_3) ions. Due to the affinity of free hydrogen ions for carbonate, the overall net effects of *Equation 1* result in an increase in bicarbonate and decrease in carbonate ions (Zeebe and Wolf-Gladrow 2001, Feely et al. 2004a). Carbonate is of special interest from a biological point of view as it is a necessary building block for calcium carbonate ($CaCO_3$), a material used widely in marine organisms for both structure and function (Feely et al. 2004a, Orr et al. 2005, Fabry et al. 2008, Hofmann et al. 2010). Calcium carbonate occurs in several crystal morphs, most commonly either calcite or aragonite. Its chemical stability is influenced by temperature, pressure, and concentrations of reactants (Ca^{2+} and CO_3^{2-}). It is common for surface waters to be at over saturation (Ω) with calcite and/or aragonite, but the decreased concentration of carbonate from ocean acidification processes can contribute to undersaturation (Zeebe and Wolf-Gladrow 2001). As a consequence of the above predicted pH drop of approximately 0.4 units, it has been calculated there would be a 50% decrease in ocean carbonate ion concentration (Orr et al. 2005, Feely et al. 2008). The significance of this point will be discussed further in the physiology section.

1.2 Regional Drivers of Ocean Acidification and Hypoxia

The California Current Large Marine Ecosystem (CCLME) is of particular interest due to the natural incursion of acidified and hypoxic water (Snyder et al. 2003, Chan et al. 2008, Feely et al. 2008). The CCLME stretches from the Northern end of Vancouver Island in Canada down to Punta Eugenia in Mexico. The spatial focus of this project is the Northern section of the CCLME extending from Southern Washington to Northern California (Feely et al. 2008). The

Northern CCLME is characterized by seasonal cycles of upwelling and downwelling (Feely et al. 2008). Coastal upwelling and downwelling are wind driven processes, in this case resulting from the seasonal migration of the Pacific High. Changes in wind direction combined with the Coriolis Effect cause upwelling of intermediate depth (100-300m) water during the summer, and strong downwelling in winter months that act with storm-driven mixing to ventilate the shelf waters (Huyer 1983, Bakun and Nelson 1991). Of most concern are the chemical properties of water being upwelled onto the shelf which has high dissolved inorganic carbon (DIC) (>2190 μmol kg^{-1}, pH $\cong$ 7.75), low oxygen, and low calcium carbonate saturation ($\Omega_{\text{aragonite}} < 1.0$) (Chan et al. 2008, Feely et al. 2008, Harris et al. 2013, Somero et al. 2015). This water last interacted with the atmosphere approximately 50 years ago in the western Pacific, and is observed to hold an anthropogenic carbon dioxide load of approximately 37-63 μmol kg^{-1} depending on the location, depth, and upwelling phase (Feely et al. 2008, 2016). Further DIC elevation occurs as a result of remineralization processes both at depth and, of greater consequence, on the continental shelf (Feely et al. 2016).

Upwelled water brings nutrients to the surface, supporting highly productive food webs (Snyder et al. 2003). While this is highly beneficial to biota in the region, increased productivity also results in increased rates of remineralization of organic carbon into DIC (Feely et al. 2016). The breakdown of organic matter results in a further decrease in oxygen level, creating local zones of hypoxia or even anoxia well up on the continental shelf (Chan et al. 2008). It is for this reason that in this area ocean acidification and hypoxia (OAH) are inherently linked.

Strong evidence already exists to document of both ocean acidification and hypoxia within the CCLME. Since 1750, surface pH in the CCLME has on average fallen, with some spatial variability, from 8.12 ± 0.03 to 8.04 ± 0.03 (Gruber et al. 2012, Hauri et al. 2013, Chan et al. 2017). While highly seasonally dependent, the range of aragonite saturation state has fallen from $\Omega = (1.0 \pm 0.1, 4.7 \pm 0.1)$ to $\Omega = (0.66 \pm 0.04, 3.9 \pm 0.04)$ since the preindustrial era (Gruber et al. 2012, Harris et al. 2013). Global ocean acidification acts synergistically with the natural incursion of acidified, hypoxic water. Models project that due to the combination of these factors, the bottom shelf area of the CCLME will be almost permanently undersaturated with aragonite by the year 2050, making it a corrosive, metabolically stressful environment for calcifying organisms (Gruber et al 2012). Evidence also exists identifying areas of hypoxia and anoxia, with one studying finding up to 80% of mid- to inner- shelf waters of central Oregon remaining hypoxic for approximately five months of the year, and some even becoming anoxic (Chan et al. 2008).

Because of these conditions, the CCLME has become known as an early impact system. While it may be challenging for many types of marine life to persist, it is also acting as a natural laboratory to study the future effects and outcomes of increasing incidence of OAH.

1.3 Economic Impacts of Ocean Acidification and Hypoxia

One of the effects of primary concern in the Pacific Northwest is the combined impact of ocean acidification and hypoxia on commercially valuable species. The molluscan shellfish mariculture industry is an important sector of the marine economy, bring in over $270 million a year and supporting over 3,000 jobs (Barton et al. 2015). Between 2007 and 2009 a dramatic failure occurred in multiple shellfish hatcheries which acted as a wake-up call on the potential adverse consequences of ocean acidification. Through study of the phenomena at Whiskey Creek Oyster Hatchery (Netarts Bay, Oregon), it was determined that the intrusion of acidified, low saturation state water was killing oyster spat. Remediation measures were eventually put in place, but economic damage was already done; one of the largest hatcheries- Taylor Shellfish Inc.- moved part of their operation to Hawaii in an attempt to avoid future challenges present in the region (Boehm et al. 2015).

Future expansion of the impacts of OAH is uncertain, especially with regard to other commercially and ecologically valuable species. For this reason, two such species were selected for this study: Dungeness crab (*Cancer magister*) and red abalone (*Haliotis rufescens*). Dungeness crab is a large fishery in the CCLME, bringing in over $214 million dollars in ex-vessel value in California, Oregon, and Washington during the 2016-2017 season (ODFW, CDFW, WDFW, 2017). Additionally, this fishery provides employment for thousands of fishermen, processors, and supply chain workers throughout the region.

While Dungeness crabs are potentially less susceptible to ocean acidification than molluscan shellfish due to the chemical components of their shells, they are still impacted by the low oxygen conditions accompanying acidified water, as has become apparent in recent years, with large die-offs impacting landings (Grantham et al. 2004, Barth et al. 2018). Oxygen concentration appears to have a large effect size on both biomass and species diversity within this region (Keller et al. 2010). This can have immediate effects on landings; Under severe hypoxic conditions, Dungeness crabs in crab pots exhibited a mortality rate of over 75% as opposed to the usual 0% mortality (Grantham et al. 2004). Additionally, Dungeness crab have been observed moving into shallower, more heavily fished areas during bottom low oxygen events which could add another source of stress to the population (Froehlich et al. 2015). Interactive effects of OAH on Dungeness crab, especially at their vulnerable juvenile stage, are not fully known, thus it is difficult to predict the full impacts to the commercial fishery.

Red abalone (*Haliotis rufescens*) is the last remaining abalone species with commercial viability on the U.S. West Coast. The United States currently produces approximately 220 metric tons of red abalone per year (*Farmed Abalone Report* 2017). There are six major abalone farms in California, all of which produce large amounts of abalone (*Farmed Abalone Report* 2017). These farms brought in an estimated average of $7.16 million dollars a year between 2000 and 2008, although it should be noted that the farm production was at times more than double what it is today (Moore and Moore 2008). Until the closure in 2018, there was quite a large recreational fishery in California for red abalone as well. In 2013, it was calculated that approximately 31,000 individuals participated in harvesting abalone recreationally, spending between $24 million and $44 million on the activity (Reid et al. 2016). Unfortunately, in late 2017 the 2018 season was declared canceled by California Fish and Game due to poor abalone counts (*'California rec…',* 2017).

A smaller recreational fishery for red abalone exists in Oregon. As a precautionary measure, the red abalone fishery in Oregon was closed as well due to low densities and unfavorable ocean conditions (Temp. Admin. Order, ODFW 156-2017). Due to fishing pressures in California along with the potential for climate migration, southern Oregon may become an important refugia for red abalone (Hobday and Tenger 2002). In addition to these challenges, little is known about the effects of OAH stress on red abalone populations. As the regulatory bodies work to resuscitate the fishery, it will be necessary to consider resiliency of the species to multiple stressors.

1.4 Ocean Acidification and Hypoxia in Policy

As is apparent both from oceanographic data and industry experience, the CCLME will experience stronger repercussions from OAH sooner than many other places. Because it is an early impact region, the governing bodies of the US West Coast are entering unknown territory when it comes to policy and management. Part of the goal of this research project has been to examine how information resulting from this research can fit into current legislative and managerial action. This effort stems from one of the core concepts in the field of ecosystem based management, which states that best management practices require accurate, up to date science and efficient communication between specialists, managers, and decision makers (McLeod and Leslie 2009, Boehm et al. 2015).

Sharing of knowledge is important especially in this case as OAH provides multiple challenges to decision makers. First is the issue of scale; although global ocean acidification trends are in large led by atmospheric changes in CO_2, local scale processes, such as upwelling and various types of runoff, can also contribute and must be accounted for (Kelly et al. 2011). It is also important to note that acidification conditions are spatially heterogeneous, and vary over multiple scales (Chan et al. 2017). OAH is also a difficult issue to communicate to policy-makers and the public, as it is complex and intangible to many people (Busch et al. 2015). Lastly, there are also still many unknowns with regards to what degree ecosystems will react to these chemical changes, leading to difficulty in evaluating risk and incentivizing action (Strong et al. 2014, Busch et al. 2015).

Additionally, there is currently no effective national OAH policy, leaving action up to the states. The only codified federal legislation concerning ocean acidification was created 2009: The Federal Ocean Acidification Research and Monitoring Act (FOARM) (33 U.S.C. 50 § 3701-3708) was passed by congress in order to coordinate an interagency response to ocean acidification. In §3705, the National Oceanic and Atmospheric Administration (NOAA) was tasked with creating an Ocean Acidification Program to help guide research on impacts and adaptive strategies (Strong et al. 2014). Management of different aspects are spread across different departments, and there is not currently a specific mechanism to organize cross-department communication with regards to OAH.

On a regional level, the U.S. West Coast has put forth several efforts to coordinate and collaborate with regard to ocean health, but none yet binding. In 2006, the West Coast Governor's Alliance on Ocean Health was created in order to foster collaborative knowledge gathering and distribution. Two years later in 2008, a binational partnership was formed in the Pacific Coast Collaborative (PCC) which also included the Canadian province of British Columbia. Together, the group drafted the Pacific Coast Action Plan on Climate and Energy (2013), but nothing binding was created. In accordance with the National Ocean Policy, in 2015, Washington, Oregon, and California chartered the West Coast Regional Planning Body (WCRPB), a partnership between tribal, state, and federal representatives. The WCRPB, "is not a regulatory body and has no independent legal authority to regulate" any involved entities (Charter, 2015).

Other networking and communication attempts have been initiated as well. To assist with information gathering and distribution in the region, the California Current Acidification Network (C-CAN) was established in 2010. C-CAN is an industry, scientist, and manager collaborative that works in collaboration with the Integrated Ocean Observing System (IOOS), especially the Northwest Association of Networked Ocean Observing Systems (NANOOS), in order to host and make accessible data on ocean acidification and hypoxia. Additionally, the California Ocean Science Trust, at the request of the California Ocean Protection Council convened the West Coast Ocean Acidification and Hypoxia Science Panel in 2012 which incorporated a wide range of expert opinion from the West Coast states and British Columbia. As opposed to other collaboration efforts, the panel focused more closely on how to advise and engage regional policy makers (Cooley et al. 2015). A report was produced that has been used to help inform state governments.

Because there has not yet been binding policy creation at the federal level, movement towards addressing OAH has been occurring primarily at the state level. Although the three states that make up the U.S. Western seaboard have all begun to address OAH in their own ways but for the sake of this project, focus will be turned to the state of Oregon. Just as at the federal level, state level managerial and regulatory responsibility for OAH impacts are split between multiple entities.

The Oregon Department of Fish and Wildlife (ODFW) and the Department of Environmental Quality (DEQ) are highly involved in implementing policy regarding OAH. ODFW, under the *Developmental Fisheries* Act (§ 506.450–.465) holds, "exclusive jurisdiction over all fish, shellfish, and all other animals living intertidally on the bottom, within the waters of this state", as well as the right to manage both threatened and unlisted species (§ 496). The DEQ deals with analysis of water quality and runs the Oregon Nonpoint Source Pollution Program which was established in 2014.

It is also important to understand the existing legislative framework. There are three key pieces of legislation that have the potential to become the legal backbone for either policy action. The first is the Coastal Zone Management Act of 1972 which granted states the ability to create management plans for their shorelines. It contains an important provision known as Federal Consistency, which gives states the ability to examine and rule on federal actions in regards to compliancy with their own Coastal Zone Management Plans.

Another essential component of Oregon's environmental and marine legal structure is the Public Trust Doctrine (PTD) and Oregon Beach Bill. The PTD is codified in Oregon State Constitution in Chapter VIII, Section 5 which mandates that state lands should be managed, "with the object of obtaining the greatest benefit for the people of this state, consistent with the conservation of this resource under sound techniques of land management". In alignment with Oregon Supreme Court rulings, the PTD in OR includes submerged lands beneath navigable waters (habitat), wildlife (shellfish), and water quality (potentially OAH); therefore, the Oregon State Government holds all of these items in trust for the public (Blumm and Doot 2012). This means that the government should be responsible for maintaining the current quality and functionality of the coastal ocean and many of its resources. Interestingly, this mission does not always seem to line up with the public's perception of their own rights to utilize natural resources. The Beach Bill (1967 HB 1601) guarantees public access to the coast, and due to the intertidal nature of some shellfish, public access and the goals of the PTD may come into conflict.

Third, and perhaps the most underutilized, is the Clean Water Act (CWA) (33 U.S.C. § 1251 *et seq*). Language in the CWA allows for regulation of the quality of water of "use and value for… propagation of fish and wildlife" (33 U.S.C. § 1313(c)), which would arguably include pH, carbonate chemistry, and oxygen levels. Carbon dioxide is currently not included as a waterborne pollutant as it is emitted as an airborne pollutant and disperses in water as a waterborne pollutant, blurring the lines between Clean Water and Clean Air Act (42 U.S.C. § 7401 *et seq.)* jurisdiction. Carbon dioxide has, however, been classified as an airborne pollutant via *Massachusetts v. EPA* (549 U.S. 497, 2007); it has been suggested that language in the CWA could be modified or interpreted to include carbon dioxide, allowing it to be regulated as a non-point source pollutant via section 303(d) which deals with the Total Daily Maximum Loads (TDMLs) of pollutants entering bodies of water (Craig 2015, Weisberg et al. 2016).

Along with existing, larger scale legislation, Oregon's state government has already made several steps towards creating a state policy framework for OAH. In 2015, the Shellfish Policy Bill (HB2209) was passed, codifying state policy of shellfish both wild and cultivated. One of the explicit goals of the bill was to improve water quality in order to support the propagation of shellfish, which could be interpreted as including aspects of OAH.

In 2017, the Oregon State Senate passed Bill 1039 (OR SB1039A) which established the state policy with regards to ocean acidification and hypoxia. This bill recognizes the fact that the effects of OAH are being felt within, "nearshore and estuaries, causing marine waters within this state's jurisdiction to be especially vulnerable to ocean acidification" (SB1039). It formally codifies the state policy as working in close collaboration with local and regional research groups to and sets the structure of the Oregon Coordinating Council on Ocean Acidification and Hypoxia. The council is comprised of representatives for state agencies, local academic institutions, stakeholder groups, and tribal interests. Together, they have been tasked with laying out strategies to monitor the environment, remediate, and evaluate economic repercussions, with the goal of producing an OAH Action Plan by 2019.

Although the agencies and jurisdictional boundaries are important, it is also incredibly important to consider heterogeneity and distribution of stakeholders throughout Oregon when crafting legislation or regulations. The bulk of the concern and action so far has been with regards to shellfish hatcheries in Oregon and Washington as hatchery failure severely impacted the shellfish industry (Cooley et al. 2015). Other groups often have not yet been directly impacted by OAH, and may not even know what it is or what it means (Busch et al. 2015). As conditions continue to change, the pool of direct stakeholders will likely continue to grow, including workers on commercial shellfish plats, fishermen, recreational harvesters, processors, and other supply chain jobs. Additionally, cultural ecosystem services provided by impacted species must be considered. Many tribes along the West Coast traditionally used and ate shellfish, and despite the government trust, they are at risk of further losing important traditions and cultural identity (Lynn et al. 2013, Bertelsen 2016).

1.5 Physiological Effects of Ocean Acidification and Hypoxia

In this section, physiological coping mechanisms for dealing with OAH in the two species of focus - Dungeness crab (*Cancer magister*) and red abalone (*Haliotis rufescens*)- will be discussed to provide a base for interpretation of results.

From a general, physiological perspective, ocean acidification is a serious challenge to organisms via multiple pathways (Kelly and Hofmann 2013, Breitburg et al. 2015, Somero et al. 2015). The two most commonly studied impacted processes are maintaining internal acid-base balance homeostasis and preserving/creating new calcified material (Orr et al. 2005, Kroeker et al. 2013a). Acidification alters the concentrations of various ions in seawater, and since many marine invertebrates respire using gas exchange over thin membranes, constant internal adjustment may be necessary to counteract ambient concentrations of ions (Fabry et al. 2008, for review). Correcting this imbalance can be an energetically expensive process, potentially diverting metabolic capacity from other processes (Burnett 1997, Pörtner 2008).

Calcification is the synthesis of calcium carbonate minerals. A wide range of marine organisms use calcification to create material for either structure or function, including the shells and skeletal structures of many marine invertebrates, calcareous algae, and even some balance and orientation structures in fish (Orr et al. 2005, Hofmann et al. 2010). The process of calcification is most favorable when water is over saturated with regards to the calcium carbonate mineral of interest ($\Omega_{sp} \geq 1.0$), although the exact range of required values varies both by mineral type, taxa, and developmental stage of individuals (Feely et al. 2004a, Orr et al. 2005, Fabry et al. 2008, Waldbusser et al. 2015a). Undersaturation of calcium carbonate can lead to degradation or even dissolution of these structures (Orr et al. 2005, Fabry et al. 2008, Hofmann et al. 2010). The stability of calcium carbonate in seawater is defined by the temperature and pressure-sensitive solubility constant K^{*}_{sp} (*Equation 2)*.

$$\Omega = [Ca^{2+}]\,[CO_3^{2-}] \,/\, K^{*}_{xp} \qquad \textit{Eq. 2.}$$

As depth increases, conditions of alkalinity, DIC concentration, temperature, and pressure become unfavorable for the stability of calcium carbonate. This is commonly known as the carbonate compensation depth, or the saturation horizon (Zeebe and Wolf-Gladrow 2001). Depletion of carbonate ions by anthropogenic processes discussed in *Section 1.1* can also contribute to shoaling of the saturation horizon, contributing to the intrusion of corrosive waters up onto the continental shelf (Feely et al. 2008, Gruber et al. 2012).

1.5.1 Dungeness Crab (*Cancer magister)*

Dungeness crabs (*Cancer magister*) are motile benthic arthropods meaning their habitats are subject to exposure to low pH, hypoxic waters during upwelling in the summer. They also have “shells”, but unlike mollusks, use chitin, a much less soluble carbohydrate, to build their exoskeleton. In certain places the chitin is reinforced with calcite, another, less soluble calcium carbonate species using a method similar to both bivalves and gastropods (Whiteley 2011). In order to form this part of the exoskeleton, it is necessary for the crab to have an alkaline exoskeletal compartment which is created by taking up calcium and bicarbonate over the gills (Wood and Cameron 1985). It seems as though many crabs are very good at maintaining this alkaline compartment, as calcification rates across several species have been found to stay the same even after carbon dioxide exposure; it is hypothesized that they are either very effient at acid-base management or that they have a very effective epicuticle seperating the area of mineral deposition from the environment (Ries et al. 2009). Some of this calcium carbonate is highly soluble, and it has been proposed that it acts as a transient source of calcium and bicarbonate ions, perhaps aiding in acid-base regulation in times of need (Whiteley 2011).

Like many marine invertebrates, Dungeness crabs respire by running water over gills, leaving them open to influx of hydrogen ions in an acidifying ocean. Excess hydrogen ions diffuse into the hemolymph and can disrupt metabolism, protein synthesis, ionoregulation, and cell volume control (Wheatly and Henry 1992). Crabs deal with this in several ways. First, they are able to buffer some of the incoming hydrogen ions with bicarbonate hemolymph proteins such as hemocyanin (oxygen binding pigment), but this can only do so much. Most pH regulation is done via electroneutral ion exchange in the gills, bringing bicarbonate in from the environment in exchange for chloride ions (Cl^-) (Whiteley 2011). Crabs that are more tolerant of high carbon dioxide tend to have more bicarbonate in their systems (Pörtner et al. 2004). In experiments, Dungeness crabs have been able to fully regulate their hemolymph pH in hypercapnic conditions over the course of twenty-four hours by accumulating bicarbonate from the water around them (Pane and Barry 2007). The build-up of lactate in extracellular space increases the oxygen affinity of transport proteins, signaling a need to increase oxygen consumption and up-regulate these expensive acid-base regulating strategies. Eventually this accumulation of buffering material must be excreted, generally by the H^+-ATPase pathway which is energetically expensive (Pane and Barry 2007).

This is not to say that many species of crabs are not adept at dealing with changes in environmental pH; other species have been shown to maintain or even increase their hemolymph pH during hypercapnic conditions (Hill et al. 1991, Pane and Barry 2007, Long et al. 2013, Glandon and Miller 2017). The ability to actually increase internal pH helps to increase hemocyanin oxygen affinity, possibly creating a physiological response capable of dealing with both acidification and low oxygen conditions (Burnett 1997). Without a strategy like this, it has been calculated that in a Dungeness crab, a decrease in hemolymph pH by 0.4 would create a

Bohr shift causing a 50% increase in the oxygen level at which hemocyanin is half saturated (P_{50}) (Johansen et al. 1970). This would mean a significant decrease in oxygen carrying capacity on top of already low oxygen conditions, exacerbating the negative effects.

Unfortunately, at this point, there appears to be limited research studying the effects of multiple stressors on the physiological response of Dungeness crabs. Because both lowered oxygen concentrations and increased pH have been shown to increase physiological stress, it seems likely that the presence of both stressors could have an interactive effect.

1.5.2 Red Abalone *(Haliotis rufescens)*

Red abalone (*Haliotis rufescens*) are large, herbivorous gastropods with a biogeographical range that extends from southern Oregon to Baja California (Estes et al. 2005). Unlike the Dungeness crab, their shell is composed primarily of calcium carbonate in its aragonite phase; this is important as aragonite is 50% more soluble in seawater than calcite, leaving it more vulnerable to dissolution (Feely et al. 2004a, Miller et al. 2009, Zippay and Hofmann 2010). Like other marine mollusks, red abalone form their shells in a closed extrapallial cavity between the outer mantle and shell in which the concentrations of aragonite can be concentrated either by passive or active transport (Weiner and Dove 2003, Gazeau et al. 2013). In order for this to be efficient, the aragonite saturation state (Ω_{arag}) must be at or over 1 (Hofmann et al. 2010, Ries 2011, Gazeau et al. 2013). If it is not, the rate of shell dissolution may exceed the rate of shell synthesis, and dissolution becomes thermodynamically favorable (Gazeau et al. 2013). It is also thought that shelled mollusks utilize carbonic anhydrase, which aids in the balance of carbon dioxide and bicarbonate (Gazeau et al. 2013). There does not appear to be a large amount of literature focusing on the calcification of red abalone. One study on *Haliotis iris* in New Zealand found a very thin diffusion boundary layer at the shell-water interface, suggesting a higher chance of dissolution, but the authors postulated that due to their internal calcification process, the rate of calcification itself may not be impacted by decreases in pH (Hurd et al. 2011).

Red abalone must also compensate for acid-base balance. The increased concentration of hydrogen ions from acidification causes greater membrane diffusion, and upon entry into the body, H^+ reactions with intra and extracellular fluid, potentially creating internal acidosis (Seibel and Walsh 2003). While some compensation can be achieved using passive buffering via non-bicarbonate buffers such as calcium carbonate, in order to fully remediate the situation it is generally necessary to use active ion exchange mechanisms (Seibel and Walsh 2003, Pörtner et al. 2004). Marine mollusks generally regulate their inner acid-base balance by both removing hydrogen ions and accumulating bicarbonate. This process is mediated either by or via transport proteins including Na^+/K^+ and H^+ ATP-ase, making it a potentially energetically expensive process (Pörtner et al. 2004, Fabry et al. 2008). One study has found that red abalone in the late veliger stage are more sensitive to low pH in combination with higher temperature, perhaps in part due to the amount of energy that is required to metamorphose and settle (Zippay and Hofmann 2010).

Fortunately, some work has been done on combined effects of stressors on red abalone. Prolonged exposure of juvenile red abalones to lowered pH (7.5) and oxygen (5mg/L) lead to reduction of shell growth (Kim et al. 2013). White (2011) showed evidence of decreased net calcification, presence of dissolution, but limited soft-tissue changes under low oxygen and pH, suggesting that there was a negative impact on the shell, but perhaps energy was being shifted to maintaining the internal tissue and workings of the organism. It is important to note, however, that multiple studies have documented evidence of genetic plasticity or adaptation to challenging conditions in red abalone, suggesting that they may have an inherent ability to deal with OAH stressors (De Wit and Palumbi 2013, Kim et al. 2013).

1.6 Thresholds and Adaptation

While individual organisms' response to OAH stressors is useful, in order to predict future mid-to-large scale consequences, it is helpful to place results in the context of a population (Gaylord et al. 2015). Within a healthy population it is highly likely that there will be variability in individual response to stress. Even with standing variability, there are inherent physiological thresholds above or below which can population success can be impacted. In conventional toxicological assays, thresholds have often been defined by the single stressor exposure level necessary to elicit a 50% mortality rate (P_{50}) within a sample. Because ocean acidification may manifest as a chronic stressor, mortality may not be a descriptive measure of physiological sensitivity and tolerance. Instead, this study focuses on the use of respiration rate as a proxy for stress response. Use of this metric also allows for a non-binary (e.g. “alive” vs “dead”) quantification of stress response, allowing for better examination of variation among the sampled individuals.

Additionally, within upwelling systems, it is highly unlikely that organisms will only be exposed to a single stressor due to the tight linkages between hypoxia and acidification (Chan et al. 2008, Gruber et al. 2012). Introduction of multiple marine stressors complicates predictions, as there can be unknown interactions (Crain et al. 2008, Somero et al. 2015, Kroeker et al. 2017). These interactions can result in the shift of thresholds, and therefore are important to consider.

Once the magnitude of OAH stress passes populations’ thresholds, there are four possible outcomes: tolerate, avoid, adapt, or perish (Dawson et al. 2011). It is likely that most shellfish species will not tolerate large changes in oceanic carbonate chemistry and oxygen due to inherent physiological energy and material requirements, although it is likely that some species will perform better than others under stress (Hofmann et al. 2010, Kroeker et al. 2013a, Waldbusser et al. 2015a). Avoidance, at least in the individual lifetime scale, seems unlikely as well, as many shellfish are sessile, slow moving, or planktonic. This leaves either adaptation or extinction.

For the sake of this project, adaptation will be assumed to have a genetic component, as opposed to acclimatization, which involves only the use of existing pathways. In order for adaptation to occur, a population must display phenotypic plasticity (Sanford and Kelly 2011, Munday et al. 2013). The portion of the population able to deal with stress must not only have characteristics which allow it to succeed, but these traits must also be heritable (Kelly and Hofmann 2013). It has been found that local adaptation can occur over relatively short timescales, introducing the potential of evolutionary rescue (Schoener 2011, Ellner 2013, Munday et al. 2013). There may, however, be a limit to this adaptive capacity. As OAH stress continues to increase, fewer individuals are likely to exhibit the phenotype required to survive. This would manifest as a decline and eventual collapse of phenotypic variance. This point of variance collapse poses a limit for which evolutionary rescue can act.

1.7 Research Questions and Hypotheses

There are two overarching goals of this research. The first is to determine the physiological threshold OAH conditions for *Cancer magister* and *Haliotis rufescens*. In this case we define the threshold as the physical OAH conditions which cause a disproportionate change in mean and variance of physiological response to stress. This assumes that along a continuum of projected changes in OAH conditions, even the “top performers” will no longer be able to maintain optimal respiration rates. Quantifying these thresholds will allow us to determine just how close current population are living to their physiological thresholds, and aid in estimating how future shifts in OAH conditions will affect this. Additionally, by manipulating multiple stressors, we can begin to understand the relative contributions of individual stressors to organismal response. This is important as it could influence interpretation of current research into ocean acidification and the relative importance of various conditions.

The second goal of this study is to examine the changes in variability of response with increasing OAH stress. In a resilient population, there will likely be sufficiently high standing variability, resulting in a variety of responses to stress. This is important, because as conditions change, a wider variety of responses increases the chances that at least some individuals carry traits that allow them to be successful. It is for this reason we will look at changes in variance of response with regards to increasing exposure to OAH stressors.

As with thresholds, there are multiple possible outcomes. First is that increased stress will result in a decrease in variance, which may suggest that a species is near its “evolutionary limit” (Lande and Shannon 1996, Kelly et al. 2012, Gaitán-Espitia et al. 2017). A severe enough collapse of variance can potentially inhibiting recovery (Pörtner 2008, Dawson et al. 2010, 2011, Munday et al. 2013). The second alternative is that there could be no change to the amount of phenotypic variability with increasing stress. Because of the natural extremes in the system, a wider tolerance for pH or oxygen values could already be a favored trait (Futuyma and Moreno 1988, Lande and Shannon 1996, Sunday et al. 2011, Pespeni et al. 2013a, Vargas et al. 2017).

Given the above discussion, the following three hypotheses have been formed. First, due to prior exposure to low pH and dissolved oxygen, juvenile Dungeness crabs and red abalone will have thresholds for impacts that will not be crossed until the distant future. Along with this, co-exposure to acidification and hypoxia will have an interactive effect on stress response of both species. Lastly, variance in individual performance will scale with the intensity of stress.

2 Quantifying Sensitivity and Adaptive Capacity of Dungeness Crab (*Cancer magister)* in the Northern California Current Ecosystem to Increasing Ocean Acidification and Hypoxia

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Abstract

The severity of ocean acidification is predicted to increase sharply in the coming decades, with serious consequences for marine species- especially those reliant on calcium carbonate for structure and function (Fabry et al. 2008). The California Current Large Marine Ecosystem (U.S. West Coast) exhibits seasonal variations in upwelling and downwelling patterns which create natural episodes of hypoxia and calcium carbonate undersaturation, exacerbating global trends of increasing ocean acidification and hypoxia (OAH) (Chan et al. 2008, Gruber et al 2012). This study focuses on *Cancer magister* (Dungeness crab), a species chosen based on their economic and ecological value, as well as lack of available information on tolerance to OAH. Respirometry was used as a proxy for metabolic impacts of stress at four different scenarios mimicking preindustrial through distant future levels of dissolved oxygen and dissolved inorganic carbon (DIC) concentrations. The goal of these experiments was to identify thresholds of tolerance and quantify a point at which variance of response expression decreases. It was found that increasing OAH stress resulted in a decrease in respiration rate mean and variance. pCO_2 was found to have a variable effect on rate as well, while dissolved oxygen content drove the bulk of the effect size. Although Dungeness crab megalopae appear to be keeping up with modern OAH chemistry, if adaptation does not keep pace with future changes it is possible that there will be negative effects on the population.

2.1 Introduction

Over the past decade, it has become apparent that the increasing prevalence anthropogenically driven ocean acidification and hypoxia (OAH) have wide-ranging repercussions on the ocean and its inhabitants (Caldeira and Wickett 2005, Orr et al. 2005, Fabry et al. 2008). Although an initial view of ocean acidification focuses on the carbonate chemistry changes driven by air-sea interactions at the global scale, the distribution of the effect is not necessarily homogenous; instead it varies on a smaller scale depending on position along the ocean's gyres, local bathymetry, and coastal processes such as upwelling (Feely et al. 2008, Doney et al. 2009, Chan et al. 2017). This means that while overall the entire ocean experiences the effects of increased dissolved carbon dioxide, certain areas will be impacted earlier and to a greater magnitude. Adding to the issue, in hotspots driven by seasonal upwelling, there is a tight linkage between high dissolved inorganic carbon and low oxygen concentrations, creating a challenging environment for marine organisms with multiple, potentially synergistic stressors (Chan et al. 2008, Hauri et al. 2013, Breitburg et al. 2015, Kroeker et al. 2017, Vargas et al. 2017).

The California Current Large Marine Ecosystem (CCLME) is an OAH hotspot (Snyder et al. 2003, Chan et al. 2008, Feely et al. 2008). This region stretches from the northern end of Vancouver Island in Canada to Punta Eugenia in Mexico. As an Eastern Boundary Current, much of the CCLME is naturally prone to seasonal cycles of upwelling and downwelling, driving annual intrusion of low oxygen, high carbon dioxide intermediate depth waters onto the continental shelf (Feely et al. 2008, Gruber et al. 2012). There is already strong evidence of both ocean acidification and hypoxia occurring in the CCLME. Since 1750, surface pH in the CCLME has on average fallen, with spatial variability, from (8.12 ± 0.03) to (8.04 ± 0.03) (Gruber et al. 2012, Hauri et al. 2013, Chan et al. 2017). Tied to these changes, the range of aragonite saturation state has fallen from $\Omega = (1.0 \pm 0.1, 4.7 \pm 0.1)$ to $\Omega = (0.66 \pm 0.04, 3.9 \pm 0.04)$ since the preindustrial era, adding further physiological stress for calcifiers (Gruber et al. 2012, Harris et al. 2013, Waldbusser et al. 2015a, 2015b). Global ocean acidification acts synergistically with the natural incursion of acidified, hypoxic water; models have found that due to these factors, the bottom shelf area of the CCLME will be almost permanently undersaturated with respect to aragonite by the year 2050, making it a corrosive, metabolically stressful environment for calcifying organisms (Gruber et al. 2012, Waldbusser et al. 2015a, 2015b). There is also evidence for seasonal areas of low oxygen, with one studying finding up to 80% of mid- to inner- shelf waters of central Oregon remaining hypoxic for approximately five months of the year, and some areas even becoming anoxic (Chan et al. 2008).

The CCLME is widely recognized as an early impact area, acting as a natural laboratory to study the current and future effects of increasing incidence of OAH (Hauri et al. 2013, Pespeni et al. 2013a, Chan et al. 2017, Vargas et al. 2017). From a physiological perspective, ocean acidification and hypoxia presents a serious challenge to organisms, especially calcifiers (Kelly and Hofmann 2013, Breitburg et al. 2015, Somero et al. 2015, Waldbusser et al. 2015b). The two primary physiological challenges are regulating internal acid-base balance and maintaining calcified material (Orr et al. 2005, Pörtner 2008, Kelly and Hofmann 2013, Kroeker et al. 2013a). When combined with the additional stress of oxygen depletion, organisms may need to reallocate energy, potentially resulting in decreased survivorship, lowered reproductive capacity, reduced growth, or structural integrity (Fabry et al. 2008, Ries et al. 2009, Gazeau et al. 2013, Kim et al. 2013, Pan et al. 2015).

The adaptive capacity to deal with increased OAH stress varies both among and within populations and taxa (Futuyma and Moreno 1988, Lande and Shannon 1996, Sunday et al. 2011, Kelly and Hofmann 2013). Because stressors relating to OAH are projected to become more challenging in the coming decades, it is important to understand both how population are dealing with current conditions and their potential to deal with changes predicted in the future. This involves the measurement of physiological thresholds as well exploration of interaction between stressors and their combined effect on tolerance. Additionally, it is important to look at changes in variation of response to stress as a proxy for standing phenotypic variability. Locating the limits of top-performers can help indicate at what point physiological conditions may start affecting the entire population.

This study focuses on how these concepts apply to the Dungeness crab (*Cancer magister*), a benthic crustacean present in the coastal eastern Pacific between the Aleutian Islands and central California (Jensen and Armstrong 1987). This species was chosen primarily due to its high economic value in the region; it is a large single species fishery along the northern CCLME, regionally bringing in $214 million dollars in commercial landings for the 2016-2017 season and providing employment for hundreds of workers in California, Oregon, and Washington (NMFS Annual Commercial Landings Statistics, 2016).

Additionally, this species was chosen due to the level of uncertainty associated with its responses to ocean acidification. Previous studies of decapod reaction to decreased pH and hypercapnia have yielded mixed results (summarized in: Kroeker et al. 2013, Wittmann and Pörtner 2013). Due to their primarily chitin carapaces, decapods are theoretically less vulnerable to the high-profile threat of shell dissolution, but they may still struggle with internal acid-base balance in low pH environments (Whiteley 2011, Wittmann and Pörtner 2013, Miller et al. 2016).

It is also likely that vulnerability changes throughout development; it is for this reason that it was chosen to look not at adult Dungeness crabs, but at megalopae. Previous studies have found evidence of slowed development and increased mortality in zoeae and larvae, but no data on vulnerability just before settlement was found in the literature (Miller et al. 2016). Determining the vulnerability of megalopae is important as it has been shown that success at this stage has the strongest influence on adult age class size (Shanks and Roegner 2007).

The extent of Dungeness crabs' sensitivity to low oxygen is fairly well established; in experiments, adult Dungeness crabs have been able to fully regulate their hemolymph pH in hypercapnic conditions over the course of twenty-four hours, but there is undoubtedly a limit (Airriess and McMahon 1994, McMahon 2001, Pane and Barry 2007). During late summer of 2017, adult Dungeness crabs were recorded suffocating in low oxygen conditions off the coast of Oregon by Oregon Department of Fish and Wildlife (Barth et al. 2018) (http://oregonmarinereserves.com/ 2017/09/06/hypoxia-central-coast). Additionally, a positive *in situ* correlation has been found with dissolved oxygen and adult Dungeness crab density, suggesting movement away from zones of low oxygen, but it is not known if megalopae are also capable of such movement driven by oxygen (Shanks 1986, Keller et al. 2010, Froehlich et al. 2015).

For the purposes of this study, adaptive capacity of Dungeness crab megalopae is explored using two approaches: (1) how increasing and concurrent acidification and low oxygen stress impact physiological thresholds, and (2) how an increase in OAH stress effects variance of response within a population. Rate of oxygen use was monitored as a proxy for metabolic response to stress resulting from manipulated pCO_2 and oxygen conditions. We hypothesize that due to prior exposure to low pH and dissolved oxygen, Dungeness crab megalopae have thresholds for impacts that will not be crossed until the distant future. Additionally, because both hypoxia and hypercapnia have been shown to have negative effects on Dungeness crabs, it seems likely that co-exposure to acidification and low oxygen will trigger a stress response in megalopae that will be of a greater magnitude than either stressor by itself. Lastly, we hypothesize that the variance in megalopae stress response will decrease as the magnitude of stress from acidification and hypoxia increases, suggesting loss of the top performers and eventual decline in collective adaptive capacity.

2.2 Methods

Juveniles of the crab *Cancer magister* were collected on nine separate occasions during May and June of 2018 using a light trap on the pier of Oregon State University's Hatfield Marine Science Center dock in Yaquina Bay, Oregon (44°37'32.7"N 124°02'43.7"W). All individuals were megalopae, the last planktonic phase before settlement.

In order to examine the gradient of intra-species variance correlated with the strength of acidification and hypoxia stress, we investigated respiration rates as a proxy for performance under pCO_2 and O_2 scenarios. Individuals were held no more than two days in ambient sea water before being transferred to manipulated seawater, and were generally transferred much quicker (Seibel et al. 2012, Howes et al. 2014). Because of the dynamic environmental conditions of eastern boundary current upwelling systems, it was assumed that a relatively quick transition from ambient to manipulated seawater was approximately analogous to conditions experienced off the coast of Oregon. Individual organisms were then added to manipulated seawater samples with no headspace and stored in 13mL or 15mL gas-tight, ground-glass vials for the duration of the trial. Controls for microbial respiration were kept without a target species individual. Respiration within the container was monitored at 1 second intervals for approximately 30 second periods at the beginning and several times during the experiment using the Fibox 3 Oxygen Meter and corresponding oxygen-sensitive optode sensor dots manufactured by PreSens Precision Sensing (Regensburg, Germany) (*Figure 2-1*). Data was acquired using Fibox 3 for PSt3 (version 6.02) software, also from PreSens.

Two separate types of assays were performed. On five of the collection dates, individuals were transported back to Oregon State University in Corvallis, OR. In these assays, crabs were subject to four levels of exposure with date serving as a block. These are henceforth referred to as the "four level" assays. Approximately 36-37 megalopae were used for each replication, with 7-8 individuals randomly chosen for each of four $p\mathrm{CO}_2$ and dissolved O_2 scenarios (see below and *Table 2-1*). Conditions for the "four level" $p\mathrm{CO}_2$ and O_2 scenarios (*Table 2-1*) were back calculated from the relationship between dissolved oxygen and dissolved inorganic carbon with an added anthropogenic load (Chan et al. 2017, Feely et al. 2018). Relationships between dissolved oxygen and dissolved inorganic carbon were determined using guidelines from Feely et al (2004b). The four conditions were designed to mimic pre-industrial (non-upwelling), baseline upwelling, contemporary upwelling, and distant future upwelling (approximately year 2100). Pre-industrial had a target pH of 8.17 and was 100% saturated with oxygen at 297 μatm. The second treatment (baseline upwelling) had a target pH of 7.56 and upper-limit hypoxic conditions (target 75 μM O_2). At this level, the water becomes undersaturated with regards to aragonite. Contemporary upwelling has a pH of 7.50 and DO of 40 μM, and could represent either modern seawater conditions at a deeper depth or future conditions; note that in this level the saturation state for calcite drops below 1. The final level, distant future, represents future worst case scenario for the year 2100 with a pH of 7.27 and very low oxygen levels ($\approx$20 μM). After incubation, each vial was flushed with low $p\mathrm{CO}_2$ ($\approx$285 μatm), high O_2 ($\approx$297 μmol/kgSW) water. After allowing individual crabs to recover, they were then sampled again for respiration rates. Any samples with missing data were not included in the analysis, resulting in a total n=152 for this set of assays.

On the other four dates, 34 individuals were transported to Oregon State University in Corvallis, OR and 11-12 were assigned to a "low", "medium", or "high" $p\text{CO}_2$ treatment. These values were allowed to vary slightly for each of the replications, resulting in twelve different $p\text{CO}_2$ bins (*Figure 2-2)*. Within each bin, the oxygen concentration was allowed to vary; these assays will henceforth be referred to as the "orthogonal range" assays. Any individuals missing data were removed from the data set, resulting in a total n=147 for this set of assays.

An additional assay was also run as a pilot test of July of 2017 in which 45 Dungeness crab megalopae were divided amongst three $p\text{CO}_2$ treatments ($p\text{CO}_2$=2972, 1079, 446 μatm). Identical procedures were followed as outlined above in the "orthogonal range" assays, except that respirometry testing was done using 5mL vials.

Seawater for manipulation was collected from the flow-through system at Hatfield Marine Science Center (Newport, OR) and then additionally filtered to 400 μm in order to remove detritus, microorganisms, and other contaminates. pH of the water was manipulated by bubbling nitrogen through the sample in order to scrub out dissolved oxygen and carbon dioxide. A small sub-sample was bubbled vigorously with carbon dioxide and slowly re-added to achieve the desired pH. Oxygen was re-added via either agitation or diffusion. Measurement of pH was done using a SAMI pH meter (Sunburst Sensors LLC, Missoula, MT, USA) which functions using a spectrophotometric technique adapted from Dickson et al. for small volumes (2007). Changes in dissolved inorganic carbon and alkalinity were back-calculated using pH and temperature on CO_2Sys (CO2SYS Program, version 2.3, written by Ernie Lewis) to determine full changes in carbonate chemistry, as previously demonstrated by Gray et al (2011). Total alkalinity (2250 μM), salinity (33.5 psu), and temperature (8 °C) were kept constant for these calculations. Manipulated water was stored in 60mL sealed syringes and stored in a cold water bath at 6°C until use in order to prevent changes in dissolved gas content.

It should be noted that despite best intentions, oxygen was difficult to manipulate with precision across multiple samples. Starting oxygen target values were likely altered during the process of transfer from manipulation vessel to respirometry vial, resulting in some scatter of starting oxygen values. This was not an issue in the orthogonal range assays, but it did make it difficult to achieve constant, target starting oxygen values in the four level assays.

Data was collated and organized using the statistical package R (R Core Team, 2016, https://www.R-project.org/) as well as Microsoft Excel (2017).

For the orthogonal range assays, data was organized by starting oxygen and $p\text{CO}_2$. Data was checked for normality and heteroskedasticity before being fit to a Generalized Linear Model with a Gamma distribution and identity link. Variables were chosen via forwards selection and comparison of AIC values. To improve AIC score and eliminate risk of heteroskedasticity of residuals, various transformations were considered. Outliers were identified using Cook's distance and tested for removal in order to significantly improve the model. Assumptions on normality and heteroskedasticity were checked with a Wilks-Shapiro test and a Breush-Pagan test. Model skewness and kurtosis were also checked. The best fit model adhering to these assumptions was run through a two-way ANOVA and effect size was calculated the corresponding eta^2 value. The R package 'changepoint' (Killick R, Haynes K and Eckley IA, 2016, https://CRAN.R-project.org/package=changepoint) was used to check for change points in the data set using the binary segmentation method (Edwards and Cavalli-Sforza 1965).

Orthogonal data was also separated into 13 equal length bins by starting oxygen value. The shift in range of respiration rates was compared across starting oxygen values using a linear regression in order to look at the differences in variance of response.

Originally it was assumed that all megalopae being caught at the same time would be approximately the same size, therefore mass would not need to be corrected for. As research moved forward, this was found to not necessarily be true. It was observed that some of the megalopae appeared to be much larger than others, so it was decided to account for this difference in size moving forward. For the four level assays, wet weight was recorded for each individual, and respiration rates were divided by wet weight in order to better normalize response.

The means of the four level data by conditions and trial were compared using one-way ANOVA and post-hoc Tukey Honest Square Difference testing.   then Kolmogorov Smirnov tests (KS tests) were run to compare distributions during the treatment and recovery. Coefficients of variation were also calculated in order to compare changes in sample variability.

2.3 Results

The techniques outlined above successfully quantified the respiration rates of individual Dungeness crab megalopae. During the original pilot run in July of 2017, respiration rates were found to be much higher than any assay done in 2018, with rates ranging from 0 to approximately 1,800 μmol O_2 individual^{-1} day$^{-1.}$ (*Figure 2-3)*. Although this data is not included in other analysis In this study, it is important to note that individuals in medium to high pCO_2 treatments displayed in increasing trend in rate of respiration as starting oxygen levels increased, while the group in low pCO_2 treatment displayed decreasing respiration rates as starting oxygen increased. This sensitivity to pCO_2 levels was not found in 2018 assays.

Individuals in the orthogonal range assays exhibited a wide range of respiration rates, ranging from 0 to approximately 200 μmol O_2 individual^{-1} day^{-1} (*Figure 2-4).* When arranged versus starting oxygen concentration, there appears to be a good deal of clustering around lower respiration values when there is a decreased starting oxygen concentration. The range of respiration rates seems to increase as oxygen increases, but eventually appears to level out between 125-175 μmol starting O_2. Based on changes to both the mean and the variance of the data, it was found that the most likely change point was occurring at 134 μmol starting O_2 (penalty = 16.82, asymptotic) (*Figure 2-4*).

Data from the orthogonal range assays proved difficult to model due to its distribution. A Gamma family generalized linear model with an identity link was fit to assess the relation of the starting oxygen, pCO_2 level, and the interaction between these terms to the respiration rate measured (*Figure 2-5*). Four outliers were removed to improve the fit of the model; this was deemed acceptable as this portion of the data is focusing more on population reaction to changes in pCO_2 and O_2. Despite a high AIC score (AIC=1274), residuals passed all tests for normality (Shapiro-Wilks W=0.9912, p=0.5198), skewness (-0.1732 > -0.8), platykurtosis (2.85 < 3.0), and homoskedasticity (Breusch-Pagan BP=5.2027, df=3, p=0.1575).

A two-way ANOVA found that all chosen model variables were significant. Eta^2 revealed that effects stemming from starting oxygen values had the largest effect, with an effect size of

0.3390 (F=72.45, $p<0.0001$) (*Table 2-2)*. Interestingly, it appears that there a no significant effect by $p\text{CO}_2$ level ($\text{eta}^2= 0.0004$, p=0.7741) or the interaction term ($\text{eta}^2=0.0054$, p=0.2837). The residuals did take up a majority of the total, with an eta^2 of 0.6493.

The four level data was combined and adjusted to account for wet weight of individuals. For conditions representing upwelling baseline, contemporary, and distant future, the mean respiration rate appeared to be depressed compared to the mean of the same group during benign conditions. During treatment, there appears to be an overall decreasing trend in mean respiration rate (*Figure 2-6)*. A linear regression found a negative relationship between respiration rate, decreasing oxygen, and increasing $p\text{CO}_2$ (slope = 358.99, p-value = 0.05, $R^2_{adj} = 0.8486$).

Looking at 95% confidence intervals, there appears to be evidence for a significant change between the mean respiration rates during treatment and control runs for Upwelling baseline and Distant future treatments, but not evidence of such in Pre-industrial and Contemporary (*Figure 2-7*). A one-way ANOVA comparing the conditions found evidence for differences between groups ($F_{3,144}=3.545$, p-value=0.0162). A post-hoc Tukey HSD determined that the only pairing significantly different from the other was Pre-industrial and Distant future. A one-way ANOVA on the benign condition data found no significant difference between group means ($F_{3,125}=1.773$, p-value=0.156).

Changes in value distribution of respiration rates between the treatment conditions and benign conditions was evaluated visually using density plots, then statistically using Kolmogorov Smirnov testing (*Figure 2-8*). Visually, there does seem to be a shift in median respiration rate value towards zero, especially for the upwelling baseline and distant future treatments, but the only condition that yielded a significant shift in distribution was distant future (KS test, D=0.5389, p-value < 0.0001). This concept was further explored by comparing changes in respiration rate range as a function of starting oxygen concentration (*Figure 2-9)*. A potential decreasing trend in range was observed as the values of the starting oxygen decreased. A general linear regression was fit to the points, but the slope p-value was not significant (p=0.3247), indicating that the slope is not statistically different from zero.

2.4 Discussion

One of the more interesting findings of this research was the high relative importance of starting oxygen concentration on Dungeness crab megalopae respiration rate. This indicates that despite pre-exposure, Dungeness crabs are already exhibiting a reaction to hypoxia stress even within contemporary conditions. It is interesting no note, however, that despite the overall decreasing trend, the only significant difference among condition means was between pre-industrial and distant future. This could potentially indicate that although OAH is exerting some impact, it may not be fully reflected in respiration rate until the distant future.

There was no significant difference between the mean rates for any of the post-treatment runs in benign conditions. This suggests that even though there is a response to OAH stress, there recovery period afterwards is very short, and that there is little to no carry over effect from the acute exposure time. This would be highly beneficial to Dungeness crab in the CCLME, as conditions can change quickly.

Examining the mean rate, however, only addresses part of the issue; this study also seeks to find the effects of OAH stress on the distribution of response. Because there was no support of a trend in respiration rate range as oxygen decreased, it seems as though increased stress from low oxygen was not causing a decrease in response variance.

It is also important to note that the respiration rates collected in these assays were for constrained megalopae in small volumes. While observationally the megalopae appeared to be moving about without significant duress, it is likely that they were not as active as they may be naturally in the water column. It should also be noted that megalopae did vary quite a bit by size and activity level, and this study was not able to fully quantify all of these characteristics. Further studies may find it useful to develop a rating scale for behaviors and activity levels of individual megalopae and compare it to respiration rate.

It was also possible to start to tease apart contributions of the multiple stressors on the respiration rate response. The largest take-away was the difference in effect that $p\mathrm{CO}_2$ had on respiration rate in the 2017 pilot versus the 2018 data. While there have been a wide variety of observed responses to elevated pCO_2 in decapods, it was unexpected that there would be such a wide difference of response by year in individuals from the same system (Ries et al. 2009, Whiteley 2011, Jackson et al. 2018). The pilot data exhibited a clear effect from pCO_2; the medium and high pCO_2 level treatments displayed a trend of increasing respiration rate while respiration rates under low pCO_2 decreased with increasing oxygen, suggesting an interaction effect between oxygen and pCO_2 (*Figure 2-3*). It is possible that the difference in effect size between years could be a result of differences in cohort sensitivity. Studies have found that there are interannual shifts in both Dungeness crab recruitment and population genetic structure, and this data supports the theory that there may be interannual alterations in adaptive capacity as well (Shanks and Roegner 2007, Malley et al. 2017, Jackson et al. 2018). This could potentially be a result of shifts in larval distribution range, illustrating the importance of considering the stability of a species' responses to environmental changes across different cohorts (Jackson et al. 2018).

While this experiment was able to successfully capture respiration rates of megalopae in different pCO_2 and O_2 regimes, the stress was acute and, discounting short acclimation periods, respiration rates could be considered instantaneous. Miller et al (2016) observed increased mortality and decreased rate of development in larval Dungeness crabs over a longer period of time in response to hypoxia and hypercapnia, consequently, it is likely that the metabolic proxies in this experiment do not capture the longer term scale repercussions of exposure to these conditions (Sunday et al. 2014). Growth, for example, was not examined in this study, and could potentially be an issue for Dungeness crabs under hypercapnic conditions. Results on growth in decapods under hypercapnic conditions have been mixed, however, and appear to vary between species, providing an unclear indication of potential time integrated impacts of OA on Dungeness crabs (Long et al. 2013, Glandon and Miller 2017).

Tolerance of hypercapnic conditions has been documented in adult Dungeness crabs, so perhaps these traits are already developing at the megalopae stage (Airriess and McMahon 1994). This is not totally unprecedented in crustaceans; a similar outcome was observed with the blue crab, *Callinectes sapidus,* which showed no influence of pCO_2 on growth nor food consumption, most likely indicating no change in standard metabolic function (Glandon and Miller 2017). It is possible this apparent resiliency to hypercapnia is the result of efficient regulation of internal compartments, either by the development of a tough epithelial layer or the use of existing bicarbonate to buffer internal pH (Pörtner et al. 2004, Pane and Barry 2007, Whiteley 2011).

A potential threshold for oxygen concentrations was found using change point analysis. In modeled data there appears to be a change in mean and variance reaction at approximately 134 μmol (*Figure 2-4*). There is noticeable grouping of respiration rates towards the lower end of starting oxygen concentrations, suggesting depression of respiration under stress. Above 134 μmol, while there may be some grouping around the mean, there appears to be a much more spread. This threshold was unexpectedly high, equivalent to approximately 47% saturation at experimental conditions. Oxygen levels lower than this are quite common at depth in the CCLME, so Dungeness crab megalopae could frequently be interacting with water masses below their optimum oxygen levels. As Pane & Barry (2007) found, adult Dungeness crabs did not start to stabilize their internal acid-base balance until after approximately 75 minutes of exposure to 40 μmol (14% at 10°C) oxygen saturation. If this is approximately the same in megalopae, then the duration of the trial should encapsulate the acclimation period.

2.5 Conclusion

Although the seasonal carbonate chemistry and oxygen extremes of the CCLME can present a challenge to marine animals, it has been proposed that many have experienced significant local adaptations to allow them to tolerate a wide variety of stress magnitudes and durations (Sanford and Kelly 2011, Pespeni et al. 2013a, Vargas et al. 2017). In this experiment I found that although Dungeness crab megalopae respiration rate had a negative relationship with decreasing oxygen and increasing $p\text{CO}_2$, there was not a statistically significant alteration in mean rate until distant future conditions were reached, indicating that the threshold has not yet been reached.

There are, however, several things to consider. The first is that this experiment accounted only for brief, acute exposure to conditions and does not account for long term scale metabolic requirements such as growth and reproduction. It is possible that although overall metabolic demands were being met that energetic production was being shifted towards homeostatic function rather than digestion, reproduction, or growth (Bernatis et al. 2007, Kroeker et al. 2013b, Long et al. 2013, Miller et al. 2016, Frieder et al. 2018). Use of both longer term experiments in conjunction with individual respiration rate techniques would certainly be of use in quantifying change with regards to exposure length, and use of genetic analysis could help identify local standing variability and adaptation in progress (Sunday et al. 2011, 2014, De Wit and Palumbi 2013, Munday et al. 2013, Pespeni et al. 2013b).

The second is the reality of changing conditions in the CCLME. Although it may appear that Dungeness crabs have some tolerance for hypoxia and hypercapnia, there are physiological limits. From the results of this experiment, $p\mathrm{CO}_2$ may play a variable role in the overall stress of OAH, but oxygen consistently has a strong effect. This makes issues such as shelf-wide severe hypoxia or anoxia even more concerning (Chan et al. 2008). Even if Dungeness crabs are able to tolerate prolonged hypercapnia with minimal effect, there is no doubt that large anoxic areas would prove fatal without methods to escape, such as has occurred off the coast of Newport, OR in recent years (ODFW, http://oregonmarinereserves.com/ 2017/09/06/hypoxia-central-coast).

While it seems that not all adult crabs may not be able to escape these anoxic water masses, it is unknown whether Dungeness megalopae possess any ability to avoid these areas. Other species of crab megalopae have been found to practice diurnal migration indicating capability to control position in the water column; if megalopae are able to come up into the mix layer, they could avoid deeper anoxic conditions and practice physical avoidance, but it remains to be seen if they could incorporate oxygen levels into their sensory signals to drive migration (Cronin and Forward 1983, Shanks 1986, Olmi 1994, Kingsford et al. 2002). This ability needs to be mapped and considered for Dungeness crabs in order to determine population vulnerability to seasonal anoxia in the CCLME.

Although this study found the potential for standing variability and adaptive capacity in Dungeness crab megalopae in the CCLME, there is still much to learn. The realities of OAH are presenting themselves already, but if projections are correct, this is only the beginning and new repercussions may begin to emerge (Snyder et al. 2003, Gruber et al. 2012, Barton et al. 2015, Ekstrom et al. 2015, Feely et al. 2018). It will be essential to monitor not only the populations of potentially vulnerable species, but also the intrapopulation diversity and phenotypic reservoir available to contribute to tolerance and adaptation (Sunday et al. 2011, 2014, Pespeni et al. 2013a, Vargas et al. 2017).

2.6 Works Cited

Airriess, C., and B. McMahon. 1994. Cardiovascular adaptations enhance tolerance of environmental hypoxia in the crab Cancer magister. Journal of Experimental Biology 190:23–41.

Barth, J., J. Fram, E. Dever, C. Risien, C. Wingard, R. Collier, and T. Kearney. 2018. Warm Blobs, Low-Oxygen Events, and an Eclipse: The Ocean Observatories Initiative Endurance Array Captures Them All. Oceanography 31:90–97.

Barton, A., G. G. Waldbusser, R. A. Feely, S. B. Weisberg, J. A. Newton, B. Hales, S. Cudd, B. Eudeline, C. J. Langdon, I. Jefferds, T. King, A. Suhrbier, and K. McLaughlin. 2015. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography 28:146–159.

Bernatis, J. L., S. L. Gerstenberger, and I. J. Mcgaw. 2007. Behavioural responses of the Dungeness crab , Cancer magister , during feeding and digestion in hypoxic conditions:941–951.

Breitburg, D. L., J. Salisbury, J. M. Bernhard, W.-J. Cai, S. Dupont, S. C. Doney, K. J. Kroeker, L. A. Levin, W. C. Long, L. M. Milke, S. H. Miller, B. Phelan, U. Passow, B. A. Seibel, A. E. Todgham, and A. M. Tarrant. 2015. And on top of that... Coping with ocean acidification in the midst of many stressors. Oceanography 28:48–61.

Caldeira, K., and M. E. Wickett. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. Journal of Geophysical Research 110:1–12.

Chan, F., J. A. Barth, C. A. Blanchette, R. H. Byrne, F. Chavez, O. Cheriton, R. A. Feely, G. Friederich, B. Gaylord, T. Gouhier, S. Hacker, T. Hill, G. Hofmann, M. A. McManus, B. A. Menge, K. J. Nielsen, A. Russell, E. Sanford, J. Sevadjian, and L. Washburn. 2017. Persistent spatial structuring of coastal ocean acidification in the California Current System. Scientific Reports 7:1–7.

Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California Current Large Marine Ecosystem. Science 319:920.

Cronin, T. W., and R. B. Forward. 1983. Vertical Migration Rhythms of Newly Hatched Larvae of the Estuarine Crab, Rhithropanopeus harrisii. The Biological Bulletin 165:139–153.

Cullison Gray, S. E., M. D. DeGrandpre, T. S. Moore, T. R. Martz, G. E. Friederich, and K. S. Johnson. 2011. Applications of in situ pH measurements for inorganic carbon calculations. Marine Chemistry 125:82–90.

Dickson, A. G., C. L. Sabine, and J. R. Christian. 2007. Guide to Best Practices for Ocean CO 2 Measurements.

Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas. 2009. Ocean Acidification: The Other CO 2Problem. Annual Review of Marine Science 1:169–192.

Edwards, A. W. F., and L. L. Cavalli-Sforza. 1965. A Method for Cluster Analysis. Biometrics 21:362–375.

Ekstrom, J. A., L. Suatoni, S. R. Cooley, L. H. Pendleton, G. G. Waldbusser, J. E. Cinner, J. Ritter, C. Langdon, R. Van Hooidonk, D. Gledhill, K. Wellman, M. W. Beck, L. M. Brander, D. Rittschof, C. Doherty, P. E. T. Edwards, and R. Portela. 2015. Vulnerability and adaptation of US shellfisheries to ocean acidification. Nature Climate Change 5:207–214.

Fabry, V. J., B. A. Seibel, R. A. Feely, and J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal Marine Science 65:414–432.

Feely, R. A., R. R. Okazaki, W. J. Cai, N. Bednaršek, S. R. Alin, R. H. Byrne, and A. Fassbender. 2018. The combined effects of acidification and hypoxia on pH and aragonite saturation in the coastal waters of the California current ecosystem and the northern Gulf of Mexico. Continental Shelf Research 152:50–60.

Feely, R. A., C. L. Sabine, J. M. Hernandez-ayon, D. Ianson, and B. Hales. 2008. Evidence for Upwelling of Corrosive “Acidified” Water on to the Continental Shelf. Science 320:1490–1493.

Feely, R. A., C. L. Sabine, R. Schlitzer, J. L. Bullister, S. Mecking, and D. Greeley. 2004. Oxygen utilization and organic carbon remineralization in the upper water column of the Pacific Ocean. Journal of Oceanography 60:45–52.

Frieder, C. A., S. L. Applebaum, and F. Pan. 2018. Shifting Balance of Protein Synthesis and Degradation Sets a Threshold for Larval Growth Under Environmental Stress.

Froehlich, H. E., S. Barbara, T. E. Essington, and A. H. Beaudreau. 2015. Movement Patterns and Distributional Shifts of Dungeness Crab Movement Patterns and Distributional Shifts of Dungeness Crab (Metacarcinus magister) and English Sole (Parophrys vetulus) During Seasonal Hypoxia.

Futuyma, D. J., and G. Moreno. 1988. The Evolution of Ecological Specialization Author. Annual Review of Ecology and Systematics 19:207–233.

Gazeau, F., L. M. Parker, S. Comeau, J. P. Gattuso, W. A. O’Connor, S. Martin, H. O. Portner, and P. M. Ross. 2013. Impacts of ocean acidification on marine shelled molluscs. Marine Biology 160:2207–2245.

Glandon, H. L., and T. J. Miller. 2017. No effect of high $p\mathrm{CO}_2$on juvenile blue crab, Callinectes sapidus, growth and consumption despite positive responses to concurrent warming. ICES Journal of Marine Science 74:1201–1209.

Gruber, N., C. Hauri, Z. Lachkar, D. Loher, T. L. Frölicher, and G.-K. Plattner. 2012. Rapid progression of ocean acidification in the California Current System. Science (New York, N.Y.) 337:220–3.

Harris, K. E., M. D. Degrandpre, and B. Hales. 2013. Aragonite saturation state dynamics in a coastal upwelling zone. Geoph 40:2720–2725.

Hauri, C., N. Gruber, M. Vogt, S. C. Doney, R. A. Feely, Z. Lachkar, A. Leinweber, A. M. P. McDonnell, and M. Munnich. 2013. Spatiotemporal variability and long-term trends of ocean acidification in the California Current System. Biogeosciences 10:193–216.

Howes, E. L., N. Bednaršek, J. Budenbender, S. Comeau, A. Doubleday, S. M. Gallager, R. R. Hopcroft, S. Lischka, A. E. Maas, J. Bijma, and J. P. Gattuso. 2014. Sink and swim: A status review of thecosome pteropod culture techniques. Journal of Plankton Research 36:299–315.

Jensen, G. C., and D. A. Armstrong. 1987. Range Extensions of Some Northeastern Pacific Decapoda. Crustaceana 52:215–217.

Keller, A. A., V. Simon, F. Chan, W. W. Wakefield, M. E. Clarke, J. A. Barth, D. A. N. Kamikawa, and E. L. Fruh. 2010. Demersal fish and invertebrate biomass in relation to an offshore hypoxic zone along the US West Coast:76–87.

Kelly, M. W., and G. E. Hofmann. 2013. Adaptation and the physiology of ocean acidification. Functional Ecology 27:980–990.

Kim, T. W., J. P. Barry, and F. Micheli. 2013. The effects of intermittent exposure to low-pH and low-oxygen conditions on survival and growth of juvenile red abalone. Biogeosciences 10:7255–7262.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities, and local self-recruitment. Bulletin of Marine Science 70:309–340.

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, and J. P. Gattuso. 2013a. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. Global Change Biology 19:1884–1896.

Kroeker, K. J., R. L. Kordas, and C. D. G. Harley. 2017. Embracing interactions in ocean acidification research: confronting multiple stressor scenarios and context dependence. Biology Letters 13:20160802.

Kroeker, K. J., F. Micheli, and M. C. Gambi. 2013b. Ocean acidification causes ecosystem shifts via altered competitive interactions. Nature Climate Change 3:156–159.

Lande, R., and S. Shannon. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437.

Long, W. C., K. M. Swiney, C. Harris, H. N. Page, and R. J. Foy. 2013. Effects of Ocean Acidification on Juvenile Red King Crab (Paralithodes camtschaticus) and Tanner Crab (Chionoecetes bairdi) Growth, Condition, Calcification, and Survival. PLoS ONE 8.

McMahon, B. R. 2001. Respiratory and circulatory compensation to hypoxia in crustaceans. Respiration Physiology 128:349–364.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, and P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (Cancer magister). Marine Biology 163:1–11.

Munday, P. L., R. R. Warner, K. Monro, J. M. Pandolfi, and D. J. Marshall. 2013. Predicting evolutionary responses to climate change in the sea. Ecology Letters 16:1488–1500.

Olmi, E. J. 1994. Vertical migration of blue crab Callinectes sapidus megalopae: Implications for transport in estuaries. Marine Ecology Progress Series 113:39–54.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. G. Najjar, G.-K. Plattner, K. B. Rodgers, C. L. Sabine, J. L. Sarmiento, R. Schlitzer, R. D. Slater, I. J. Totterdell, M.-F. Weirig, Y. Yamanaka, and A. Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–6.

Pan, T.-C. F., S. L. Applebaum, and D. T. Manahan. 2015. Experimental ocean acidification alters the allocation of metabolic energy. Proceedings of the National Academy of Sciences 112:4696–4701.

Pane, E. F., and J. P. Barry. 2007. Extracellular acid – base regulation during short-term hypercapnia is effective in a shallow-water crab , but ineffective in a deep-sea crab 334:1–9.

Pespeni, M. H., F. Chan, B. A. Menge, and S. R. Palumbi. 2013a. Integrative and Comparative Biology Signs of Adaptation to Local pH Conditions across an Environmental Mosaic in the California Current Ecosystem. Integrative and Comparitive Biology 53:857–870.

Pespeni, M., E. Sanford, B. Gaylord, T. Hill, J. Hosfelt, H. K. Jaris, M. LaVigne, E. Lenz, A. Russell, M. Young, and S. Palumbi. 2013b. Evolutionary change during experimental ocean acidification. Proceedings of the National Academy of Sciences 110:6937–6942.

Pörtner, H.-O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology Progress Series 373:203–217.

Pörtner, H. O., M. Langenbuch, and A. Reipschlager. 2004. Biological Impact of Elevated Ocean CO2 Concentrations: Lessons from Animal Physiology and Earth History. Journal of Oceanography 60:705–718.

Ries, J. B., A. L. Cohen, D. C. Mccorkle, J. B. Ries, A. L. Cohen, and D. C. Mccorkle. 2009. Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. Geology 37:1131–1134.

Sanford, E., and M. W. Kelly. 2011. Local Adaptation in Marine Invertebrates. Annual Review of Marine Science 3:509–535.

Seibel, B. A., A. E. Maas, and H. M. Dierssen. 2012. Energetic plasticity underlies a variable response to ocean acidification in the pteropod, Limacina helicina antarctica. PLoS ONE 7.

Shanks, A. L. 1986. Vertical migration and cross-shelf dispersal of larval Cancer spp. and Randallia ornata (Crustacea: Brachyura) off the coast of southern California. Marine Biology: International Journal on Life in Oceans and Coastal Waters 92:189–199.

Shanks, A. L., and G. C. Roegner. 2007. Recruitment limitation in Dungeness crab populations is driven by variation in atmospheric forcing. Ecology 88:1726–1737.

Snyder, M. A., L. C. Sloan, N. S. Diffenbaugh, and J. L. Bell. 2003. Future climate change and upwelling in the California Current. Geophysical Research Letters 30:1823.

Somero, G. N., J. M. Beers, F. Chan, T. M. Hill, T. Klinger, and S. Y. Litvin. 2015. What Changes in the Carbonate System, Oxygen, and Temperature Portend for the Northeastern Pacific Ocean: A Physiological Perspective. BioScience 66:14–26.

Sunday, J. M., P. Calosi, S. Dupont, P. L. Munday, J. H. Stillman, and T. B. H. Reusch. 2014. Evolution in an acidifying ocean. Trends in Ecology and Evolution 29:117–125.

Sunday, J. M., R. N. Crim, C. D. G. Harley, and M. W. Hart. 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. PLoS ONE 6:1–8.

Vargas, C. A., N. A. Lagos, M. A. Lardies, C. Duarte, P. H. Manríquez, V. M. Aguilera, and B. Broitman. 2017. Species-specific responses to ocean acidification should account for local adaptation and adaptive capacity. Nature Ecology and Evolution 1:1–7.

Waldbusser, G. G., B. Hales, C. J. Langdon, B. A. Haley, P. Schrader, E. L. Brunner, M. W. Gray, C. A. Miller, and I. Gimenez. 2015a. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nature Climate Change 5:273–280.

Waldbusser, G. G., B. Hales, C. J. Langdon, B. A. Haley, P. Schrader, E. L. Brunner, M. W. Gray, C. A. Miller, I. Gimenez, and G. Hutchinson. 2015b. Ocean acidification has multiple modes of action on bivalve larvae. PLoS ONE 10.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. Marine Ecology Progress Series 430:257–271.

De Wit, P., and S. R. Palumbi. 2013. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. Molecular Ecology 22:2884–2897.

Wittmann, A. C., and H. O. Pörtner. 2013. Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change 3:995–1001.

2.7 Appendix

Figure 2-1 Illustration of respirometry equipment. Individual megalopae were placed in gas tight vials and in vitro *changes in oxygen concentration were measured using an oxygen sensitive optode (represented by pink dot in vial) and a Fibox 3 Oxygen Sensor. Measurements were taken at multiple points during the assay in order to calculate respiration rate, and between measurements vials were kept in a cold water bath maintained at 10℃*

Table 2-1 Target four-level treatment conditio

Treatment	pH
Pre-Industrial	8.17

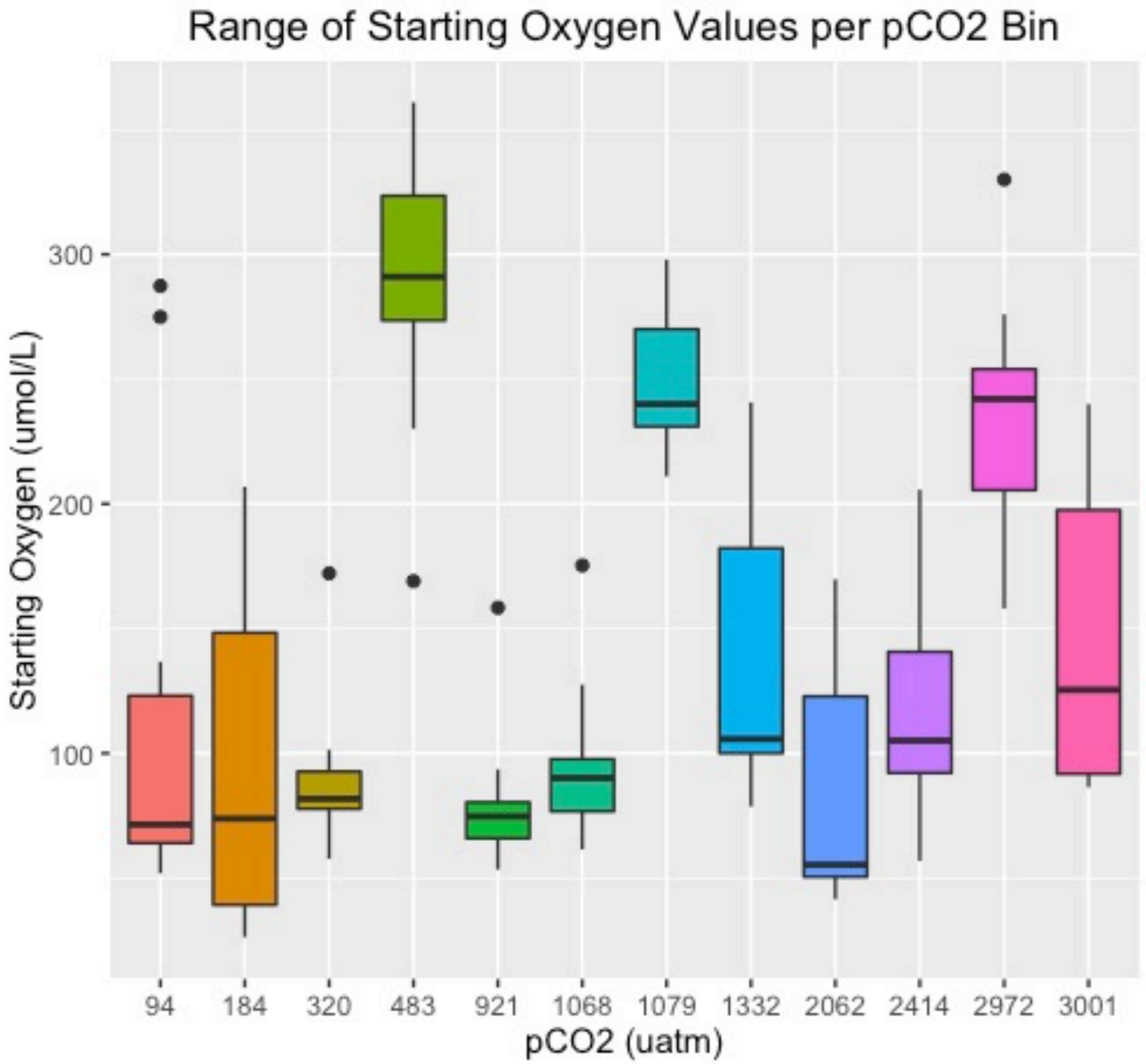


Figure 2-2 Starting oxygen concentration range across pCO_2 *values for orthogonal range assays.*

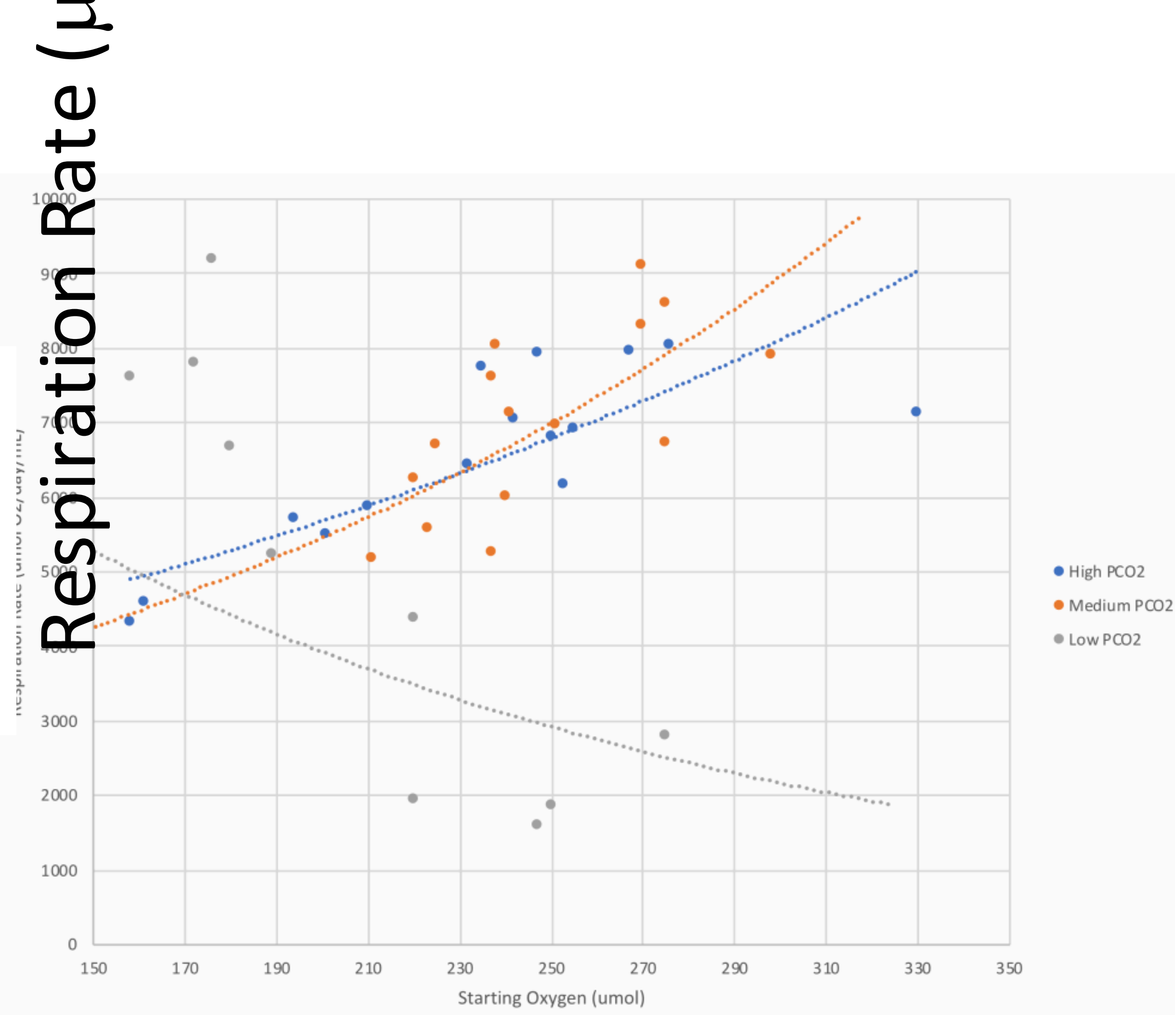


Figure 2-3 Respiration rates for Dungeness crab megalopae during an initial pilot study in July of 2017. Note that not only are the respiration rates much higher, there appears to be a change in response to oxygen concentration shift as a function of pCO_2*, a pattern not reflected in the data collected during the 2018 sampling season. Note that these values have not been adjusted for volume, and true respiration rates per individual need to be divided by five.*

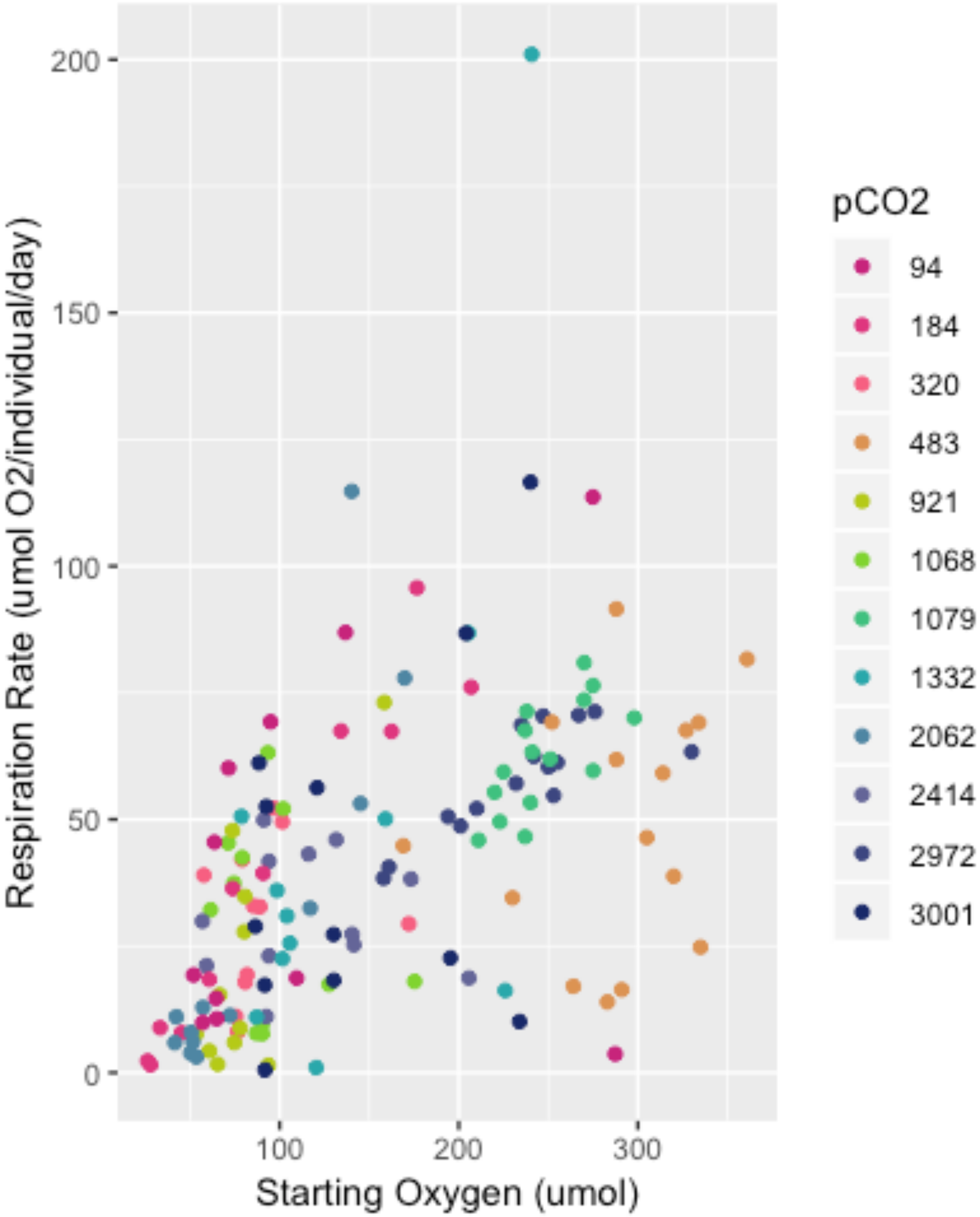


Figure 2-4 Starting oxygen concentration versus the respiration rate of Dungeness crab megalopae in the orthogonal range assays coded by pCO_2. *Note that this data is not mass adjusted.*

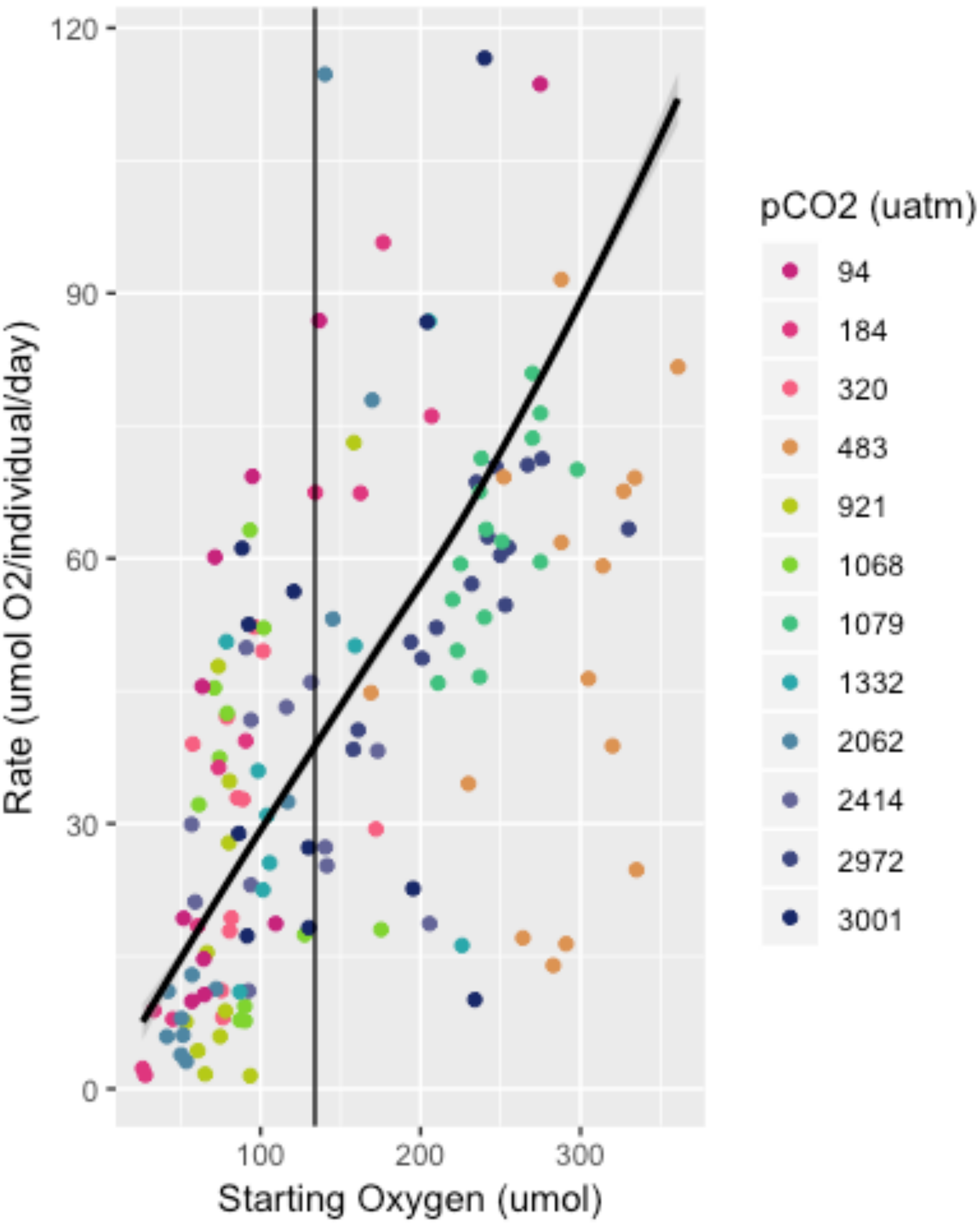


Figure 2-5 Generalized Linear Model fit to un-transformed orthogonal range data (AIC 2823). Vertical line represented calculated change point in data at 134 µmol O_2. Note clustering of data to the left of the change point, and more random distribution to the right.

Table [illegible] Eta squared values for modeled varia

Variable	η^2
Starting Oxygen	0.3390

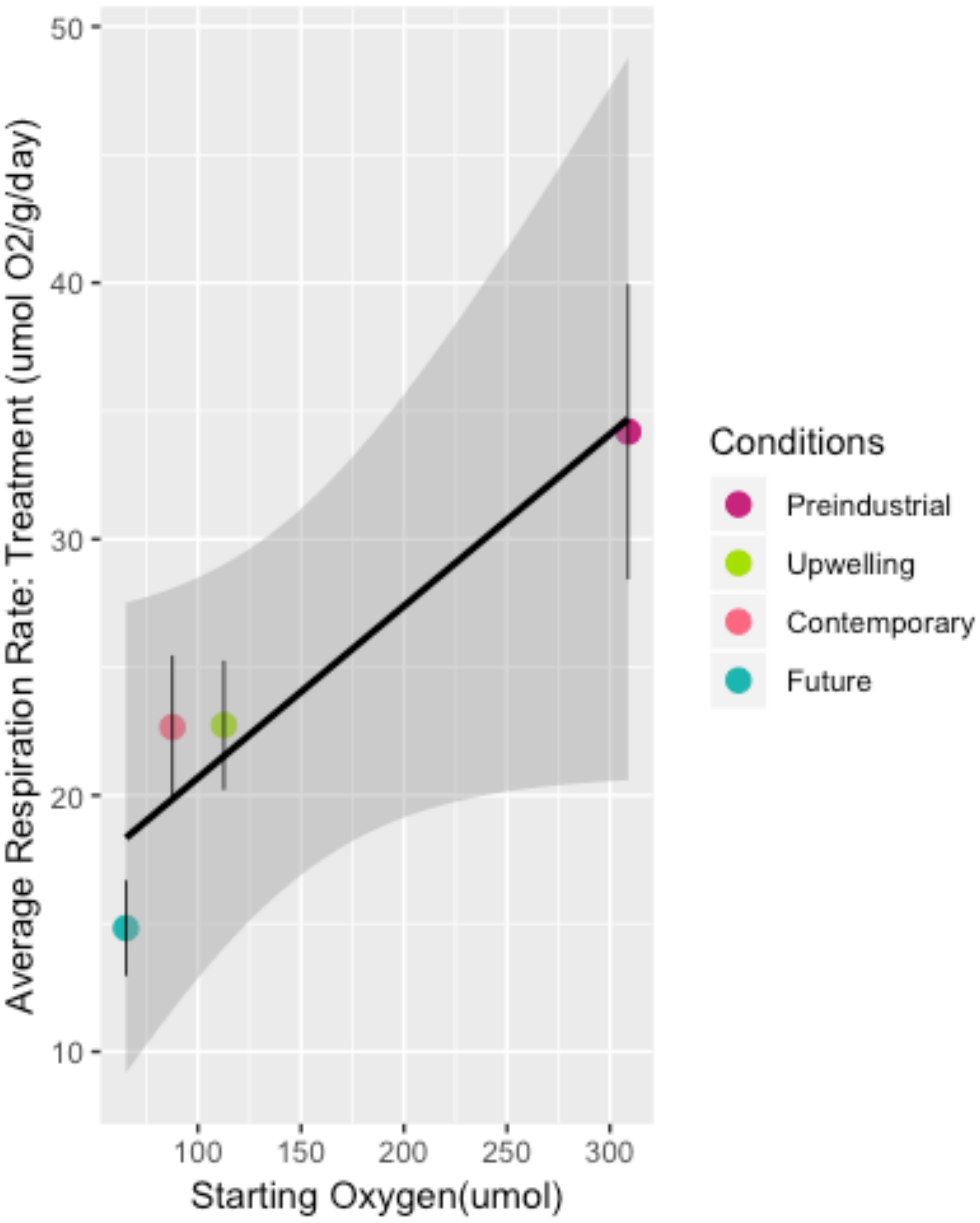


Figure 2-6 Linear regression of the means for each of four conditions plotted against starting oxygen. Gray shading represents standard error. The slope of the line is 358.99 with a p-value of 0.05. The R^2_{adj} *of the fit is 0.8486. Note that the conditions are in order by starting oxygen, not temporally; the trend temporally moves from right to left.*

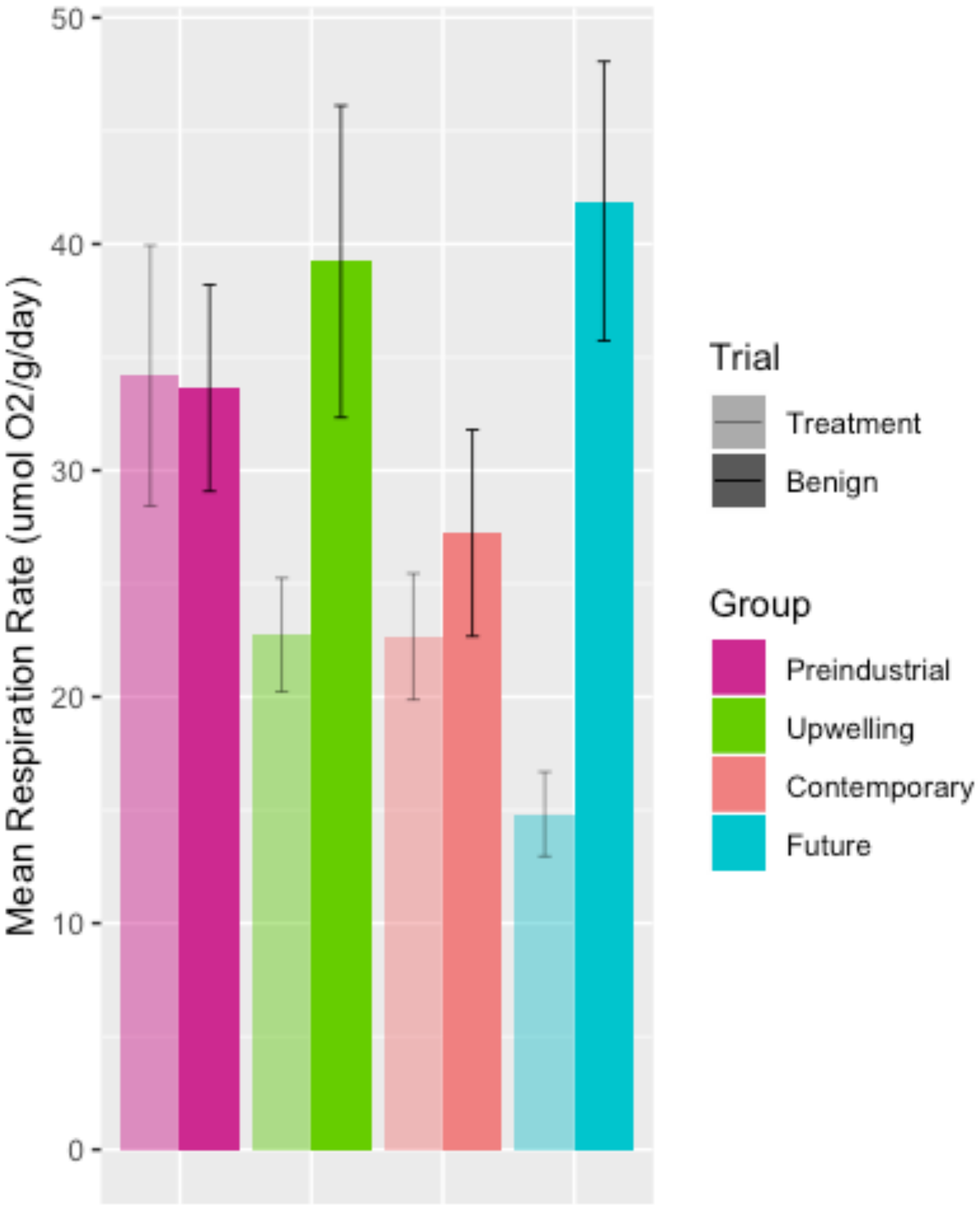
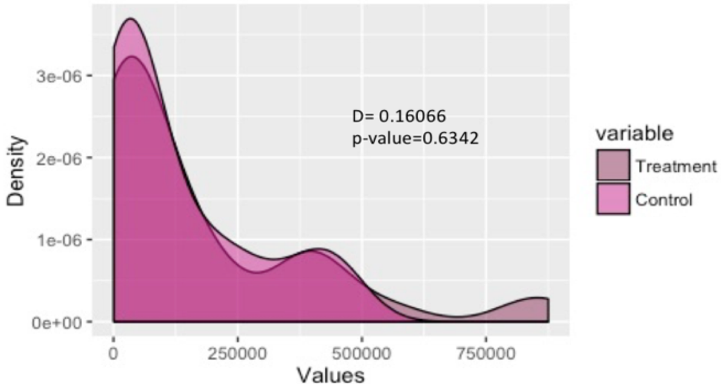


Figure 2-7 Mean respiration rates by level and treatment. Standard error bars represent 95% confidence interval. The treatment trial represents the mean respiration rate under each one of the four conditions outlined in Table 2-1. *The control is the mean respiration rate for the same individuals subjected to benign conditions. The only statistically significant pair for the treatment trials was between Preindustrial and Distant future. There were no significant differences between control runs.*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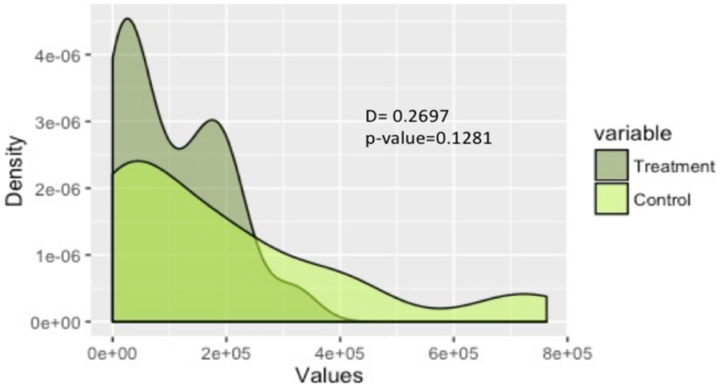
variable
Treatment
Control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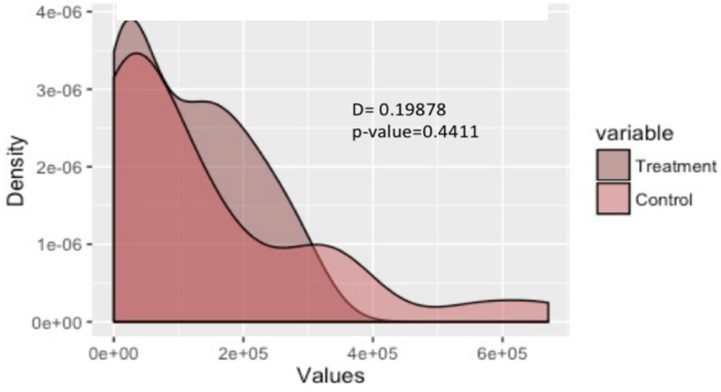
Treatment
Control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1e-06
0e+00
0e+00
2e+05
4e+05
6e+05
Values
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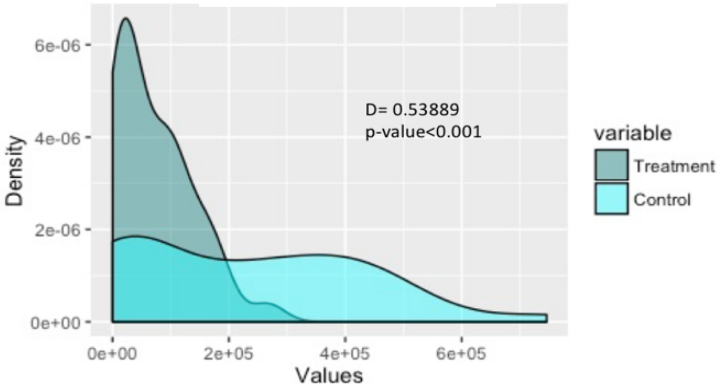
p-value=0.4411
variable
Treatment
Control


Density
6e-06
4e-06
2e-06
0e+00
0e+00
2e+05
4e+05
6e+05
Values
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p-value<0.001
variable
Treatment
Control

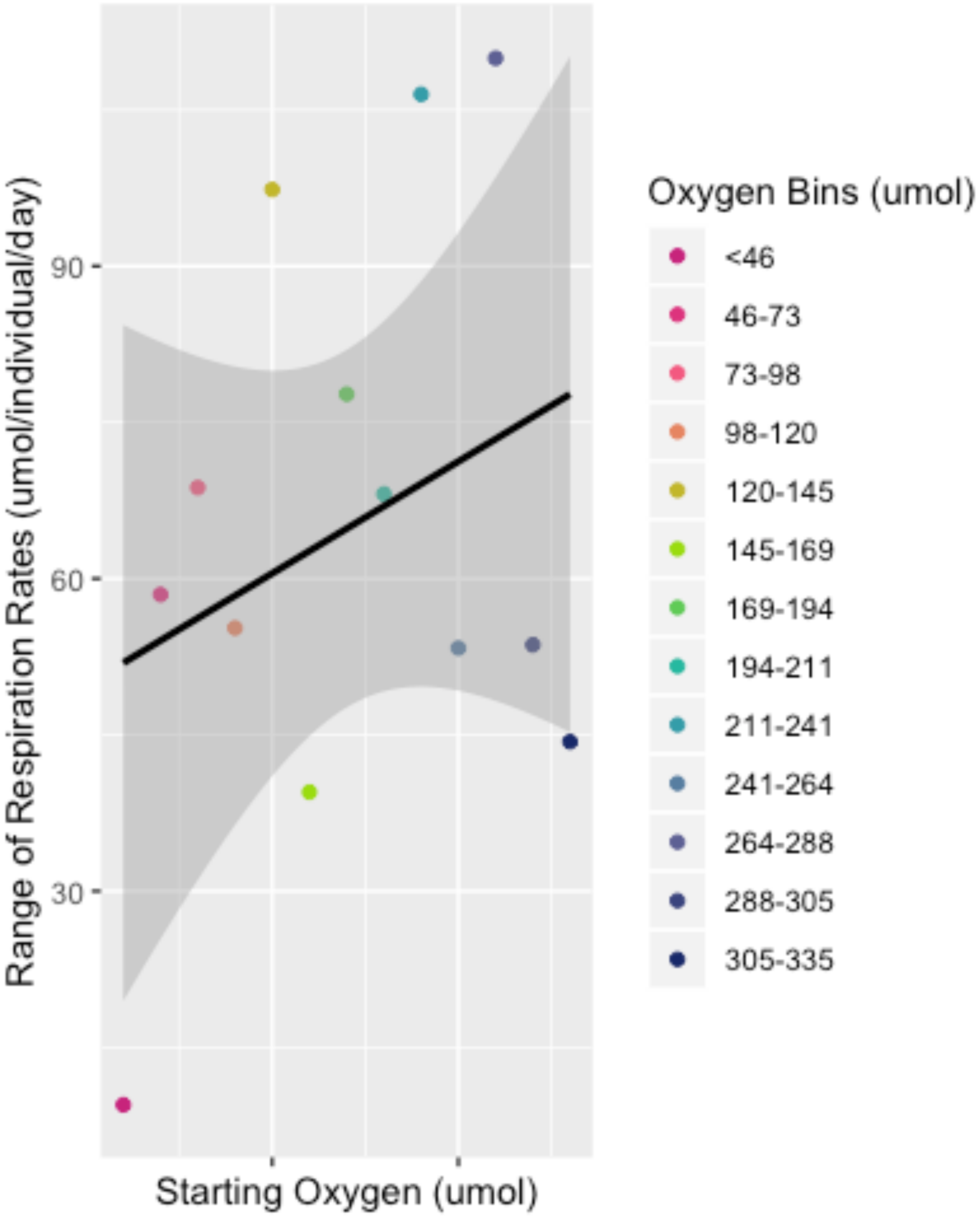

Figure 2-9 Alterations in range of Dungeness crab megalopae respiration rate in response to changes in oxygen. The line represents a linear regression, with shading symbolizing standard error. The slope of the regression line was 2.084 (p-value=0.3247, R^2_{adj}=0.0052)

3 Sensitivity and Resilience of red abalone (Haliotis rufescens) to projected hypercapnic and hypoxic conditions in the California Current

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Abstract

Stress to organisms stemming from ocean acidification and hypoxia (OAH) are projected to worsen over the next century, resulting in non-analog conditions which challenge many marine animals. Local to regional scale phenomena results in hotspot of OAH exposure in the California Current Large Marine Ecosystem (CCLME). A combination of stress resulting from exposure to both hypercapnia and hypoxia presents a challenge to marine organisms. In order to understand and better model future outcomes of species, it is important to understand not only the mean response to changes in conditions and where thresholds lie, but also how variability within the population changes and translates into long term adaptive capacity. This study focuses on *Haliotis rufescens*, or the red abalone, a large marine gastropod native to the southern portion of the CCLME, and currently the center of a struggling industry. Using respirometry as a proxy for metabolic exertion, individual aerobic respiration rates at four regimes (pre-industrial, modern, modern-poor, predicted for 2100) of oxygen and carbon dioxide were recorded and compared to individual rates in high oxygen, low carbon dioxide conditions. It was found that *H. rufescens* showed evidence of a synergistic, negative effect of lowered oxygen and hypercapnia resulting in aerobic metabolism depression and a shift in respiration rate value density. It was also observed that there was no significant difference in response to modern, upwelling, or future scenarios, possibly indicating a strong ability to acclimate to less than idea situations. Lastly, effect size of variables in a best fit model remained quite low which suggests a sizable phenotypic plasticity within the group sampled. Although this study only examines a snapshot response to changes in conditions, high variability in red abalone could be an indicator of adaptive capacity to changing ocean conditions.

3.1 Introduction

Over the last decade it has become apparent that stress to organisms stemming from increased ocean acidification and hypoxia (OAH) are in urgent need of further investigation (Feely et al. 2004a, Caldeira and Wickett 2005, Orr et al. 2005). On the largest scale, ocean acidification stems from increased anthropogenic carbon dioxide emissions, but it can also be affected by regional to local scale processes such as upwelling, runoff, and excessive remineralization (Caldeira and Wickett 2005, Orr et al. 2005, Doney et al. 2007, Hauri et al. 2013). This leads to hotspot locations where the repercussions of OAH are felt stronger and earlier, such as in the California Current Large Marine Ecosystem (CCLME) (Gruber et al. 2012, Hauri et al. 2013, Chan et al. 2017). The CCLME serves as a natural laboratory for OAH experiments, producing a wide range of extremes in conditions (Feely et al. 2008, Then et al. 2016, Gaitán-Espitia et al. 2017). During the summer months, strong upwelling conditions can result in widespread hypoxia or even anoxia well up onto the continental shelf along with hypercapnic, low pH conditions (Chan et al. 2008, Somero et al. 2015, Feely et al. 2018).

Physiologically, these conditions are challenging to marine organisms (Widdicombe and Spicer 2008, Portner 2010, Kelly and Hofmann 2013). Low oxygen triggers a stress reaction by reducing capacity to produce ATP and putting a strain on metabolic function, while hypercapnia can interfere with internal acid base balance (Guppy and Withers 1999). Hypercapnia also presents an extra challenge to organisms that use calcium carbonate ($CaCO_3$) for structure or function, as lowered pH and carbonate availability decreases the saturation state (Ω) of seawater with regards to calcium carbonate. Because calcification moves forward most readily in an environment at or over saturation ($\Omega \geq 1$) with $CaCO_3$, lowered saturation state can result in metabolic exertion or even dissolution of calcified material (Orr et al. 2005, Hofmann et al. 2010, for review).

The strength of these effects varies both between and within species and populations of organisms depending on lifestyle, structural components, and exposure (Orr et al. 2005, Widdicombe and Spicer 2008, Parker et al. 2012, Kroeker et al. 2013a). Understanding differences in response on an individual level is important as it is essential for determining how increasing stress can affect the capacity of the population to rebound from severe episodes and potentially contribute to evolutionary rescue (Dawson et al. 2011, Ellner 2013, Kelly and Hofmann 2013, Sunday et al. 2014).

This study focuses on the species *Haliotis rufescens*, or the red abalone. The red abalone is the last remaining abalone species with commercial viability on the U.S. West Coast (Reid et al. 2016). Despite a beleaguered past, the United States currently produces approximately 220 metric tons of commercially grown red abalone per year (Karpov et al. 2000, *Farmed Abalone Report* 2017). Until the closure in 2018, there was quite a large recreational fishery in California for red abalone as well. In 2013, it was calculated that approximately 31,000 individuals participated in harvesting abalone recreationally, spending between $24 million and $44 million on the activity (Reid et al. 2016). Unfortunately, in late 2017 the 2018 season was declared canceled by California Fish and Game due to poor conditions and low abalone density counts (*'California rec…',* 2017, https://cdfgnews.wordpress.com/2017/12/08/california-recreational-abalone-fishery-to-be-closed-in-2018/). Oregon was soon to follow, also closing their recreational fishery (*'Oregon postpones…',* 2017, https://www.dfw.state.or.us/news/2017/12_dec/122817.asp). As the regulatory bodies work to resuscitate the fishery, it will be necessary to consider resiliency of this species to OAH.

The red abalone is a large, marine gastropod present in the CCLME from southern Oregon to Baja California (Estes et al. 2005). As gastropods, their shell is composed primarily of aragonite, a fairly soluble $CaCO_3$ morph, leaving the mineral more vulnerable to dissolution (Fabry et al. 2008, Zippay and Hofmann 2010, Miller et al. 2016). Effect of low pH on calcification in a related species *Haliotis iris* yielded mixed results due to the balance between a thin surface diffusion boundary layer combined with extrapallial calcification, but the intricacies of the effect on *Haliotis rufescens* calcification is not yet known (Hurd et al. 2011, Tripp et al. 2017).

This is not the only effect of OAH on abalone; prolonged exposure of juvenile (5-10mm) red abalones to lowered pH (7.5) and oxygen (5mg/L) can lead to reduction of shell growth (Kim et al. 2013). There is also evidence of synergistic effects of changing ocean conditions, as it has been shown that red abalone in the late veliger stage are more sensitive to low pH in combination with higher temperature and sensitivity changed with life stage (Portner 2010, Zippay and Hofmann 2010). This is not to say that the red abalone have no adaptive capacity for these conditions; Several studies have found genetic or physiological evidence of local adaptation and variance within red abalone living in the CCLME (De Wit and Palumbi 2013, Kim et al. 2013).

In order to further test the sensitivity and variance of response of the red abalone to OAH, this experiment seeks to determine stress response to four different oxygen and carbon dioxide exposure conditions representing pre-industrial (>200μmol O_2, $p$$CO_2$=285 μatm), baseline upwelling conditions (75μmol O_2, $p$$CO_2$=1238 μatm), contemporary upwelling conditions (40μmol O_2, $p$$CO_2$=1508 μatm), and values projected for the distant future (30μmol O_2, $p$$CO_2$=2628 μatm) (Feely et al. 2004b, 2018, Chan et al. 2017). Respiration rates were recorded for approximately 120 abalone first in a selected treatment condition, then again afterwards in O_2 rich and low $p$$CO_2$ conditions. The juvenile stage was selected for study as it has been found to be the age which has the largest leverage on future cohort size (Rogers-Bennett et al. 2004, Kim et al. 2013).

There two main goals of this project were to (1) determine the physiological threshold of juvenile red abalone to increasing exposure to OAH stress, and (2) examine the relationship between variance of response and the intensity of OAH stress. Due to prior exposure to upwelling conditions in their natural habitat, I hypothesized that juvenile red abalone would not reach their physiological threshold to OAH stressors until distant future conditions are reached. Along with this, I expected that co-exposure to both low oxygen and high $p$$CO_2$ would have an interactive effect on the respiration rate. Lastly, I hypothesized that variance in individual performance will decrease as the stress from hypoxia and hypercapnia increases.

3.2 Methods

The *Haliotis rufescens* (red abalone) samples for this experiment were reared in Moss Landing, CA and maintained temporarily at Monterey Bay Aquarium Research Institute, also in Moss Landing, CA. In August 2018, approximately 250 juvenile (size 10-15mm) individuals were shipped overnight to Corvallis, OR in an insulated box containing ice packs. Abalone were placed in shellfish bags with fresh *Palmaria palmata* at a density of 125 juveniles per bag and held in outdoor flow-through tanks at Hatfield Marine Science Center in Newport, OR until their use in experiments. The bags were checked every other day to ensure sufficient seaweed and monitor health of subjects.

A total of four experiments were run during August of 2018 with each run utilizing approximately 32 individuals divided among four treatment conditions (*Table 3-1)*. Each experiment represented a block and data was pooled, and any individual missing data was removed, resulting in a total sample size of 128.

The four different target conditions were calculated by selected oxygen values representative of preindustrial, upwelling baseline, contemporary upwelling, and distant future conditions (Chan et al. 2017, Feely et al. 2018). Dissolved inorganic carbon (DIC) was then back-calculated using the published relationship between dissolved oxygen and DIC in Feely et al (2004b). An additional subsidy of anthropogenic DIC was added to current and future scenarios. All calculations were adjusted for the target temperature of the respiration assays (10 ± 0.1 °C), total alkalinity was assumed to be 2250 μmol/kgSW, and salinity 33.5 psu.

Seawater for all experimentation was collected from the flow-through system at Hatfield Marine Science Center (Newport, OR). Before manipulation, water was filtered to 400 μm to ensure removal of any large cells and detritus. Carbonate chemistry was manipulated by first stripping the water using nitrogen gas. Additional filtered seawater was bubbled vigorously with carbon dioxide gas, then slowly added until the desired $p\text{CO}_2$ was reached. Oxygen was manipulated in general via diffusion and re-scrubbing with nitrogen. Approximate oxygen saturation percentages were checked using a Hach Luminescent Dissolved Oxygen probe (Hach Industries, Colorado, USA). Precision pH measurement was done using a Durafet pH probe (Honeywell, Morris Plains, NJ, USA) which measures pH by ion-sensitive field-effect transistor. Readings were taken with software from MadgeTech (New Hampshire, USA). From this, extended carbonate chemistry values were calculated using CO_2SYS (CO2SYS Program, version 2.3, written by Ernie Lewis), a technique demonstrated by Gray et al (2011). All pH manipulations were done to within ± 0.006 units of target values. After verification of pH and oxygen, seawater was stored in 60mL syringes in a cold water bath maintained at 6°C for no longer than 18 hours.

Four replications of assays were performed, each with approximately 32 abalone chosen at random from the holding tanks. These abalone were randomly divided into four groups to coincide with the four condition levels. Once selected for a treatment, individuals were placed in 15mL ground glass vials which were filled with manipulated seawater and then sealed with no headspace (*Figure 3-1)*. It should be noted that despite best intentions, target oxygen values were not always achieved with great precision due to intrusion of oxygen during transfer from manipulation container to experimental respiration chambers.

Samples were then incubated in a cold water bath maintained at 10 ± 0.1 °C and allowed to equilibrate for approximately 15 minutes. Vials had been outfitted with an oxygen sensor optode which was read using a Fibox 3 Oxygen Meter (PreSens Precision Sensing, Regensburg, Germany). Data output was acquired using Fibox 3 PSt3 (v. 6.02 software). A first oxygen reading was taken post temperature equilibration, then a second approximately 40 minutes later. Individuals were then removed from the bath, allowed to recover for approximately 30 minutes, then the vials were flushed with high oxygen, low $p\mathrm{CO}_2$ (O_2 >200 μmol, pH $\cong$ 8.1), re-equilibrated, and the sampling process was repeated. While abalone individuals encompassed a relatively narrow range in size, respiration rates were adjusted for size by dividing rates by the wet weight of the individual.

Data was organized and analyzed using the statistical package R (R Core Team, 2016, https://www.R-project.org/) and Microsoft Excel (2016). Means among treatment conditions were examined and compared using two-way ANOVA testing followed by Wilcoxon Rank Sum paired differences verified by post-hoc with Tukey’s Honest Square Differences (HSD). Kolmogorov-Smirnov (KS) tests were used to compare relatedness of distribution between the treatment and control for each of the four levels.

In order to look at relative contributions of OAH stressors to response, respiration data from the four treatment assays was used. The response variable was log-transformed and modeled using a Generalized Linear Model with a Gaussian distribution and an identity link. The final model was chosen using forwards selection based on AIC scores. Heteroskedascity was tested with a studentized Breush Pagan test, and normality tested using Wilks-Shapiro. The model was then run through a two-way ANOVA in order to determine the eta^2 effect size of both level, starting oxygen, and an interaction term on respiration rate during treatment.

To examine the relationship between increasing OAH stress and variance of response, the treatment data was then binned into 13 different sections by length, and the range of values was calculated for each section. A linear regression was then run through the data to test for trends. Lastly, the R package 'changepoint' (Killick R, Haynes K and Eckley IA, 2016, https://CRAN.R-project.org/package=changepoint) was used to check for change points in the treatment data set based on alterations in mean and variance using the binary segmentation method (Edwards and Cavalli-Sforza 1965).

3.3 Results

Individual abalone respiration rates were captured and varied widely, ranging from 0 to approximately 750 μmol O_2 g^{-1} d^{-1} (*Figure 3-2*). A significant slope was calculated relating the decrease in average red abalone respiration rate and the worsening of OAH stressors over time (Slope=1.6059, p-value=0.004, R^2_{adj}= 0.9866) (*Figure 3-3*).

Comparison of paired mean respiration rates between treatment conditions revealed a large difference between the mean respiration rate for preindustrial conditions and all other treatments (*Figure 3-4)*. A one-way ANOVA ($F_{4,142}$=82.19, p value= <0.0001) confirmed dissimilarities between the means, and a post-hoc Tukey HSD confirmed that the preindustrial mean respiration rate is statistically different from all other means (all p-values <0.0001). Baseline upwelling, contemporary upwelling, and distant future mean respiration rates were not considered statistically different from each other. While not displayed in *Figure 3-4*, the microbial respiration controls were not considered statistically similar to any of the treatment averages.

There does not appear to be any obvious pattern to the mean respiration rates during benign conditions (*Figure 3-4)*. A one-way ANOVA ($F_{4,141}$=19.69, p<0.0001) suggested differences between this group of means, but a post-hoc Tukey HSD found the only significant differences were between the each group and the microbial respiration control (all p-values <0.0001), allowing us to assume that there is no significant difference among the means during the control runs.

Density curves of respiration data during treatment and benign conditions were compared to visually compare changes in response distribution (*Figure 3-5*). For each level, there seems to be a more visually striking median during the control run, suggesting a tighter grouping. In the baseline upwelling, contemporary upwelling, and distant future, the magnitude of the median is higher than the median of the control. Kolmogorov-Smirnov testing was done for each level between the treatment and control, and it was confirmed that the distributions differed significantly for all four levels.

Changes in the range across 13 binned starting oxygen sections appeared to have a trend showing a decrease in variance of response as OAH stress, in the chart represented by oxygen, increased. A linear regression revealed that the slope of the line was not statistically significant (p= 0.889) (*Figure 3-6*).

Respiration data from the treatment portion of the assays was fit with a generalized linear model using the log-transformed respiration rate as the dependent variable and treatment level, starting oxygen, and interaction term as the explanatory variables (*Figure 3-7)*. Four outliers were tested and removed in order to satisfy normality assumptions (Shapiro-Wilks test p-value= 0.04312 with outlier and p-value= 0.2258 without). A gaussian distribution was chosen and stepwise selection was used to verify the suitability of the model. The identity link was chosen for the model both based on AIC (129) and simplicity of interpretation. Post hoc testing was conducted to examine normality and homoscedasticity of residuals. No evidence of heteroscedasticity was found with a studentized Breush Pagan test (p-value= 0.0675), and residuals met assumptions for normality (Shapiro Wilks, W=0.9777, p-value=0.0542).

A two-way ANOVA was run on the variables in the in order to calculate the eta^2 effect size *(Table 3-2)*. All three variables were found to be statistically significant, but the effect size was greatest for the interaction term (0.0958). Starting oxygen and level had medium effect sizes of 0.0347 and 0.0430 respectively.

3.4 Discussion

The fact that mean respiration rate decreased as the intensity of OAH stress increased from preindustrial to distant future conditions suggests that red abalone are already experiencing negative consequences of low oxygen and increased $p\mathrm{CO}_2$. Although there was a slight difference between the means of baseline upwelling, contemporary upwelling, and distant future conditions, it is not statistically significant, meaning the average response of juvenile red abalone does not change significantly as OAH conditions worsen. This could indicate that the physiological threshold for OAH stress has already been passed.

This study also confirmed that there was indeed an interactive term effect between hypoxia and hypercapnia (Tripp et al. 2017). This is echoed in the effect sizes calculated from the fitted model where the interaction term had the largest effect size at 0.09. This seems to be a common finding in red abalone and other marine invertebrates (Portner 2010, Kim et al. 2013). Kim et al (2013) found that prolonged hypoxia resulted in increased mortality, while chronic low pH had a strong effect on growth; the net result of both stressors was less and smaller abalone surviving to adulthood. Due to the tight coupling of hypoxia and hypercapnia in upwelling areas, this could have serious consequences for red abalone populations, especially if temperatures increase as well (Tripp et al. 2017).

While the fitted model may indicate a medium size effect from the interactive term, the effect sizes of starting oxygen and level were both small but still significant ($\eta^2 = 0.03$ and 0.04). There was a residual η^2 of 0.24, suggesting that either there are factors influencing the response not included in the model, or possibly indicating variance of response in the population. The later would fall in line with the findings of De Wit and Palumbi (2013) as well as Kim et al (2013) who found evidence for standing phenotypic variance in the red abalone population in northern California. It should be noted, however, that the abalone used in this study came from a breeding program and may or may not reflect population variance in the wild. These results can, however, serve as a basis for studying the phenotypic diversity and health of cultured abalones compared to wild populations.

Looking at the comparison of value densities between treatment and control, it seems as though there is a change in respiration rate distribution especially in baseline upwelling, contemporary upwelling, and distant future conditions is visually apparent (*Figure 3-5*). This is reflected in the p-values for the Kolmogorov-Smirnov test (*Table 3-2*); all p-values were significant, but baseline upwelling, contemporary upwelling, and distant future conditions were <0.001, while preindustrial was 0.01- still significant but not as extreme. The significant change in distribution in the preindustrial, which was essential two runs of the same treatment, could be a result of variations in oxygen. Looking at the true, not targeted, values of the mean starting oxygen, the average for the treatment was 241.80 ± 16.02 versus the control of 234.06 ± 13.67 (95% confidence interval). While the confidence intervals overlap indicating likely lack of statistical difference, the average in the control was slightly lower, and if oxygen sensitivity is as extreme as other literature suggests, perhaps it could have contributed to the shift in median respiration rate (Kim et al. 2013, Boch et al. 2017, Tripp et al. 2017).

Despite best intentions, oxygen proved to be the variable most difficult to modify with precision. The true, average starting oxygen value for preindustrial was 241.80 μmol, baseline upwelling was 62.98 μmol, contemporary upwelling 58.23 μmol, and distant future 70.02 μmol. While differences were not statistically significant, the pattern of mean respiration rate mimics the pattern of mean starting oxygen. In future studies, improvement of oxygen manipulation techniques could greatly assist in ensuring target values are met and help to reduce scatter of results due to variations in oxygen.

It should also be noted that this experiment is only looking at one size class of red abalone and does not account for potential differences in effect across age or size classes. For instance, high temperature and low pH act synergistically against fertilization success, and different larval stages have been found to have varying tolerance to low pH (Zippay and Hofmann 2010, Boch et al. 2017). In order to better predict future population changes, it may be helpful to acquire survivorship data for red abalone at different life stages in different conditions.

Further future studies should also focus on the effects of chronic versus acute exposure. Due to sampling techniques and available technology, the respiration rates collected for each individual are more or less instantaneous, capturing a moment in time of exposure response. A sister study to this project is currently underway comparing the physiological data collected here with variations in genotype of the same individuals. This additional information may be able to start separating the impacts of individual acclimation versus genetic adaptation, as well as the potential heritability of traits that increase resilience.

3.5 Conclusion

In this study we were able to use individual respiration rates of juvenile red abalone (*Haliotis rufescens*) to verify distinct changes in both value and distribution of stress responses across four pCO_2 and dissolved oxygen regimes. These rates give a valuable clue about the variance in phenotypic expression of a vulnerable species when exposed to a naturally occurring, multi-stressor scenario. While this study was only able to capture an instantaneous image of metabolic activity, it was hopefully able to capture evidence of acclimation and phenotype expression in action.

Red abalone are particularly vulnerable to interactive effects between low oxygen and acidification, and that even current conditions can elicit an impact on stress response. This is especially important due to the low species densities of red abalone observed in the CCLME in conjunction with the popularity of the recreational fishery. Further studies should look into possible interactions between this sensitivity and potential fishing pressures if California and Oregon are to reopen their fisheries.

Ocean acidification and hypoxia in the CCLME stem from a long-running issue, and even with remediation actions, it will likely continue to grow worse over the next few centuries (Snyder et al. 2003, Caldeira and Wickett 2005, Orr et al. 2005, Feely et al. 2018). Calcifying organisms, such as the red abalone, are especially at risk as chemical changes increase energetic expenditure to maintain calcium carbonate structure (Orr et al. 2005, Miller et al. 2009). It is important to determine not only mean population response, but also the amount of standing genetic variability and its distribution in populations when considering projections for future impacts (Futuyma and Moreno 1988, Lande and Shannon 1996, Sunday et al. 2014).

3.6 Works Cited

Boch, C. A., S. Y. Litvin, F. Micheli, G. De Leo, E. A. Aalto, C. Lovera, C. B. Woodson, S. Monismith, and J. P. Barry. 2017. Effects of current and future coastal upwelling conditions on the fertilization success of the red abalone (Haliotis rufescens). ICES Journal of Marine Science 74:1125–1134.

Caldeira, K., and M. E. Wickett. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. Journal of Geophysical Research 110:1–12.

Chan, F., J. A. Barth, C. A. Blanchette, R. H. Byrne, F. Chavez, O. Cheriton, R. A. Feely, G. Friederich, B. Gaylord, T. Gouhier, S. Hacker, T. Hill, G. Hofmann, M. A. McManus, B. A. Menge, K. J. Nielsen, A. Russell, E. Sanford, J. Sevadjian, and L. Washburn. 2017. Persistent spatial structuring of coastal ocean acidification in the California Current System. Scientific Reports 7:1–7.

Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California Current Large Marine Ecosystem. Science 319:920.

Cullison Gray, S. E., M. D. DeGrandpre, T. S. Moore, T. R. Martz, G. E. Friederich, and K. S. Johnson. 2011. Applications of in situ pH measurements for inorganic carbon calculations. Marine Chemistry 125:82–90.

Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond predictions: biodiversity conservation in a changing climate. Science 332:53–58.

Doney, S. C., N. Mahowald, I. Lima, R. a Feely, F. T. Mackenzie, J.-F. Lamarque, and P. J. Rasch. 2007. Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system. Proceedings of the National Academy of Sciences of the United States of America 104:14580–14585.

Ellner, S. P. 2013. Rapid evolution: From genes to communities, and back again? Functional Ecology 27:1087–1099.

Estes, J. A., D. R. Lindberg, and C. Wray. 2005. Evolution of large body size in abalones (Haliotis): patterns and implications. Paleobiology 31:591–606.

Fabry, V. J., B. A. Seibel, R. A. Feely, and J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal Marine Science 65:414–432.

Farmed Abalone Report. 2017.

Feely, R. A., R. R. Okazaki, W. J. Cai, N. Bednaršek, S. R. Alin, R. H. Byrne, and A. Fassbender. 2018. The combined effects of acidification and hypoxia on pH and aragonite saturation in the coastal waters of the California current ecosystem and the northern Gulf of Mexico. Continental Shelf Research 152:50–60.

Feely, R. A., C. L. Sabine, J. M. Hernandez-ayon, D. Ianson, and B. Hales. 2008. Evidence for Upwelling of Corrosive “Acidified” Water on to the Continental Shelf. Science 320:1490–1493.

Feely, R. A., C. L. Sabine, K. Lee, W. Berelson, J. Kleypas, V. J. Fabry, F. J. Millero, and Anonymous. 2004a. Impact of anthropogenic CO2 on the CaCO3 system in the oceans. Science 305:362–366.

Feely, R. A., C. L. Sabine, R. Schlitzer, J. L. Bullister, S. Mecking, and D. Greeley. 2004b. Oxygen utilization and organic carbon remineralization in the upper water column of the Pacific Ocean. Journal of Oceanography 60:45–52.

Futuyma, D. J., and G. Moreno. 1988. The Evolution of Ecological Specialization Author. Annual Review of Ecology and Systematics 19:207–233.

Gaitán-Espitia, J. D., D. Marshall, S. Dupont, L. D. Bacigalupe, L. Bodrossy, and A. J. Hobday. 2017. Geographical gradients in selection can reveal genetic constraints for evolutionary responses to ocean acidification. Biology Letters 13:20160784.

Glass, G. V, P. D. Peckham, and J. R. Sanders. 1972. Consequences of Failure to Meet Assumptions Underlying the Fixed Effects Analyses of Variance and Covariance. Review of Educational Research 42:237–288.

Gruber, N., C. Hauri, Z. Lachkar, D. Loher, T. L. Frölicher, and G.-K. Plattner. 2012. Rapid progression of ocean acidification in the California Current System. Science (New York, N.Y.) 337:220–3.

Guppy, M., and P. Withers. 1999. Metabolic depression in animals: Physiological perspectives and biochemical generalizations. Biological Reviews 74:1–40.

Hauri, C., N. Gruber, M. Vogt, S. C. Doney, R. A. Feely, Z. Lachkar, A. Leinweber, A. M. P. McDonnell, and M. Munnich. 2013. Spatiotemporal variability and long-term trends of ocean acidification in the California Current System. Biogeosciences 10:193–216.

Hofmann, G. E., J. P. Barry, P. J. Edmunds, R. D. Gates, D. A. Hutchins, T. Klinger, and M. A. Sewell. 2010. The Effect of Ocean Acidification on Calcifying Organisms in Marine Ecosystems: An Organism-to-Ecosystem Perspective. Annual Review of Ecology, Evolution, and Systematic 41:127–147.

Hurd, C. L., C. E. Cornwall, K. Currie, C. D. Hepburn, C. M. McGraw, K. A. Hunter, and P. W. Boyd. 2011. Metabolically induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: A mechanism for differential susceptibility? Global Change Biology 17:3254–3262.

Karpov, K. a, P. L. Haaker, I. K. Taniguchi, and L. Rogers-Bennett. 2000. Serial depletion and the collapse of the California abalone (Haliotis spp .) fishery. Workshop on Rebuilding Abalone Stocks in British Columbia 200:11–24.

Kelly, M. W., and G. E. Hofmann. 2013. Adaptation and the physiology of ocean acidification. Functional Ecology 27:980–990.

Kim, T. W., J. P. Barry, and F. Micheli. 2013. The effects of intermittent exposure to low-pH and low-oxygen conditions on survival and growth of juvenile red abalone. Biogeosciences 10:7255–7262.

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, and J. P. Gattuso. 2013. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. Global Change Biology 19:1884–1896.

Lande, R., and S. Shannon. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437.

Lix, L. M., J. C. Keselman, and H. J. Keselman. 1996. Consequences of Assumption Violations Revisited: A Quantitative Review of Alternatives to the One-Way Analysis of Variance "F" Test. Review of Educational Research 66:579–619.

Miller, A. W., A. C. Reynolds, C. Sobrino, and G. F. Riedel. 2009. Shellfish face uncertain future in high CO2 world: Influence of acidification on oyster larvae calcification and growth in estuaries. PLoS ONE 4.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, and P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (Cancer magister). Marine Biology 163:1–11.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. G. Najjar, G.-K. Plattner, K. B. Rodgers, C. L. Sabine, J. L. Sarmiento, R. Schlitzer, R. D. Slater, I. J. Totterdell, M.-F. Weirig, Y. Yamanaka, and A. Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–6.

Parker, L. M., P. M. Ross, W. A. O'Connor, L. Borysko, D. A. Raftos, and H. O. Pörtner. 2012. Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology 18:82–92.

Pespeni, M., E. Sanford, B. Gaylord, T. Hill, J. Hosfelt, H. K. Jaris, M. LaVigne, E. Lenz, A. Russell, M. Young, and S. Palumbi. 2013. Evolutionary change during experimental ocean acidification. Proceedings of the National Academy of Sciences 110:6937–6942.

Portner, H.-O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213:881–893.

Reid, J., L. Rogers-Bennett, F. Vasquez, M. Pace, C. A. Catton, J. V. Kashiwada, and I. K. Taniguchi. 2016. The economic value of the recreational red abalone fishery in northern California. California Fish and Game 102:119–130.

Seibel, B. A., A. E. Maas, and H. M. Dierssen. 2012. Energetic plasticity underlies a variable response to ocean acidification in the pteropod, Limacina helicina antarctica. PLoS ONE 7.

Snyder, M. A., L. C. Sloan, N. S. Diffenbaugh, and J. L. Bell. 2003. Future climate change and upwelling in the California Current. Geophysical Research Letters 30:1823.

Somero, G. N., J. M. Beers, F. Chan, T. M. Hill, T. Klinger, and S. Y. Litvin. 2015. What Changes in the Carbonate System, Oxygen, and Temperature Portend for the Northeastern Pacific Ocean: A Physiological Perspective. BioScience 66:14–26.

Sunday, J. M., P. Calosi, S. Dupont, P. L. Munday, J. H. Stillman, and T. B. H. Reusch. 2014. Evolution in an acidifying ocean. Trends in Ecology and Evolution 29:117–125.

Then, A. Y., J. M. Hoenig, N. G. Hall, and D. A. Hewitt. 2016. Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. ICES Journal of Marine Science 73:582–595.

Tripp, M., C. Bock, M. A. Tripp-valdez, C. Bock, M. Lucassen, and S. E. Lluch-cota. 2017. Metabolic response and thermal tolerance of green abalone juveniles (Haliotis fulgens : Gastropoda) under acute hypoxia and hypercapnia Journal of Experimental Marine Biology and Ecology Metabolic response and thermal tolerance of green abalone juveniles (Haliotis fulgens : Gastropoda) under acute hypoxia and hypercapnia. Journal of Experimental Marine Biology and Ecology 497:11–18.

Widdicombe, S., and J. I. Spicer. 2008. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? Journal of Experimental Marine Biology and Ecology 366:187–197.

De Wit, P., and S. R. Palumbi. 2013. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. Molecular Ecology 22:2884–2897.

Zippay, M. L., and G. E. Hofmann. 2010. Effect of pH on Gene Expression and Thermal Tolerance of Early Life History Stages of Red Abalone (Haliotis rufescens). Journal of Shellfish Research 29:429–439.

Table 3-1 Target values for four exposure co
pressure of CO2, total CO2, and saturation s

Conditions	pH
Preindustrial	8.13
Baseline	

Figure 3-1 Experimental set up for measuring individual respiration rates of juvenile red abalone. Individuals were placed in sealed ground glass vials with an oxygen sensitive optode. A Fibox 3 Oxygen Sensor by PreSens was used to measure the in situ *concentration of oxygen at the beginning and end of the assay in order to calculate rate of oxygen usage. Between measurements, vials were stored in a 10℃ water bath.*

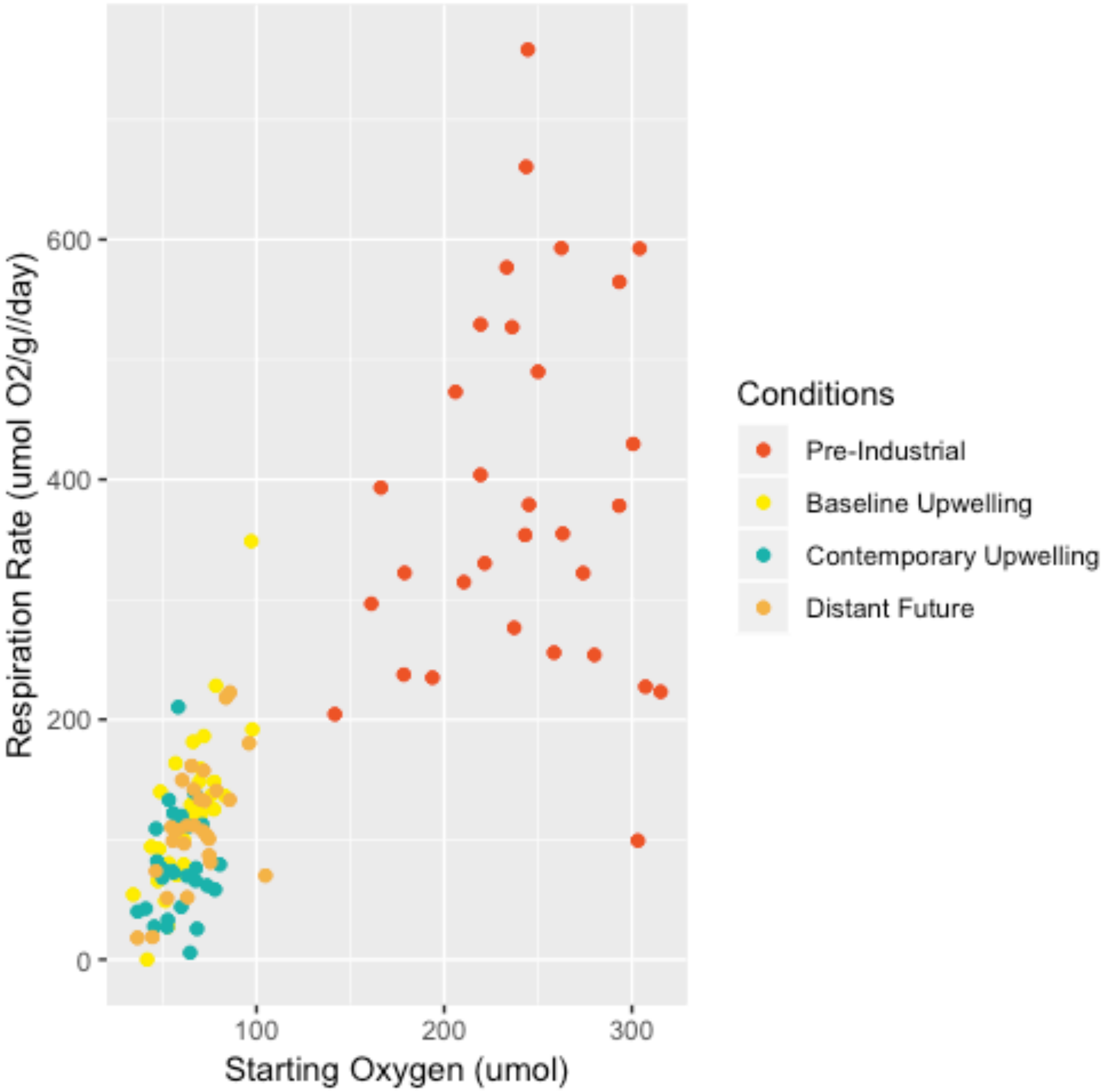

Figure 3-2 Respiration rates of red abalone plotted against the starting oxygen concentration and color coded by treatment condition.

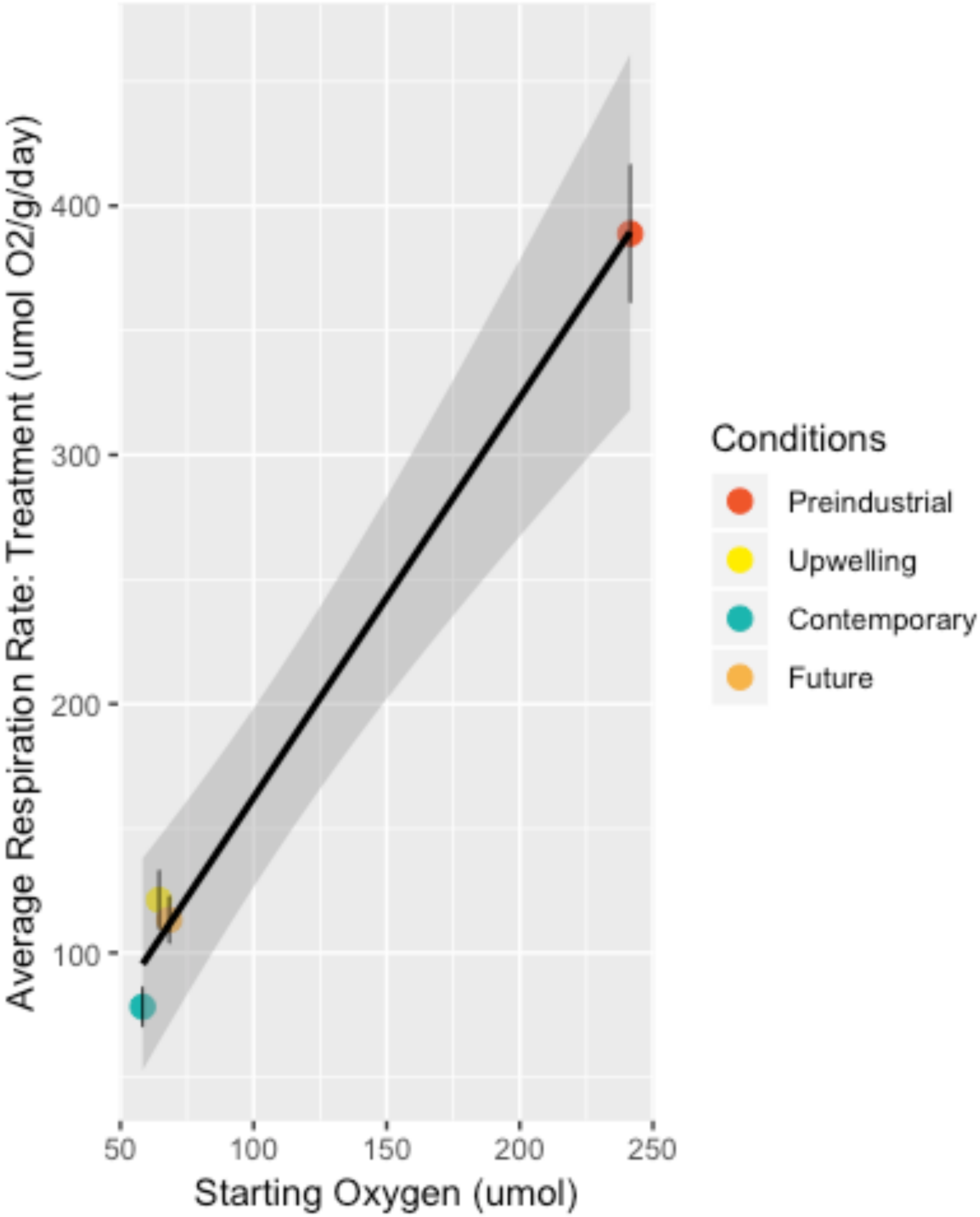

Figure 3-3 Average red abalone respiration rate at each of four treatment conditions plotted against average starting oxygen concentration. Rates have been normalized by wet weight of individuals. The line represents a linear regression through the four points (Slope=1.6059, p-value=0.004, R^2_{adj}= 0.9866). Error bars show 95% confidence intervals.

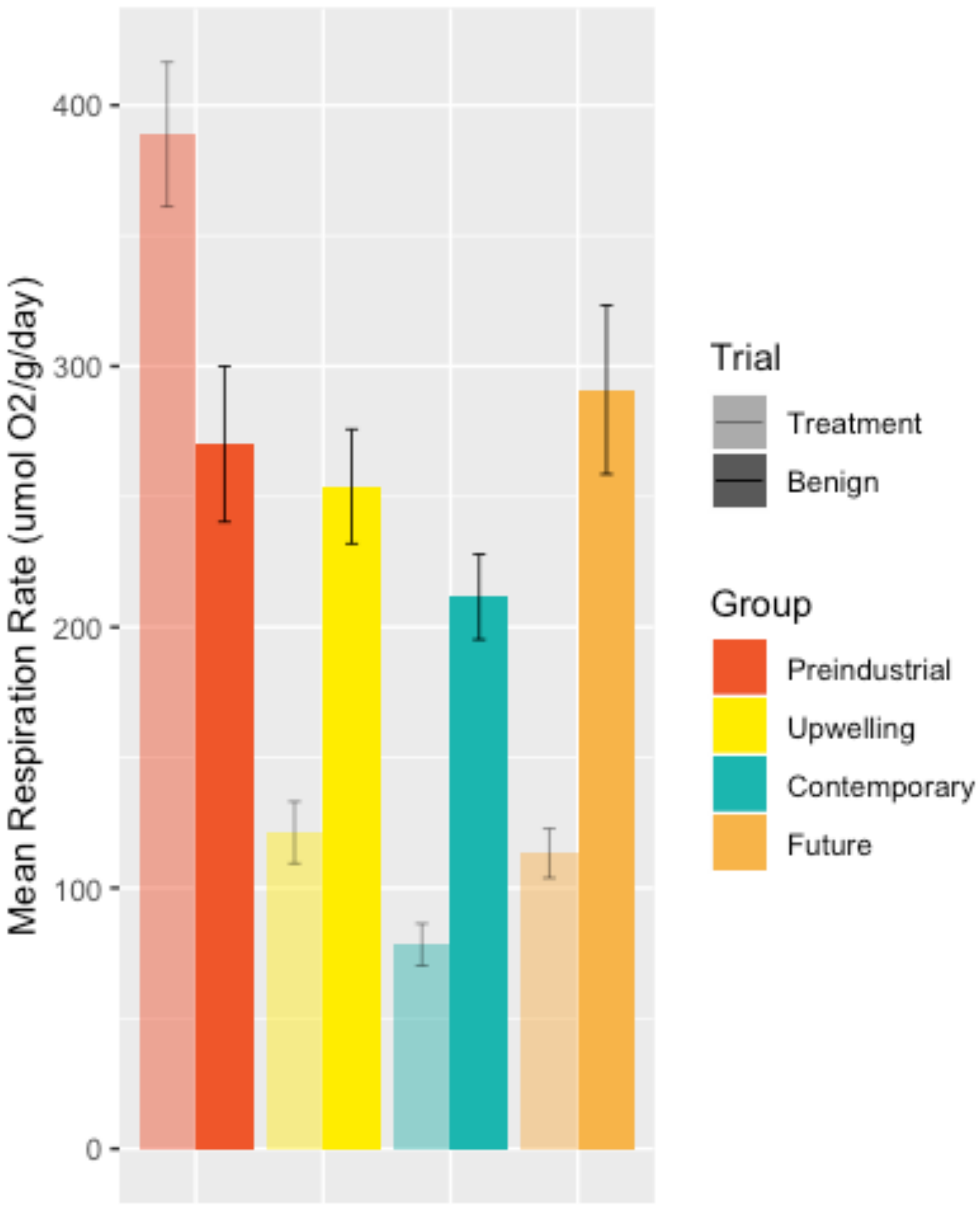

Figure 3-4 Bar chart of red abalone mass-corrected mean respiration rate in for each of the four conditions during selected treatment and benign conditions.

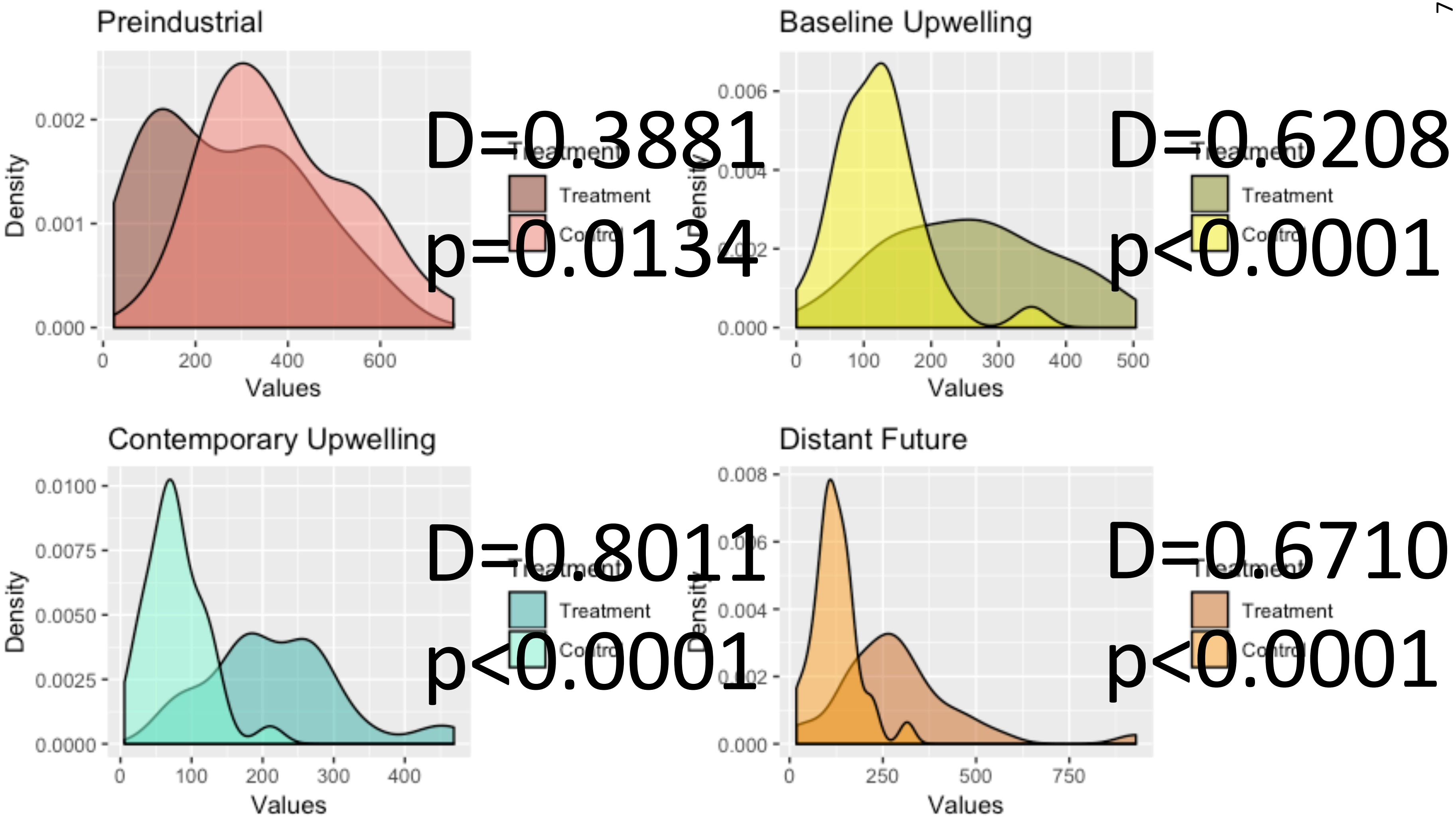

Figure 3-5 Comparison of red abalone respira
all control trials except for the pre-industrial r

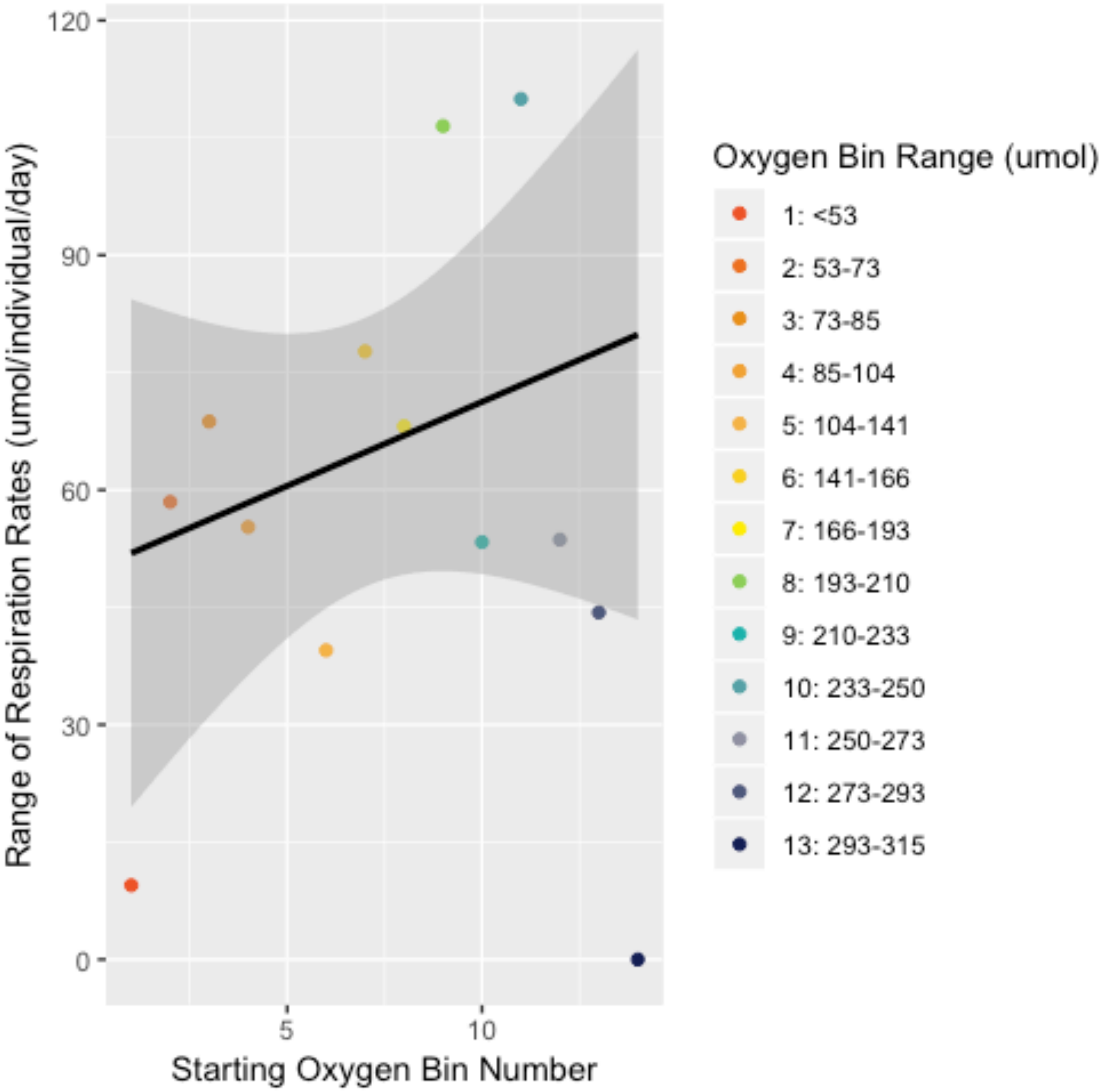

Figure 3-6 Ranges of binned red abalone respiration rate values as a function of increasing starting oxygen concentration. Line is a linear regression with a slope of 0.366 (p=0.889)

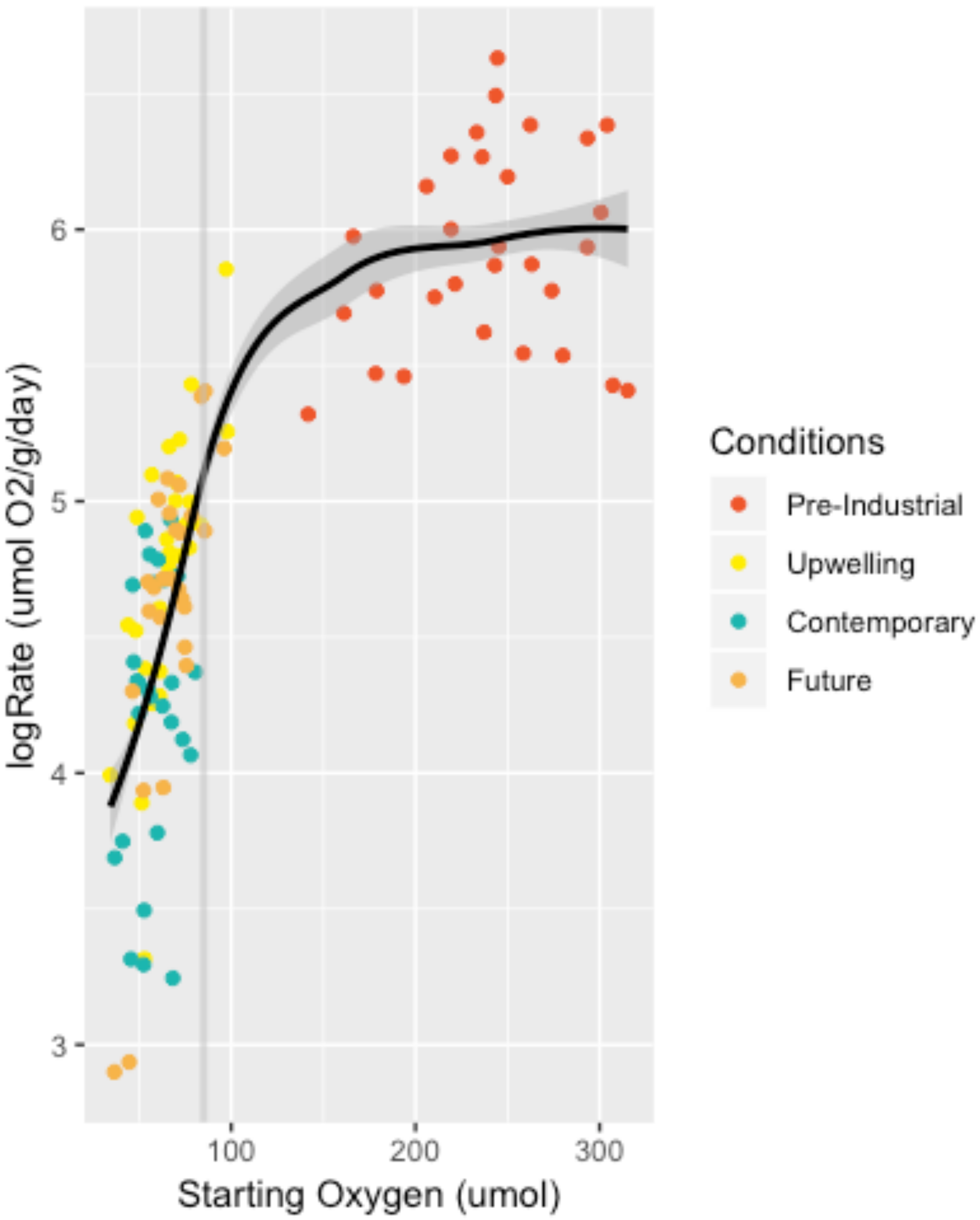

Figure 3-7 Log-transformed red abalone treatment respiration rate plotted against starting oxygen. Color coded by condition groupings. Line overlay represents LOESS smoothed predicted values from a Generalized Linear Model with a Gaussian distribution and Identity link (AIC=155) with shading representing standard error. Notice the grouping of data points of the baseline upwelling, contemporary, and distant future conditions. Also marked is a vertical line through 82 μmol O_2, representing the estimated change point.

Table 3-2 Eta squared and ANOVA table values f
function of starting oxygen, experimental condit

Variable	η^2
Starting Oxygen	0.0347
Conditions	0.0430

Sour and Out of Breath: A Policy Briefing on Ocean Acidification and Hypoxia in Oregon

3.8 Executive Summary

Oregon's coast is a hotspot for experiencing ocean acidification and hypoxia (OAH). These changes in ocean chemistry have a detrimental effect on shellfish, which has the potential to impact coastal economy. While legislation has been passed establishing a state policy on OAH, no enforceable regulations or policies have been passed to deal with the repercussions of the changing oceans.

3.9 Background

The root cause of ocean acidification is carbon dioxide (CO_2) entering the ocean from the atmosphere. As CO_2 concentrations in the atmosphere continue to rise due to human combustion of fossil fuels, greater levels of CO_2 gas are being pushed into the ocean.[1] Once dissolved, the CO_2 becomes a weak acid, lowering the pH of the ocean.[2] This dissolution of CO_2 triggers a chain reaction, resulting in an increase in bicarbonate ions (HCO_3^-) and decrease in carbonate ions (CO_3^{2-}). With less carbonate to form calcium carbonate (shell material), shellfish are unable to grow as well and may even experience shell dissolution.[3]

Oregon is especially at risk for experiencing ocean acidification due to a natural occurrence known as upwelling which acts synergistically with the mechanism described above. Seasonal changes in wind patterns pull up deep ocean water into the coastal waters. Although this brings nutrients necessary for productive waters, deep sea waters generally have higher CO_2 and therefore lower pH as well as low oxygen levels known as hypoxia.[4] This is why most current legislation in the state focuses on both ocean acidification *and* hypoxia.

Ocean acidification and hypoxia provide multiple challenges to decision makers. First is the issue of scale; although global ocean acidification trends are in large led by atmospheric changes in CO_2, local scale processes, such as upwelling and various types of runoff, can also contribute and must be accounted for.[5] It is also a difficult issue to communicate, as it is complex and intangible to many people. There are also still many unknowns about to what degree ecosystems will react to these chemical changes, leading to difficulty in evaluating risk and taking action. Ocean acidification is an important issue, however, and due to its potential impacts on commercially valuable species and coastal economies, it must be taken into consideration in coastal planning.

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[1] Sabine et al 2004

[2] Zeebe & Wolf-Gladrow 2001

[3] Feely et al 2004

[4] Chan et al 2008

[5] Kelly et al 2011

3.10 Policy Alternatives

- **Clean Water Act of 1972**- gives states the ability to create Total Daily Maximum Loads as per **Section 303(d)** or manage pollution sources via the **National Pollution Discharge Elimination System.** Does not yet cover carbon dioxide, but could be amended to do so. Needs updated monitoring criteria.

- **Coastal Zone Management Act**- gives states the power of *Federal Consistency* and the ability to create and enforce their own Coastal Zone Management Plans. Oregon's current iteration is the Territorial Sea Plan. Potential amendments could be used as basis for regulation.

- **Public Trust Doctrine**- includes submerged lands beneath navigable waters (habitat), wildlife (shellfish), and water quality (potentially OA); therefore, the Oregon State Government holds all of these items in trust for the public

- **Oregon House Bill 2209**- 2015 shellfish policy bill with a central goal of improving water quality in order to safeguard shellfish- both wild and cultivated- in the state.

- **Oregon Senate Bill 1039**- passed in 2017, this bill created state policy on OAH. Established the Oregon Coordinating Council on OAH to help identify areas for research and action.

3.11 Conclusions

While ocean acidification and hypoxia are problems on a global scale, Oregon will be experiencing these problems much sooner and with a much greater magnitude than many other areas. This is especially concerning due to the economic and social importance of potentially vulnerable shellfish to these changes in ocean chemistry. While Oregon has been progressive in setting policy regarding shellfish (HB2209) and ocean acidification and hypoxia (SB1039), there is yet no real concrete plan for dealing with OAH related changes. It will be necessary to work with the Coordinating Council on OAH in order to determine areas of need and create policy to address them.

3.12 Further Reading

Gruber et al 2012. Rapid progression of ocean acidification in the California Current System. Science 337:220–3. http://science.sciencemag.org/content/337/6091/220.full?casa_token=ShNHCX-p-zQAAAAA:le5nn_HNYRC4EUxh3rMVY8fXIVLVeQPCBnPVD6gSHZtNqV39vB2kwwj8UETP55sHT8K0_9-A3rdH2g

Klinger et al. 2017. Using integrated, ecosystem-level management to address intensifying ocean acidification and hypoxia in the California Current large marine ecosystem. Elementa: Science of the Anthropocene 5(0):16. https://www.elementascience.org/articles/10.1525/elementa.198/

3.13 Extended Commentary

While the above briefing focused on management of OAH within the state of Oregon, it is may be necessary to examine the problem with a wider lens. Because it is largely driven by global anthropogenic carbon emissions, to address the root of the problem it would be necessary to achieve global cooperation in reduction of greenhouse gas emissions. While current efforts under the United Nations Framework Convention for Climate Change, including the *Paris Agreement* (United Nations, 2016) have been valiant, due to the long residence time of carbon dioxide in the ocean, the effects of OAH would continue to be felt even if emissions were to be reduced to zero immediately (Strong et al. 2014). Because of this and the challenges of reaching global consensus, at this point in time it is perhaps best to focus on remediating the situation on a local or regional scale and creating more adaptive and resilient communities and ecosystems.

The U.S. West Coast is primed to address this issue both due to its local saliency and legal structures already in place. Between the West Coast Regional Planning Body (WCRPB) and the Pacific Coast Collaborative (PCC), it seems as if the basic infrastructure is in place to move forwards with regional-scale actions to manage and remediate OAH. There are, however, several large barriers remaining: funding, scale, and implementation. There is little funding allocated for the WCRPB, and surely even less for the PCC. Additionally, the role of both of these entities is seemingly more to act as a conduit for OAH information between states and provinces. Lastly, even if they put forth recommendations, there is no binding mechanism to guarantee implementation. Therefore, it is perhaps a viable route for a regional body to use existing federal or even state legislation instead of trying to create something new.

There are currently several potential legislative vehicles that could be used by regional bodies to address OAH, but each of them has both pros and cons. The most discussed option in literature is to utilize the 303(d) section of the Clean Water Act (Craig 2015, Weisberg et al. 2016). This section establishes state's abilities to create Total Maximum Daily Loads of nonpoint source pollutants entering a waterway and establish protocol to reduce loading. The Center for Biological Diversity has attempted to push the EPA to consider waters impacted by ocean acidification as impaired under 303(d) with a 2013 petition. When the EPA replied via a memo recommending the use of pH level monitoring to determine impaired waters, CBD filed a lawsuit regarding the deferral of regulations for anthropogenic carbon dioxide emissions (*Center for Biological Diversity v. EPA*, 2015, US Dist. LEXIS 25945). The court determined that the EPA had no statutory authority to determine sources of carbon dioxide, and removed the referral rule.

Another issue arises due to the large scale of the root causes; the leading cause of OAH globally is anthropogenic carbon emissions into the air, but it only becomes an issue when that gas diffuses into water. This blurs the lines between jurisdiction of the federal Clean Air Act and Clean Water Act, creating a bureaucratic stalemate (Craig 2015). Acidification is also difficult because most components of the carbonate system are measured indirectly with varying levels of accuracy. This does not align with the US EPA's Redbook limits for water quality which only uses a pH range- and a broad one at that. On top of that, the recommended bracket of values has not been updated since 1976, and is out of date with regards to modern oceanographic technology and scientific thinking (Weisberg et al. 2016).

It is also important to note that acidification conditions are spatially heterogeneous, and vary over multiple scales (Chan et al. 2017). California, for instance, has not yet experienced OAH to the degree Washington has even though they are both adjacent to the CCLME (Hauri et al. 2013). To better guide the West Coast as well as other regions, the EPA should consider updating their protocol for measuring water quality with regards to acidification, likely to be more tailored to local conditions and take into account calcium carbonate saturation measurements, a better indicator of biological corrosiveness (Craig 2015).

In order to successfully use the Clean Water Act 303(d) listings, a clear opinion would need to be delivered either through legislation or the judicial branch that carbon dioxide should be considered a waterborne pollutant and regulated as a non-point source contributor to OAH (Craig 2015, Weisberg et al. 2016). While it may be nearly impossible for three states to impose carbon dioxide emission limits on a global scale, action can still be taken on a smaller scale. Washington, Oregon, and California have all made actions towards monitoring and reducing non-point source pollutants, but due to the scale of the CCLME, it may be beneficial to create a regional maximum load just as the northeastern states did with mercury emissions. In order to tackle power plant emissions in 1967, New England created NESCAUM, a 501(c)(3) nonprofit to coordinate mercury emission reductions in the region; it is possible a similar pattern could be followed on the West Coast to deal with carbon dioxide.

Another tool that could be useful would be the revision of National Pollution Discharge Elimination System (NPDES) under the Clean Water Act (33 U.S.C. § 1342). NPDES allows states to regulate point-sources including both industry and wastewater treatment facilities which introduce effluent into the nation's waterways. This is likely not the most direct or efficient route to go. Language would need to be revised or amended to include carbon dioxide as a waterborne pollutant in order for it to qualify under this statute. While other pollutants do contribute to OAH on a local scale, the effects in the CCLME are largely driven by upwelling. Even though it may not be top priority for the West Coast, eventual consideration of point source contributors through revision of NPDES is still well within reason (Kelly et al. 2011).

Through the Coastal Zone Management Act, states may also be able to coordinate and manage actions through revisions of their Coastal Zone Management Plans (Strong et al. 2014). While it would still be up to each state individually to modify their plans, coordination via the WCRPB to ensure alignment of priorities and outcomes could help to ensure cohesion of actions across the ecosystem scale. Due to Federal Consistency, states could create new management targets and check federal actions within or adjacent to their waters which may impact those targets.

As the effects of OAH continue to worsen, it may be advisable to create new Marine Protected Areas (MPAs), likely on the state level, or federally designated National Estuarine Research Reserves (NERRs) in order to preserve refuge habitat or relieve excess stress on ecosystems to better guarantee resiliency. This would likely be quite difficult to do the variety of codification and legal application of the public trust doctrine, public access, and right to fish across the three states (Obegi 2008, Sagarin and Turnipseed 2012). Although challenging, if done with tact, protected areas can be an incredibly effective tool for ensuring ecosystem resilience in the face of OAH. Protection strategies involving Marine Spatial Planning and restrictions of this type require careful consideration of stakeholder expectations and needs which is best achieved through the framework of Ecosystem Based Management (EBM)

(McLeod and Leslie 2009, Lester et al. 2010, Levin et al. 2018). EBM considers connections across multiple scales, explicit trade-offs, socio-ecological interactions, and embraces change, all of which are necessary to create lasting management programs, especially considering the scale and stakeholder demographic along the West Coast.

The US West Coast has a very unique opportunity to model regional scale policy and management approaches to dealing with the emerging issue of OAH. Although California, Oregon, and Washington are all in different places in the planning process and have distinct legal structures, they all have common goals and desired outcomes. The best route forward at time of writing is likely through revision and consolidation of current legislation and legal framework, as well as continuation of communication and data sharing networks. Current regional structures need to be strengthened and allocated more funding in order to better address this problem. Through using a nesting arrangement of local, state, regional, and federal legislation, information, and considerations, it is very likely that an exemplary system of management could be set up to act as a template for other regions and issues around the globe (Sievanen et al. 2011).

3.14 Bibliography

Chan, F., J. A. Barth, C. A. Blanchette, R. H. Byrne, F. Chavez, O. Cheriton, R. A. Feely, G. Friederich, B. Gaylord, T. Gouhier, S. Hacker, T. Hill, G. Hofmann, M. A. McManus, B. A. Menge, K. J. Nielsen, A. Russell, E. Sanford, J. Sevadjian, and L. Washburn. 2017. Persistent spatial structuring of coastal ocean acidification in the California Current System. Scientific Reports 7:1–7.

Craig, R. K. 2015. Dealing With Ocean Acidification: the Problem, the Clean Water Act, and State and Regional Approaches. Page Washington Law Review.

Hauri, C., N. Gruber, M. Vogt, S. C. Doney, R. A. Feely, Z. Lachkar, A. Leinweber, A. M. P. McDonnell, and M. Munnich. 2013. Spatiotemporal variability and long-term trends of ocean acidification in the California Current System. Biogeosciences 10:193–216.

Kelly, R. P., M. M. Foley, W. S. Fisher, R. A. Feely, B. S. Halpern, G. G. Waldbusser, and M. R.

Caldwell. 2011. Mitigating local causes of ocean acidification with existing laws. Science 332:1036–1037.

Lester, S. E., K. L. McLeod, H. Tallis, M. Ruckelshaus, B. S. Halpern, P. S. Levin, F. P. Chavez, C. Pomeroy, B. J. McCay, C. Costello, S. D. Gaines, A. J. Mace, J. A. Barth, D. L. Fluharty, and J. K. Parrish. 2010. Science in support of ecosystem-based management for the US West Coast and beyond. Biological Conservation 143:576–587.

Levin, P. S., T. E. Essington, K. N. Marshall, L. E. Koehn, L. G. Anderson, P. S. Levin, A. Bundy, C. Carothers, F. Coleman, L. R. Gerber, J. H. Grabowski, J. N. Sanchirico, and A. D. M. Smith. 2018. Building effective fishery ecosystem plans. Marine Policy In Press.

McLeod, K. L., and H. M. Leslie. 2009. Why Ecosystem Based Management? Pages 3–12Ecosystem Based Management for the Oceans.

Obegi, D. 2008. Is there a Constitutional Right to fish in a Marine Protected Area? An analysis of the California Constitution’s right to fish provision and its impact on the state’s power to create marine reserves and other types of Marine Protected Areas (MPAs). West-Northwest Journal of Environmental Law & Policy 1419:1–29.

Sagarin, R. D., and M. Turnipseed. 2012. The Public Trust Doctrine: Where Ecology Meets Natural Resources Management. Annual Review of Environment and Resources 37:473–496.

Sievanen, L., H. M. Leslie, J. M. Wondolleck, S. L. Yaffee, K. L. McLeod, and L. M. Campbell. 2011. Linking top-down and bottom-up processes through the new U.S. National Ocean Policy. Conservation Letters 4:298–303.

Strong, A. L., K. J. Kroeker, L. T. Teneva, L. A. Mease, and R. P. Kelly. 2014. Ocean acidification 2.0: Managing our Changing Coastal Ocean Chemistry. BioScience 64:581–592.

Weisberg, S. B., N. Bednaršek, R. A. Feely, F. Chan, A. B. Boehm, M. Sutula, J. L. Ruesink, B. Hales, J. L. Largier, and J. A. Newton. 2016. Water quality criteria for an acidifying ocean: Challenges and opportunities for improvement. Ocean and Coastal Management 126:31–41.

<u>*Policy & Reports Cited*</u>

Assembly Bill No 2139 (2016) 352 AB 2139. Sacramento, CA.

Clean Air Act (1970) 42 U.S.C. § 7401 *et seq.*

Clean Water Act (1972) 33 U.S.C. § 1251 *et seq***.**

Section 303(d): Impaired Waters and Total Maximum Daily Loads.

Exec. Order No. 12-07 (2012) WA EO 12-07: "Washington's Response to Ocean Acidification". Office of the Governor. Olympia, Washington.

Federal Ocean Acidification Research and Monitoring Act of 2008 (2007) 33 U.S.C. 50 § 3701-3708. 110th Congress. Washington, DC

Massachusetts v. EPA (549 U.S. 497, 2007)

Oregon Shellfish Policy Bill (2015) HB 2209. 78th Leg., Reg Sess. Salem, OR.

Pacific Coast Action Plan on Climate and Energy (2013) http://pacificcoastcollaborative.org/Documents/Pacific%20Coast%20Climate%20Action%20Plan.pdf (Last visited 18 Mar 2018)

Senate Bill 1039A (2017) 79th Leg., Reg. Sess. Salem, OR.

Senate Bill 1363 (2016) 2015-2016 Regular Sess. Sacramento, CA.

Senate Bill 5603 (2013) 63rd Leg., Reg. Sess. Olympia, WA.

Codified: Wash Rev Code 43.06.338 (2016)

Shellfish Protection Act of 1993 (1993) California Water Code § 14950-19958

Washington State Blue Ribbon Panel on Ocean Acidification (2012): Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response. H. Adelsman and L. Whitely Binder (eds). Washington Department of Ecology, Olympia, Washington. Publication no. 12-01-015.

West Coast Regional Planning Body Charter (2015) (Last visited 18 Mar 2018) https://static1.squarespace.com/static/535fdf12e4b071a2c2e39cae/t/59a48ce3f5e231b165732007/1503956197132/WestCoastRPB_FullCharter_SignatoryDraft.pdf

2017 Addendum to Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response. (2017) *Washington Marine Resources Advisory Council*. EnviroIssues (eds). Seattle, Washington.

4 Discussion and Conclusion

4.1 Discussion

Results from this study indicate that both Dungeness crab (*Cancer magister*) and red abalone (*Haliotis rufescens*) are currently being affected to some degree by increasing stress due to changes in ocean chemistry. For the trials involving Dungeness crab megalopae, while there was general decreasing trend in respiration rate with increasing hypoxia stress, the only conditions which results in a statistically different mean represented distant future values. Likewise, this group was the only treatment with a statistically different distribution between control and treatment values. Differences in both mean rates and sampled rate distribution did not significantly vary between preindustrial, baseline upwelling, or contemporary upwelling. This could be good news for the Dungeness crab, indicating that pre-exposure to extreme conditions have resulted in adaptive capacity in current and near future conditions (Sanford and Kelly 2011, Pespeni et al. 2013a, Gaitán-Espitia et al. 2017, Vargas et al. 2017). To survive distant future conditions, Dungeness crabs will have to continue to develop adaptations at the same pace as changes in ocean chemistry are happening (Lande and Shannon 1996, Sunday et al. 2011, 2014).

Additionally, it appears that neither $p\text{CO}_2$ nor the interaction term between $p\text{CO}_2$ and oxygen has a significant effect on respiration rate on Dungeness crab megalopae, echoing results of previous studies on marine decapods (Pörtner et al. 2004, Pane and Barry 2007, Ries et al. 2009, Whiteley 2011). Dissolved oxygen was found to be the dominant forcing factor on respiration rate. While the resilience to hypercapnia is indeed good news, presence of anoxia on the continental shelf could present an insurmountable challenge without physical avoidance capabilities and strategies (Airriess and McMahon 1994, Guppy and Withers 1999, Chan et al. 2008). The high amount of residual effect size (~0.34) suggests that there may be other factors not taken into account in this experiment. Unexplained variance could also imply a degree of variability in response within the population, but this would likely need to be confirmed using genotypic methods.

Interestingly, this pattern was not initially observed in the pilot study run in July of 2017. This assay showed a change in slope direction and magnitude that varied as a function of pCO_2 across a range of oxygen values. The fact that this pattern was not repeated in 2018 suggests that there may be a cohort effect in Dungeness crab megalopae. This should be considered in future research.

Red abalone experienced a similar pattern in reaction to worsening OAH conditions, but the contributing stressors differed from Dungeness crab. Modeled red abalone data revealed that there was also a strong interaction effect between pCO_2 and oxygen, reinforcing the findings of Kim et al (2013). Both starting oxygen concentration and pCO_2 had smaller but still significant effect sizes. Again, total effect size of chosen variables left fair high residuals (0.2956), suggesting either that there are factors not encapsulated in the model or that there is phenotypic variability within in the population (De Wit and Palumbi 2013, Kim et al. 2013).

Comparison of mean red abalone respiration rates provided some insight into the timeline of potential OAH impact. Mean respiration rate for preindustrial conditions was significantly higher than levels representing baseline upwelling, contemporary upwelling, or distant future conditions during treatment. This suggests that increasing OAH stress results in metabolic depression, but the reaction to stress does not increase significantly with worsening conditions. Because there is no change to stress response across the three “stressful” conditions, it is possible that red abalone have already reached a physiological threshold. There is evidence of local adaptation occurring in wild population of red abalone, but, within the context of this experiment, there does not appear to be adaptive capacity for increased OAH stress (De Wit and Palumbi 2013, Sunday et al. 2014).

One interesting effect noted in both species was the lack of significant difference among mean respiration rates in control conditions. This indicates that both species are able to reacclimate back to benign conditions relatively quickly after exposure to acute OAH stress. Because of the rapid changes in conditions in the CCLME, this makes sense, and could prove to be a valuable trait for dealing with changes to ocean chemistry.

Although the data collected in this study provides valuable insight into the projected responses of Dungeness crabs and red abalone to worsening ocean acidification and hypoxia, the results are certainly not definitive. There are several important topics which require more study, the first being consideration of duration. This experiment was limited by the inability to alter or maintain water chemistry, especially oxygen content, once respiration vials were sealed for incubation, creating a limiting factor for the run time of the assay. While the technique used was very successful at collecting small scale individual respiration rates in order to better understand variance in distribution of responses, it would be useful to find one or more proxies for metabolic stress on individuals while maintaining a longer term experiment. This study also focused on fairly rapid acute exposure, while projected for the future suggest that medium term to chronic exposure may be more realistic. Further studies should be done to investigate the repercussions of chronic hypercapnia and hypoxia on metabolic function and as well as if and where energy reallocation occurs. It would also be helpful to look at multigenerational studies to better understand the demarcation between behavioral and inheritable adaptive strategies.

In order to fully understand the full physiological consequences of future conditions, it will be necessary to add in the cross-effects of temperature with hypoxia and hypercapnia. Temperature has been shown to be an important covariate, exacerbating effects of low oxygen and high $p\text{CO}_2$ (Wood and Cameron 1985, Hale et al. 2011, Lischka et al. 2011, Whiteley 2011, Then et al. 2016). Unfortunately, due to time restrictions and sample availability, a three-way cross between temperature, oxygen, and $p\text{CO}_2$ was not available at this time, but could be accomplished in the future following similar procedures.

Lastly, this study only looked at one life stage for both Dungeness crabs and red abalone. While the stages were selected on the basis of the ontogenetic vulnerability hypothesis, it remains to be seen how changes in ocean conditions may differently effect spawning, egg and larval success, or growth and development within either species. It is entirely possible that there are different levels of vulnerability at different life stages, particularly pre-adult, and that the age or size chosen for this study was perhaps not the most vulnerable (Whiteley 2011, De Roos and Persson 2013, Dineshram et al. 2013, Frieder et al. 2014, Miller et al. 2016). Size and age of individuals was in part affected by availability of samples. In future studies it will be important to consider tolerance at not only one life stage, but how that vulnerability changes across the life span and whether carry over effects are potentially occurring.

Despite the uncertainties presented above, there is still a need for action with regards to management and policy for ocean acidification and hypoxia. One of the most important aspects of creating effective marine planning on this issue will be to ensure continued communication between researchers, managers, and policy makers as well as a pipeline to allow for integration of new information as it becomes available. A built in adaptive capacity, if you will, for policy, allows for more timely reaction to unforeseen changes in both physical conditions and responses of marine species of concern.

An important first step in this direction is to revisit previously defined water quality criteria at both the state and federal level, and consider how better to incorporate and measure pH, carbonate chemistry measurements, and oxygen levels. It is clear that these parameters have the potential to strongly effect species which drive thriving markets, provide valuable ecosystem services, and are a part of healthy, resilient ecosystems, justifying modification or inclusion of these conditions under the umbrella of water quality. Baselines need to be established for both chemical and ecological indicators, and monitoring programs should be put in place to determine the magnitude of changes occurring.

It will also be necessary to tailor solutions to specific locations. Although local processes may in part contribute to OAH, a large portion of the CCLME, especially off the coast of Oregon, is due to seasonal upwelling, a phenomenon not so much affected by state or regional water quality management. Ongoing research on spatial patterns in OAH conditions and the potential for local scale refugia may be a key piece in ensuring healthy populations of vulnerable species (Chan et al. 2017).

Due to the interconnected nature of CCLME, regional scale collaboration and communication will continue to be essential. Artificial boundaries between states work well in the legal system, but they matter not to the movement of the ocean. Cohesion of regulatory actions and monitoring programs will help to ensure successful management of the entire system. Along these same lines, it may be useful to follow the template set forth by Northeast

States Coordinated Air Use Management (NESCAUM) in order to work towards regional emissions standards.

Overall, the states bordering the CCLME seem to be on the right track towards confronting and dealing with the consequences of OAH. We are just beginning to be able to predict ecological and economic outcomes for the region based on models of future conditions, and because of the crash of oyster hatcheries in the mid-2000’s, there seems to be more public awareness and investment here than in other areas. Despite a degree of uncertainty inherent to scientific research, the physical and political atmosphere seems to be at an ideal point for action, and it may be possible to efficiently deal with this problem along the U.S. west coast.

4.2 Conclusion

Fortunately, this study has shown that for Dungeness crabs and red abalone there appears to be some resiliency to OAH stress, but their adaptive capacity it not without limit. These two species are already showing negative impacts of increasing stress. Current management plans do not take into account the potential impacts of these environmental changes on economically and environmentally important species, but it is important they consider it going forward.

The story of OAH in the CCLME is a compelling one, a narrative that potentially has the power to act as either a warning or a beacon of hope as humanity begins to understand and react to the repercussions of a changing ocean. The three states making up the U.S. West Coast are in a critical position where action will be necessary in order to preserve the current status quo. This study is evidence that the effects are happening now, and action is needed even in the face of uncertainty.

5 Works Cited

Airriess, C., and B. McMahon. 1994. Cardiovascular adaptations enhance tolerance of environmental hypoxia in the crab Cancer magister. Journal of Experimental Biology 190:23–41.

Bakun, A., and C. S. Nelson. 1991. The Seasonal Cycle of Wind-Stress Curl in Subtropical Eastern Boundary Current Regions. Journal of Physical Oceanography 21:1815–1834.

Barth, J., J. Fram, E. Dever, C. Risien, C. Wingard, R. Collier, and T. Kearney. 2018. Warm Blobs, Low-Oxygen Events, and an Eclipse: The Ocean Observatories Initiative Endurance Array Captures Them All. Oceanography 31:90–97.

Barton, A., G. G. Waldbusser, R. A. Feely, S. B. Weisberg, J. A. Newton, B. Hales, S. Cudd, B. Eudeline, C. J. Langdon, I. Jefferds, T. King, A. Suhrbier, and K. McLaughlin. 2015. Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation strategies implemented in response. Oceanography 28:146–159.

Bernatis, J. L., S. L. Gerstenberger, and I. J. Mcgaw. 2007. Behavioural responses of the Dungeness crab , Cancer magister , during feeding and digestion in hypoxic conditions:941–951.

Bertelsen, J. M. 2016. “Fed” up with acidification: “trusting” the Federal Government to protect the Tulalip tribes’ access to shellfish beds. Washington Journal of Environmental Law & Policy 6:495–529.

Blumm, M. C., and E. Doot. 2012. Oregon’s Public Trust Doctrine: Public Rights in Waters, Wildlife, and Beaches. Environmental Law 42:375.

Boch, C. A., S. Y. Litvin, F. Micheli, G. De Leo, E. A. Aalto, C. Lovera, C. B. Woodson, S. Monismith, and J. P. Barry. 2017. Effects of current and future coastal upwelling conditions on the fertilization success of the red abalone (Haliotis rufescens). ICES Journal of Marine Science 74:1125–1134.

Boehm, A., M. Jacobson, M. O’Donnell, M. Sutela, W. Wakefield, S. Weisberg, and E. Whiteman. 2015. Ocean acidification science needs for natural resource managers of the North American west coast. Oceanography 28:170–181.

Breitburg, D. L., J. Salisbury, J. M. Bernhard, W.-J. Cai, S. Dupont, S. C. Doney, K. J. Kroeker, L. A.

Levin, W. C. Long, L. M. Milke, S. H. Miller, B. Phelan, U. Passow, B. A. Seibel, A. E. Todgham, and A. M. Tarrant. 2015. And on top of that... Coping with ocean acidification in the midst of many stressors. Oceanography 28:48–61.

Brewer, P. G. 1997. Ocean chemistry of the fossil fuel CO2 signal: The haline signla of "business as usual." Geophysical Research Letters 24:1367–1369.

Burnett, L. E. 1997. The Challenges of Living in Hypoxic and Hypercapnic Aquatic Environments. American Zoologist 37:633–640.

Busch, D. S., M. O'Donnell, C. Hauri, K. Mach, M. Poach, S. Doney, and S. Signorini. 2015. Understanding, Characterizing, and Communicating Responses to Ocean Acidification: Challenges and Uncertainties. Oceanography 25:30–39.

Caldeira, K., and M. E. Wickett. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. Journal of Geophysical Research 110:1–12.

Chan, F., J. A. Barth, C. A. Blanchette, R. H. Byrne, F. Chavez, O. Cheriton, R. A. Feely, G. Friederich, B. Gaylord, T. Gouhier, S. Hacker, T. Hill, G. Hofmann, M. A. McManus, B. A. Menge, K. J. Nielsen, A. Russell, E. Sanford, J. Sevadjian, and L. Washburn. 2017. Persistent spatial structuring of coastal ocean acidification in the California Current System. Scientific Reports 7:1–7.

Chan, F., J. A. Barth, J. Lubchenco, A. Kirincich, H. Weeks, W. T. Peterson, and B. A. Menge. 2008. Emergence of anoxia in the California Current Large Marine Ecosystem. Science 319:920.

Cooley, S. R., E. B. Jewett, J. Reichert, L. Robbins, G. Shrestha, D. Wieczorek, and S. B. Weisberg. 2015. Getting ocean acidification on decision makers' to-do lists: Dissecting the process through case studies. Oceanography 28:198–211.

Craig, R. K. 2015. Dealing With Ocean Acidification: the Problem, the Clean Water Act, and State and Regional Approaches. Page Washington Law Review.

Crain, C. M., K. Kroeker, and B. S. Halpern. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. Ecology Letters 11:1304–1315.

Cronin, T. W., and R. B. Forward. 1983. Vertical Migration Rhythms of Newly Hatched Larvae of the Estuarine Crab, Rhithropanopeus harrisii. The Biological Bulletin 165:139–153.

Cullison Gray, S. E., M. D. DeGrandpre, T. S. Moore, T. R. Martz, G. E. Friederich, and K. S. Johnson. 2011. Applications of in situ pH measurements for inorganic carbon calculations. Marine Chemistry 125:82–90.

Dawson, T. P., S. T. Jackson, J. I. House, I. C. Prentice, and G. M. Mace. 2011. Beyond predictions: biodiversity conservation in a changing climate. Science 332:53–58.

Dawson, T. P., M. D. A. Rounsevell, T. Kluvánková-Oravská, V. Chobotová, and A. Stirling. 2010. Dynamic properties of complex adaptive ecosystems: implications for the sustainability of service provision. Biodiversity and Conservation 19:2843–2853.

Devries, T., M. Holzer, and F. Primeau. 2017. Recent increase in oceanic carbon uptake driven by weaker upper-ocean overturning. Nature 542:215–218.

Dickson, A. G., C. L. Sabine, and J. R. Christian. 2007. Guide to Best Practices for Ocean CO 2 Measurements.

Dineshram, R., V. Thiyagarajan, A. Lane, Y. Ziniu, S. Xiao, and P. T. Y. Leung. 2013. Elevated CO2 alters larval proteome and its phosphorylation status in the commercial oyster, Crassostrea hongkongensis. Marine Biology 160:2189–2205.

Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas. 2009. Ocean Acidification: The Other CO 2Problem. Annual Review of Marine Science 1:169–192.

Doney, S. C., N. Mahowald, I. Lima, R. a Feely, F. T. Mackenzie, J.-F. Lamarque, and P. J. Rasch. 2007. Impact of anthropogenic atmospheric nitrogen and sulfur deposition on ocean acidification and the inorganic carbon system. Proceedings of the National Academy of Sciences of the United States of America 104:14580–14585.

Edwards, A. W. F., and L. L. Cavalli-Sforza. 1965. A Method for Cluster Analysis. Biometrics 21:362–375.

Ekstrom, J. A., L. Suatoni, S. R. Cooley, L. H. Pendleton, G. G. Waldbusser, J. E. Cinner, J. Ritter, C. Langdon, R. Van Hooidonk, D. Gledhill, K. Wellman, M. W. Beck, L. M. Brander, D. Rittschof, C. Doherty, P. E. T. Edwards, and R. Portela. 2015. Vulnerability and adaptation of US shellfisheries to ocean acidification. Nature Climate Change 5:207–214.

Ellner, S. P. 2013. Rapid evolution: From genes to communities, and back again? Functional

Ecology 27:1087–1099.

Estes, J. A., D. R. Lindberg, and C. Wray. 2005. Evolution of large body size in abalones (Haliotis): patterns and implications. Paleobiology 31:591–606.

Fabry, V. J., B. A. Seibel, R. A. Feely, and J. C. Orr. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. ICES Journal Marine Science 65:414–432.

Farmed Abalone Report. 2017. .

Feely, R. A., S. R. Alin, B. Carter, N. Bednaršek, B. Hales, F. Chan, T. M. Hill, B. Gaylord, E. Sanford, R. H. Byrne, C. L. Sabine, D. Greeley, and L. Juranek. 2016. Chemical and biological impacts of ocean acidification along the west coast of North America. Estuarine, Coastal and Shelf Science 183:260–270.

Feely, R. A., R. R. Okazaki, W. J. Cai, N. Bednaršek, S. R. Alin, R. H. Byrne, and A. Fassbender. 2018. The combined effects of acidification and hypoxia on pH and aragonite saturation in the coastal waters of the California current ecosystem and the northern Gulf of Mexico. Continental Shelf Research 152:50–60.

Feely, R. A., C. L. Sabine, J. M. Hernandez-ayon, D. Ianson, and B. Hales. 2008. Evidence for Upwelling of Corrosive “Acidified” Water on to the Continental Shelf. Science 320:1490–1493.

Feely, R. A., C. L. Sabine, K. Lee, W. Berelson, J. Kleypas, V. J. Fabry, F. J. Millero, and Anonymous. 2004a. Impact of anthropogenic CO2 on the CaCO3 system in the oceans. Science 305:362–366.

Feely, R. A., C. L. Sabine, R. Schlitzer, J. L. Bullister, S. Mecking, and D. Greeley. 2004b. Oxygen utilization and organic carbon remineralization in the upper water column of the Pacific Ocean. Journal of Oceanography 60:45–52.

Frieder, C. A., S. L. Applebaum, and F. Pan. 2018. Shifting Balance of Protein Synthesis and Degradation Sets a Threshold for Larval Growth Under Environmental Stress.

Frieder, C. A., J. P. Gonzalez, E. E. Bockmon, M. O. Navarro, and L. A. Levin. 2014. Can variable pH and low oxygen moderate ocean acidification outcomes for mussel larvae? Global Change Biology 20:754–764.

Froehlich, H. E., S. Barbara, T. E. Essington, and A. H. Beaudreau. 2015. Movement Patterns and

Distributional Shifts of Dungeness Crab Movement Patterns and Distributional Shifts of Dungeness Crab (Metacarcinus magister) and English Sole (Parophrys vetulus) During Seasonal Hypoxia.

Futuyma, D. J., and G. Moreno. 1988. The Evolution of Ecological Specialization Author. Annual Review of Ecology and Systematics 19:207–233.

Gaitán-Espitia, J. D., D. Marshall, S. Dupont, L. D. Bacigalupe, L. Bodrossy, and A. J. Hobday. 2017. Geographical gradients in selection can reveal genetic constraints for evolutionary responses to ocean acidification. Biology Letters 13:20160784.

Gaylord, B., K. J. Kroeker, J. M. Sunday, K. M. Anderson, J. P. Barry, N. E. Brown, S. D. Connell, S. Dupont, K. E. Fabricius, J. M. Hall-Spencer, T. Klinger, M. Milazzo, P. L. Munday, B. D. Russell, E. Sanford, S. J. Schreiber, V. Thiyagarajan, M. L. H. Vaughan, S. Widdicombe, and C. D. G. Harley. 2015. Ocean acidification through the lens of ecological theory. Ecology 96:2–15.

Gazeau, F., L. M. Parker, S. Comeau, J. P. Gattuso, W. A. O’Connor, S. Martin, H. O. Portner, and P. M. Ross. 2013. Impacts of ocean acidification on marine shelled molluscs. Marine Biology 160:2207–2245.

Glandon, H. L., and T. J. Miller. 2017. No effect of high pCO2on juvenile blue crab, Callinectes sapidus, growth and consumption despite positive responses to concurrent warming. ICES Journal of Marine Science 74:1201–1209.

Grantham, B. A., F. Chan, K. J. Nielsen, D. S. Fox, J. A. Barth, A. Huyer, J. Lubchenco, and B. A. Menge. 2004. Upwelling-driven nearshore hypoxia signals ecosystem and oceanographic changes. Nature 429:749–754.

Gruber, N., C. Hauri, Z. Lachkar, D. Loher, T. L. Frölicher, and G.-K. Plattner. 2012. Rapid progression of ocean acidification in the California Current System. Science (New York, N.Y.) 337:220–3.

Guppy, M., and P. Withers. 1999. Metabolic depression in animals: Physiological perspectives and biochemical generalizations. Biological Reviews 74:1–40.

Hale, R., P. Calosi, L. Mcneill, N. Mieszkowska, and S. Widdicombe. 2011. Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. Oikos 120:661–674.

Harris, K. E., M. D. Degrandpre, and B. Hales. 2013. Aragonite saturation state dynamics in a coastal upwelling zone. Geoph 40:2720–2725.

Hauri, C., N. Gruber, M. Vogt, S. C. Doney, R. A. Feely, Z. Lachkar, A. Leinweber, A. M. P. McDonnell, and M. Munnich. 2013. Spatiotemporal variability and long-term trends of ocean acidification in the California Current System. Biogeosciences 10:193–216.

Hill, A., A. Taylor, and S. RHC. 1991. Physiological and metabolic responses of the shore crab Carcinus maenas (L.) during environmental anoxia and subsequent recovery. Journal of Experimental Marine Biology and Ecology 150:31–50.

Hobday, A. J., and M. J. Tenger. 2002. The warm and the cold: Influence of temperature and fishing on population dynamics of red abalone.

Hofmann, G. E., J. P. Barry, P. J. Edmunds, R. D. Gates, D. A. Hutchins, T. Klinger, and M. A. Sewell. 2010. The Effect of Ocean Acidification on Calcifying Organisms in Marine Ecosystems: An Organism-to-Ecosystem Perspective. Annual Review of Ecology, Evolution, and Systematic 41:127–147.

Howes, E. L., N. Bednaršek, J. Budenbender, S. Comeau, A. Doubleday, S. M. Gallager, R. R. Hopcroft, S. Lischka, A. E. Maas, J. Bijma, and J. P. Gattuso. 2014. Sink and swim: A status review of thecosome pteropod culture techniques. Journal of Plankton Research 36:299–315.

Hurd, C. L., C. E. Cornwall, K. Currie, C. D. Hepburn, C. M. McGraw, K. A. Hunter, and P. W. Boyd. 2011. Metabolically induced pH fluctuations by some coastal calcifiers exceed projected 22nd century ocean acidification: A mechanism for differential susceptibility? Global Change Biology 17:3254–3262.

Huyer, A. 1983. Coastal upwelling in the California current system. Progress in Oceanography 12:259–284.

IPCC. 2014. Climate Change 2014 Synthesis Report.

Jackson, T. M., G. C. Roegner, and K. G. O’Malley. 2018. Evidence for interannual variation in genetic structure of Dungeness crab (Cancer magister) along the California Current System. Molecular Ecology 27:352–368.

Jensen, G. C., and D. A. Armstrong. 1987. Range Extensions of Some Northeastern Pacific Decapoda. Crustaceana 52:215–217.

Johansen, K., C. Lenfant, and A. T. Mecklenburg. 1970. Respiration in the Crab, Cancer magister. Z. vergl. Physiologie 70:1–19.

Karpov, K. a, P. L. Haaker, I. K. Taniguchi, and L. Rogers-Bennett. 2000. Serial depletion and the collapse of the California abalone (Haliotis spp .) fishery. Workshop on Rebuilding Abalone Stocks in British Columbia 200:11–24.

Keller, A. A., V. Simon, F. Chan, W. W. Wakefield, M. E. Clarke, J. A. Barth, D. A. N. Kamikawa, and E. L. Fruh. 2010. Demersal fish and invertebrate biomass in relation to an offshore hypoxic zone along the US West Coast:76–87.

Kelly, M. W., and G. E. Hofmann. 2013. Adaptation and the physiology of ocean acidification. Functional Ecology 27:980–990.

Kelly, M. W., E. Sanford, and R. K. Grosberg. 2012. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proceedings of the Royal Society B: Biological Sciences 279:349–356.

Kelly, R. P., M. M. Foley, W. S. Fisher, R. A. Feely, B. S. Halpern, G. G. Waldbusser, and M. R. Caldwell. 2011. Mitigating local causes of ocean acidification with existing laws. Science 332:1036–1037.

Kim, T. W., J. P. Barry, and F. Micheli. 2013. The effects of intermittent exposure to low-pH and low-oxygen conditions on survival and growth of juvenile red abalone. Biogeosciences 10:7255–7262.

Kingsford, M. J., J. M. Leis, A. Shanks, K. C. Lindeman, S. G. Morgan, and J. Pineda. 2002. Sensory environments, larval abilities, and local self-recruitment. Bulletin of Marine Science 70:309–340.

Kroeker, K. J., R. L. Kordas, R. Crim, I. E. Hendriks, L. Ramajo, G. S. Singh, C. M. Duarte, and J. P. Gattuso. 2013a. Impacts of ocean acidification on marine organisms: Quantifying sensitivities and interaction with warming. Global Change Biology 19:1884–1896.

Kroeker, K. J., R. L. Kordas, and C. D. G. Harley. 2017. Embracing interactions in ocean acidification research: confronting multiple stressor scenarios and context dependence.

Biology Letters 13:20160802.

Kroeker, K. J., F. Micheli, and M. C. Gambi. 2013b. Ocean acidification causes ecosystem shifts via altered competitive interactions. Nature Climate Change 3:156–159.

Lande, R., and S. Shannon. 1996. The role of genetic variation in adaptation and population persistence in a changing environment. Evolution 50:434–437.

Lester, S. E., K. L. Mcleod, H. Tallis, M. Ruckelshaus, B. S. Halpern, P. S. Levin, F. P. Chavez, C. Pomeroy, B. J. Mccay, C. Costello, S. D. Gaines, A. J. Mace, J. A. Barth, D. L. Fluharty, and J. K. Parrish. 2010. Science in support of ecosystem-based management for the US West Coast and beyond. Biological Conservation 143:576–587.

Levin, P. S., T. E. Essington, K. N. Marshall, L. E. Koehn, L. G. Anderson, P. S. Levin, A. Bundy, C. Carothers, F. Coleman, L. R. Gerber, J. H. Grabowski, J. N. Sanchirico, and A. D. M. Smith. 2018. Building effective fishery ecosystem plans. Marine Policy In Press.

Lischka, S., J. Budenbender, T. Boxhammer, and U. Riebesell. 2011. Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod Limacina helicina: Mortality, shell degradation, and shell growth. Biogeosciences 8:919–932.

Long, W. C., K. M. Swiney, C. Harris, H. N. Page, and R. J. Foy. 2013. Effects of Ocean Acidification on Juvenile Red King Crab (Paralithodes camtschaticus) and Tanner Crab (Chionoecetes bairdi) Growth, Condition, Calcification, and Survival. PLoS ONE 8.

Lynn, K., J. Daigle, J. Hoffman, F. Lake, N. Michelle, D. Ranco, C. Viles, G. Voggesser, and P. Williams. 2013. The impacts of climate change on tribal traditional foods. Climatic Change 120:545–556.

Malley, K. G. O., K. Corbett, T. D. Beacham, D. P. Jacobson, T. M. Jackson, and G. C. Roegner. 2017. Genetic connectivity of Dungeness crab (Cancer magister) across oceanographic regimes Genetic Connectivity of the Dungeness Crab (Cancer magister) Across Oceanographic Regimes Published By : National Shellfisheries Association.

McLeod, K. L., and H. M. Leslie. 2009. Why Ecosystem Based Management? Pages 3–12Ecosystem Based Management for the Oceans.

McMahon, B. R. 2001. Respiratory and circulatory compensation to hypoxia in crustaceans.

Respiration Physiology 128:349–364.

Miller, A. W., A. C. Reynolds, C. Sobrino, and G. F. Riedel. 2009. Shellfish face uncertain future in high CO2 world: Influence of acidification on oyster larvae calcification and growth in estuaries. PLoS ONE 4.

Miller, J. J., M. Maher, E. Bohaboy, C. S. Friedman, and P. McElhany. 2016. Exposure to low pH reduces survival and delays development in early life stages of Dungeness crab (Cancer magister). Marine Biology 163:1–11.

Moore, T. O., and J. D. Moore. 2008. Status of Fisheries Report 2008.

Munday, P. L., R. R. Warner, K. Monro, J. M. Pandolfi, and D. J. Marshall. 2013. Predicting evolutionary responses to climate change in the sea. Ecology Letters 16:1488–1500.

Obegi, D. 2008. Is there a Constitutional Right to fish in a Marine Protected Area? An analysis of the California Constitution's right to fish provision and its impact on the state's power to create marine reserves and other types of Marine Protected Areas (MPAs). West-Northwest Journal of Environmental Law & Policy 1419:1–29.

Olmi, E. J. 1994. Vertical migration of blue crab Callinectes sapidus megalopae: Implications for transport in estuaries. Marine Ecology Progress Series 113:39–54.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos, R. M. Key, K. Lindsay, E. Maier-Reimer, R. Matear, P. Monfray, A. Mouchet, R. G. Najjar, G.-K. Plattner, K. B. Rodgers, C. L. Sabine, J. L. Sarmiento, R. Schlitzer, R. D. Slater, I. J. Totterdell, M.-F. Weirig, Y. Yamanaka, and A. Yool. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–6.

Pan, T.-C. F., S. L. Applebaum, and D. T. Manahan. 2015. Experimental ocean acidification alters the allocation of metabolic energy. Proceedings of the National Academy of Sciences 112:4696–4701.

Pane, E. F., and J. P. Barry. 2007. Extracellular acid – base regulation during short-term hypercapnia is effective in a shallow-water crab , but ineffective in a deep-sea crab 334:1–9.

Parker, L. M., P. M. Ross, W. A. O'Connor, L. Borysko, D. A. Raftos, and H. O. Pörtner. 2012.

Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology 18:82–92.

Pespeni, M. H., F. Chan, B. A. Menge, and S. R. Palumbi. 2013a. Integrative and Comparative Biology Signs of Adaptation to Local pH Conditions across an Environmental Mosaic in the California Current Ecosystem. Integrative and Comparitive Biology 53:857–870.

Pespeni, M., E. Sanford, B. Gaylord, T. Hill, J. Hosfelt, H. K. Jaris, M. LaVigne, E. Lenz, A. Russell, M. Young, and S. Palumbi. 2013b. Evolutionary change during experimental ocean acidification. Proceedings of the National Academy of Sciences 110:6937–6942.

Portner, H.-O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. Journal of Experimental Biology 213:881–893.

Pörtner, H.-O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist’s view. Marine Ecology Progress Series 373:203–217.

Pörtner, H. O., M. Langenbuch, and A. Reipschlager. 2004. Biological Impact of Elevated Ocean CO2 Concentrations: Lessons from Animal Physiology and Earth History. Journal of Oceanography 60:705–718.

Reid, J., L. Rogers-Bennett, F. Vasquez, M. Pace, C. A. Catton, J. V. Kashiwada, and I. K. Taniguchi. 2016. The economic value of the recreational red abalone fishery in northern California. California Fish and Game 102:119–130.

Ries, J. B. 2011. A physicochemical framework for interpreting the biological calcification response to CO2-induced ocean acidification. Geochimica et Cosmochimica Acta 75:4053–4064.

Ries, J. B., A. L. Cohen, D. C. Mccorkle, J. B. Ries, A. L. Cohen, and D. C. Mccorkle. 2009. Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. Geology 37:1131–1134.

Rogers-Bennett, L., B. L. Allen, and G. E. Davis. 2004. Measuring abalone (Haliotis spp.) recruitment in California to examine recruitment overfishing and recover criteria. Journal of Shellfish Research 23:1201–1207.

De Roos, A., and L. Persson. 2013. Population and Community Ecology of Ontogenetic

Development. Princeton University Press.

Sabine, C. L., R. A. Feely, N. Gruber, R. M. Key, K. Lee, J. L. Bullister, R. Wanninkhog, C. S. Wong, D. W. R. Wallace, B. Tilbrook, F. J. Millero, T.-H. Peng, A. Kozyr, T. Ono, and A. F. Rios. 2004. The Oceanic Sink for Anthropogenic CO2. Science (New York, N.Y.) 305:367–371.

Sagarin, R. D., and M. Turnipseed. 2012. The Public Trust Doctrine: Where Ecology Meets Natural Resources Management. Annual Review of Environment and Resources 37:473–496.

Sanford, E., and M. W. Kelly. 2011. Local Adaptation in Marine Invertebrates. Annual Review of Marine Science 3:509–535.

Schoener, T. W. 2011. The Newest Synthesis: Understanding the Interplay of Evolutionary and Ecological Dynamics. Science 331:426–429.

Seibel, B. A., A. E. Maas, and H. M. Dierssen. 2012. Energetic plasticity underlies a variable response to ocean acidification in the pteropod, Limacina helicina antarctica. PLoS ONE 7.

Seibel, B. A., and P. J. Walsh. 2003. Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance. Journal of Experimental Biology 206:641–650.

Shanks, A. L. 1986. Vertical migration and cross-shelf dispersal of larval Cancer spp. and Randallia ornata (Crustacea: Brachyura) off the coast of southern California. Marine Biology: International Journal on Life in Oceans and Coastal Waters 92:189–199.

Shanks, A. L., and G. C. Roegner. 2007. Recruitment limitation in Dungeness crab populations is driven by variation in atmospheric forcing. Ecology 88:1726–1737.

Sievanen, L., H. M. Leslie, J. M. Wondolleck, S. L. Yaffee, K. L. McLeod, and L. M. Campbell. 2011. Linking top-down and bottom-up processes through the new U.S. National Ocean Policy. Conservation Letters 4:298–303.

Snyder, M. A., L. C. Sloan, N. S. Diffenbaugh, and J. L. Bell. 2003. Future climate change and upwelling in the California Current. Geophysical Research Letters 30:1823.

Somero, G. N., J. M. Beers, F. Chan, T. M. Hill, T. Klinger, and S. Y. Litvin. 2015. What Changes in the Carbonate System, Oxygen, and Temperature Portend for the Northeastern Pacific Ocean: A Physiological Perspective. BioScience 66:14–26.

Strong, A. L., K. J. Kroeker, L. T. Teneva, L. A. Mease, and R. P. Kelly. 2014. Ocean acidification 2.0: Managing our Changing Coastal Ocean Chemistry. BioScience 64:581–592.

Sunday, J. M., P. Calosi, S. Dupont, P. L. Munday, J. H. Stillman, and T. B. H. Reusch. 2014. Evolution in an acidifying ocean. Trends in Ecology and Evolution 29:117–125.

Sunday, J. M., R. N. Crim, C. D. G. Harley, and M. W. Hart. 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. PLoS ONE 6:1–8.

Then, A. Y., J. M. Hoenig, N. G. Hall, and D. A. Hewitt. 2016. Interpretation and design of ocean acidification experiments in upwelling systems in the context of carbonate chemistry co-variation with temperature and oxygen. ICES Journal of Marine Science 73:582–595.

Tripp, M., C. Bock, M. A. Tripp-valdez, C. Bock, M. Lucassen, and S. E. Lluch-cota. 2017. Metabolic response and thermal tolerance of green abalone juveniles (Haliotis fulgens : Gastropoda) under acute hypoxia and hypercapnia Journal of Experimental Marine Biology and Ecology Metabolic response and thermal tolerance of green abalone juveniles (Haliotis fulgens : Gastropoda) under acute hypoxia and hypercapnia. Journal of Experimental Marine Biology and Ecology 497:11–18.

Vargas, C. A., N. A. Lagos, M. A. Lardies, C. Duarte, P. H. Manríquez, V. M. Aguilera, and B. Broitman. 2017. Species-specific responses to ocean acidification should account for local adaptation and adaptive capacity. Nature Ecology and Evolution 1:1–7.

Waldbusser, G. G., B. Hales, C. J. Langdon, B. A. Haley, P. Schrader, E. L. Brunner, M. W. Gray, C. A. Miller, and I. Gimenez. 2015a. Saturation-state sensitivity of marine bivalve larvae to ocean acidification. Nature Climate Change 5:273–280.

Waldbusser, G. G., B. Hales, C. J. Langdon, B. A. Haley, P. Schrader, E. L. Brunner, M. W. Gray, C. A. Miller, I. Gimenez, and G. Hutchinson. 2015b. Ocean acidification has multiple modes of action on bivalve larvae. PLoS ONE 10.

Weiner, S., and P. M. Dove. 2003. An Overview of Biomineralization Processes and the Problem of the Vital Effect. Reviews in Mineralogy and Geochemistry 54:1–29.

Weisberg, S. B., N. Bednaršek, R. A. Feely, F. Chan, A. B. Boehm, M. Sutula, J. L. Ruesink, B. Hales, J. L. Largier, and J. A. Newton. 2016. Water quality criteria for an acidifying ocean: Challenges and opportunities for improvement. Ocean and Coastal Management 126:31–

41.

Wheatly, M. G., and R. P. Henry. 1992. Extracellular and intracellular acid-base regulation in crustaceans. Journal of Experimental Zoology 263:127–142.

Whiteley, N. M. 2011. Physiological and ecological responses of crustaceans to ocean acidification. Marine Ecology Progress Series 430:257–271.

Widdicombe, S., and J. I. Spicer. 2008. Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? Journal of Experimental Marine Biology and Ecology 366:187–197.

De Wit, P., and S. R. Palumbi. 2013. Transcriptome-wide polymorphisms of red abalone (Haliotis rufescens) reveal patterns of gene flow and local adaptation. Molecular Ecology 22:2884–2897.

Wittmann, A. C., and H. O. Pörtner. 2013. Sensitivities of extant animal taxa to ocean acidification. Nature Climate Change 3:995–1001.

Wood, C. M., and J. N. Cameron. 1985. Temperature and the physiology of intracellular and extracellular acid-base regulation in the Blue Crab Callinectes sapidus. Journal of Experimental Biology 179:151–179.

Zeebe, R., and D. Wolf-Gladrow. 2001. CO2 in Seawater-Equilibrium, Kinetics, Isotopes. Elsevier:100.

Zippay, M. L., and G. E. Hofmann. 2010. Effect of pH on Gene Expression and Thermal Tolerance of Early Life History Stages of Red Abalone (Haliotis rufescens). Journal of Shellfish Research 29:429–439.

Policy & Reports Cited

Assembly Bill No 2139 (2016) 352 AB 2139. Sacramento, CA.

Clean Air Act (1970) 42 U.S.C. § 7401 *et seq.*

Clean Water Act (1972) 33 U.S.C. § 1251 ***et seq*.**

Section 303(d): Impaired Waters and Total Maximum Daily Loads.

Exec. Order No. 12-07 (2012) WA EO 12-07: "Washington's Response to Ocean Acidification". Office of the Governor. Olympia, Washington.

Federal Ocean Acidification Research and Monitoring Act of 2008 (2007) 33 U.S.C. 50 § 3701-3708. 110th Congress. Washington, DC

Massachusetts v. EPA (549 U.S. 497, 2007)

Oregon Shellfish Policy Bill (2015) HB 2209. 78th Leg., Reg Sess. Salem, OR.

Pacific Coast Action Plan on Climate and Energy (2013) http://pacificcoastcollaborative.org/Documents/Pacific%20Coast%20Climate%20Action%20Plan.pdf (Last visited 18 Mar 2018)

Senate Bill 1039A (2017) 79th Leg., Reg. Sess. Salem, OR.

Senate Bill 1363 (2016) 2015-2016 Regular Sess. Sacramento, CA.

Senate Bill 5603 (2013) 63rd Leg., Reg. Sess. Olympia, WA.
Codified: Wash Rev Code 43.06.338 (2016)

Shellfish Protection Act of 1993 (1993) California Water Code § 14950-19958

Temporary Administrative Order (2017) 635 ODFW 156-2917

Washington State Blue Ribbon Panel on Ocean Acidification (2012): Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response. H. Adelsman and L. Whitely Binder (eds). Washington Department of Ecology, Olympia, Washington. Publication no. 12-01-015.

West Coast Regional Planning Body Charter (2015) (Last visited 18 Mar 2018) https://static1.squarespace.com/static/535fdf12e4b071a2c2e39cae/t/59a48ce3f5e231b165732007/1503956197132/WestCoastRPB_FullCharter_SignatoryDraft.pdf

2017 Addendum to Ocean Acidification: From Knowledge to Action, Washington State's Strategic Response. (2017) *Washington Marine Resources Advisory Council*. EnviroIssues (eds). Seattle, Washington.

News Articles & Press Releases

California recreational abalone fishery to be closed in 2018. (08 Dec 2017) Press Release, *California Department of Fish and Wildlife.* (Last viewed 18 Mar 2018) https://cdfgnews.wordpress.com/2017/12/08/california-recreational-abalone-fishery-to-be-closed-in-2018/

Scientists: Oregon dodges a 'dead zone' bullet in 2017; hypoxia seasons similar to wildlife. 08 Nov 2017. Press Release, *Oregon State University*. Last viewed 18 Mar 2018. http://today.oregonstate.edu/news/scientists-oregon-dodges-%E2%80%98dead-zone%E2%80%99-bullet-2017-hypoxia-season-similar-wildfire