

PHOTOTAXIS OF DUNGENESS CRAB ZOEAE IN HIGH-CO₂ SEAWATER:
IMPLICATIONS FOR COASTAL ECOSYSTEMS IN AN ACIDIFIED OCEAN

by

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ABSTRACT

Phototaxis of Dungeness crab zoeae in high-CO₂ seawater:
implications for coastal ecosystems in an acidified ocean.

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Anthropogenic carbon dioxide emissions are reducing the global average oceanic pH in a process known as ocean acidification. High levels of carbon dioxide (CO₂) in seawater are shown to increase phototaxis and impair predator avoidance based on visual cues in larval fish. However, we currently do not understand the impact of high-CO₂ seawater on the phototaxis of any larval crustacean. The present study is the first to evaluate the phototaxis of a larval crustacean in high-CO₂ seawater. Larvae of the ecologically and economically important Dungeness crab *Metacarcinus magister* were reared in three CO₂ treatments (400, 1600, 3200 μatm) and exposed individually to a directional light in a horizontal aquarium in a chronic behavioral bioassay. The response of larvae to light treatments was video recorded and analyzed for variation in phototaxis to see if acidification would impact their natural tendency to approach this light stimulus. Larvae exposed to a light treatment were significantly more likely to swim to the light than those in the control (i.e., dark) treatment, thus demonstrating positive phototaxis. Non-significant results indicate that the phototactic behavior of larval *M. magister* does not appear to be pH-sensitive. Since benthic Puget Sound organisms are evolutionarily adapted to withstand large pH fluctuations, it is possible that high-CO₂ conditions are not a threat to *M. magister* phototactic behavior. However, weak non-significant trends may suggest that animals reared in high-CO₂ seawater swam to the light the faster, spent a greater proportion of time stationary at the light, and exhibited a lower overall mean speed in the control treatment than animals from the other two CO₂ treatments. The latter behavior could be explained by a change in metabolism due to CO₂-induced acidification. Info-disruption through overexcitation of the histaminergic photoreceptor cell, which could affect fitness and survival rates, is proposed as a mechanism for a possible heightened phototactic response in *M. magister* larvae reared in high-CO₂ seawater.

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Dedication and Acknowledgements

This thesis is lovingly dedicated to the memory of my grandfather, Dr. Don Goodenough, who taught me algebra on the back of a placemat at a Chinese food restaurant, and to the memory of my uncles, Jamie Goodenough, who actively motivated me to pursue higher education, and Chuck Goodenough, who firmly believed that I could accomplish anything I put my mind to.

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1. LITERATURE REVIEW

1.1 Introduction

Anthropogenic carbon emissions are increasing the global average concentration of atmospheric carbon dioxide (CO₂) and since the oceans and the atmosphere are constantly exchanging gases and equilibrating, oceanic CO₂ is rising at the same rate as atmospheric CO₂. CO₂ and H₂O chemically react to decrease the pH of seawater in a process known as ocean acidification (OA). OA has the potential to impact marine life at the individual, species, population, and ecosystem level, thereby causing deleterious socioeconomic repercussions. The survival and fitness of marine organisms may be affected physiologically by high-CO₂ seawater via a number of processes such as fertilization, growth, and behavior. By determining possible impacts of ocean acidification on marine species through manipulative experiments exposing marine organisms to high-CO₂ seawater, models may be generated to better predict ecosystem-wide impacts.

Accordingly, the research conducted in this thesis examines the behavioral responses of Dungeness crab *Metacarcinus magister* zoeae to light, specifically phototaxis, in high-CO₂ conditions by using a chronic bioassay approach. To set the stage as to why this work is important, this literature review will first review the literature addressing the process of OA, and then analyze the current state of knowledge about larval and crustacean behavioral response to elevated levels of CO₂ in seawater along with larval crab visual phototransduction and phototaxis. Finally, it will clarify how the specific research approach employed in this study advances our understanding of the

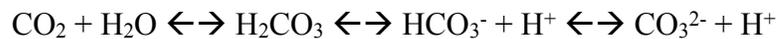
impacts of high-CO₂ seawater on larval crustacean phototaxis.

1.2 Ocean acidification

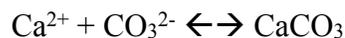
Anthropogenic carbon emissions are changing Earth's chemical balance. Since pre-industrial times, carbon emissions have elevated the atmospheric CO₂ concentrations from a global average of 287 ppm (Etheridge et al. 1996; Meehl et al. 2007) to an average in October 2013 of 394 ppm (NOAA 2013, unpublished data). When air and water come into contact, gases like CO₂ are exchanged and equilibrated, and when atmospheric CO₂ concentrations rise above oceanic CO₂ concentrations, the oceans absorb CO₂ to maintain equilibrium (Doney et al. 2009b). The global ocean is a net sink for anthropogenic CO₂ (Sabine et al. 2004). However, this relief of atmospheric CO₂ does not come without problems. Through a process known as “ocean acidification,” global marine life is threatened with an environment that may be changing too rapidly for adaptation.

Data from 30 years of oceanographic cruises show that surface concentrations of pCO₂ in the North Pacific are increasing at the same rate as atmospheric CO₂ levels (Takahashi et al. 2006). This provides evidence for a stable rate of CO₂ mixing between seawater and the atmosphere. It also provides a solid link between anthropogenic carbon emissions and the increases in dissolved inorganic carbon. Average global surface ocean pH has already decreased by about 0.1, which corresponds to a 30% increase in hydrogen ions, and the ocean pH is predicted to sink by another 0.3-0.4 units by the year 2100 (Feely et al. 2004; Orr et al. 2005; Doney et al. 2009b; Steinacher et al. 2009). Ocean

acidification (OA) has the potential to change the function of ecosystems worldwide. Analyses of global measurements of inorganic carbon show that nearly half of anthropogenic carbon emissions is absorbed by the world's oceans (Sabine et al 2004). When CO₂ enters seawater, it reacts chemically with H₂O to create carbonic acid (H₂CO₃). Carbonic acid dissociates, increasing the concentration of hydrogen ions (H⁺) in seawater and decreasing the concentration of carbonate ions (CO₃²⁻) through reactions described by the chemical equation below (Doney et al. 2009b; Feely et al. 2010).



This lowered pH is defined as an increase in hydrogen ion activity (Covington et al. 1985). A decrease in pH can cause a decrease in the saturation states of minerals aragonite and calcite because a decrease in carbonate ions leads to a decrease in saturation state. Aragonite and calcite are chemically identical since both are forms of calcium carbonate (CaCO₃), and the difference between the two is that the molecules forming aragonite are less tightly packed than those in calcite, making them more prone to dissolution in low-pH seawater (Feely and Chen 1982; Mucci 1983). Saturation state (Ω) is the thermodynamic potential for a mineral to form or dissolve. The following chemical reaction illustrates the dissolution and precipitation of calcium carbonate:



The saturation state is defined as the product of calcium and carbonate concentrations divided by the calcium carbonate concentration:

$$([\text{Ca}^{2+}] \times [\text{CO}_3^{2-}]) / [\text{CaCO}_3] = \Omega$$

A decrease in CO_3^{2-} makes CaCO_3 dissolution more likely. The ratio of the stoichiometric solubility product (K_{sp}^*) to carbonate (CO_3^{2-}) primarily dictates the saturation state of calcium carbonate minerals (Feely et al. 2010):

$$\Omega_{\text{arg}} = [\text{Ca}^{2+}][\text{CO}_3^{2-}]/K_{sp}^*_{\text{arg}}$$

$$\Omega_{\text{cal}} = [\text{Ca}^{2+}][\text{CO}_3^{2-}]/K_{sp}^*_{\text{cal}}$$

When the saturation state falls below 1 ($\Omega < 1$), minerals such as aragonite and calcite, which are vital for the formation of calcified body parts of many marine organisms, dissolve (Orr et al. 2005, Doney et al. 2009b, Feely et al. 2010). This puts marine organisms at risk of being able to adequately complete their body plans. A number of other physiological impacts, some of which may influence behavior, could occur as a result of ocean acidification.

1.3 The California Current System and Puget Sound

This study focuses on the zooplanktonic larvae of *M. magister*, which inhabit Northeast Pacific coastal surface waters within the California Current System (CCS) and the Puget Sound (Pauley et al. 1989). Puget Sound is a fjordal estuary complex that may be prone to rapid negative effects of ocean acidification since pH levels are low in comparison with the global average oceanic pH due to both natural and anthropogenic factors (Feely et al. 2010). OA in conjunction with existing chemical conditions in the Puget Sound could have compounding effects on coastal and estuarine ecosystems in the region (Feely et al. 2010). Natural factors such as coastal upwelling from the CCS into the Sound and biotic activity as well as anthropogenic factors such as nutrient inputs and atmospheric nitric and sulfuric acid emissions can each impact the pH of Puget Sound,

contributing to its vulnerability to ocean acidification (Doney et al. 2007; Feely et al. 2010). I will review each of these factors in the following paragraphs.

Natural biotic activity can lead to low pH levels in Puget Sound. Phytoplankton blooms occur with greater frequency during summer months due to nutrient and sunlight availability (Pitcher et al. 2010). Puget Sound experiences high rates of algal blooms due to naturally high nutrient concentrations (MacFadyen et al. 2008). When phytoplankton die, bacteria consume the carbon. With bacterial growth comes increased biotic respiration, and CO₂ seawater concentrations increase, leading to decreases in pH (Feely et al. 2010). These natural CO₂ production mechanisms predispose Puget Sound ecosystems to vulnerability to ocean acidification.

Anthropogenic nutrient inputs can also contribute to phytoplankton blooms and consequent increases in acidity. Due to the heavy urbanization of the Puget Sound Basin, drainage can sweep pollutants, nutrients, and organic matter into the Sound (Feely et al. 2010). Because parts of this inland sea are characterized by sluggish circulation and constrained flow, local nutrient inputs can have large impacts (Feely et al. 2010). Nutrient inputs from development can contribute to algal blooms, which may lead to localized decreases in pH as bacteria consume their biomass, as described above (Khangaonkar et al. 2012; Feely et al. 2010).

Another key factor contributing to the low average pH of Puget Sound is the natural acidity of the coastal waters of the CCS that feed this estuary. The CCS is characterized by coastal seasonal upwelling, which brings to the surface cold, nutrient-rich, low-pH, low-oxygen seawater with a low carbonate saturation state (Feely et al.

2010; Gruber et al. 2012). Deep coastal northeast Pacific waters are low in pH since the global ocean circulation system brings a deep current that downwells in the North Atlantic, accumulates CO₂ along the way, and upwells in the Northeast Pacific (Broecker and Peng 1992). Natural conditions in the CCS are already among the most corrosive in the world, with upwelling events carrying water with pH as low as 7.65 in deep coastal Washington waters (Feely et al. 2008; Hauri et al. 2013). There are large variations in pH spatially and temporally in the CCS. The current may rapidly be approaching a departure from its current pH range (Hauri et al. 2013). Based on models, the average CCS pH decreased from 8.12 to 8.04 between the years 1750 and 2005, and by the year 2050 under current emission rates, could decrease to a pH of 7.92 (Gruber et al. 2012).

In addition to these stressors to Puget Sound pH, local atmospheric non-CO₂ emissions may further acidify coastal waters. The combustion of biomass and fossil fuels leads to atmospheric deposition of nitric and sulfuric acid in coastal ecosystems and could further decrease seawater pH close to shore. This effect is likely to be more significant in coastal waters than in the open ocean due to proximity to land-based emissions. In coastal waters, atmospheric nitrogen and sulfur deposition could account for 10-50% of anthropogenic acidification (Feely et al. 2010). Atmospheric nitrogen efflux as well as nitrogen carried in freshwater to coastal oceans is estimated to increase during the next few decades (Doney et al 2009a). These mounting impacts of natural factors such as biotic activity and coastal upwelling, and anthropogenic factors such as nutrient input and non-CO₂ sources of acidification predispose Puget Sound to the harmful effects of CO₂-driven ocean acidification.

Although *M. magister* inhabits the full range of the Pacific coast, the adult specimen used in this study was collected in Puget Sound, Washington, so it is necessary to understand the past, current and future pH levels of this estuary, particularly at the surface. The range of surface pH levels in 2008 in Puget Sound was 7.72 to 7.95 (Feely et al. 2010). The average overall pH of Puget Sound may be lower than this range since it does not include deeper waters, which are generally more acidic than surface waters, and since pH may have decreased in the years since 2008, In Hood Canal, dissolved inorganic carbon was $54 \mu\text{mol kg}^{-1}$ and $18 \mu\text{mol kg}^{-1}$ higher than the average Admiralty Inlet levels in the summer and the winter, respectively (Feely et al. 2010). This corresponds to a 24-49% decrease in pH that can be accounted for by anthropogenic CO_2 input since the industrial revolution, while the rest is due to natural respiration (Feely et al. 2010). Modeling future levels of pH in this basin is difficult due to the quantity of unknown variables at hand (Feely et al. 2010). No comprehensive model of future pH variability parameters within Puget Sound exists as of December 2013.

1.4 *Metacarcinus magister* natural history

Marine life is already exhibiting the impacts of ocean acidification (Doney et al. 2009b). Quantifying ocean acidification impacts on ecosystems is a complex task due to the variability in organismal physiology and the magnitude of diversity. Some species may proliferate in a low-pH ocean (Le Quesne et al. 2012), while others may become extinct (Uthicke and Fabricius 2012; Dupont and Thorndyke 2009). In order to determine the effects of ocean acidification on *M. magister*, a holistic account of the organism's natural history and vulnerability must be considered.

The Dungeness crab *Metacarcinus magister* (Decapoda: Brachyura; formerly known as *Cancer magister*) is a benthic invertebrate that inhabits the rocky sandy-mud intertidal zone (Karpov 1983) along the west coast of North America from the Santa Barbara Channel in California to the Aleutian Islands in Alaska, including Puget Sound (Pauley et al. 1989). *M. magister* is distinguishable from crabs within its genus by a pronounced tenth marginal carapace tooth (MacKay 1934). In Washington, *M. magister* females extrude their eggs between October and December (Cleaver 1949). Females brood the eggs on their pleopods until they hatch, which occurs between January and April in Washington (Cleaver 1949). Zoeae typically hatch synchronously with high tide (DeCoursey 1979) and once liberated, swim toward the surface and are transported seaward by outgoing currents. In the zooplankton, zoeae molt through five different larval stages, called instars, until they become megalopae and then settle as juvenile crabs (Poole 1966). Megalopae and zoeae are important prey sources of Chinook salmon, pink salmon, and coho salmon, as well as rockfish and herring, hence *M. magister*'s valuable role in the pelagic ecosystem (Orcutt et al. 1976; Reilly 1983; Prince and Gotshall 1976). The vulnerability of *M. magister* zoeae to ocean acidification can be characterized in terms of larval vulnerability and crustacean vulnerability.

1.5 Vulnerability of larvae to OA

Early developmental stages of marine invertebrates are particularly vulnerable to the effects of ocean acidification. Since pH tolerance varies by life stage, it is crucial to test the response of embryonic, larval, and juvenile organisms to ocean acidification (Kurihara 2008). It is presumably advantageous for larvae to minimize each stage of

development in order to minimize time spent in the water column where these animals are vulnerable to predation (Dupont and Thorndyke 2009). Any delays in development caused by OA could lead to population-level impacts (Dupont and Thorndyke 2009). Larvae are among the most vulnerable of life stages to predation due to their low trophic level and small size. Indeed, predation is considered to be the most common cause of mortality for planktonic larvae (Morgan 1995). During early developmental stages, marine invertebrates have highly specific environmental requirements and small shifts in seawater chemistry components such as pH can have large impacts (Thorson 1950; Kurihara 2008). Many mollusk species are at risk of larval mortality or impaired development due to ocean acidification (e.g. Timmins-Schiffman et al. 2013). However, some crab species show enhanced growth and calcification in high-CO₂ seawater (e.g. Long et al. 2013), so calcification may be a lesser concern for crustacean larvae than other physiological indices (Whiteley 2011).

1.6 Vulnerability of crustaceans to OA

1.6.1 *Calcification*

One of the most-studied impacts of ocean acidification on marine life is that of impaired calcification and shell dissolution (e.g. Bednarsek et al. 2012). Studies on brachyuran crabs have shown that calcification may not be as critical as other physiological factors that could limit crustacean success in an acidified ocean (Ries et al. 2009). Cancrid crab and other crustacean exoskeletons have a higher ratio of calcite to aragonite than do those of mollusks and echinoderms (Boßelmann et al. 2007). Aragonite is more soluble than calcite, so other phyla are more susceptible to population threats

based on calcification problems than are crustaceans (Whiteley 2011). Blue crab *Callinectes sapidus* shows increased calcification with increased acidity (Ries et al. 2009). Barnacles may grow harder shells under highly acidified conditions (McDonald et al. 2009). Crustacean physiological adjustment to changes in pH, such as internal acid-base regulation and behavioral impacts, may be a factor of greater concern than calcification (Whiteley 2011). However, post-moult calcification in crustaceans could be impacted by increases in seawater CO₂. Calcification in crustaceans involves the uptake of calcium (Ca²⁺) and bicarbonate (HCO₃⁻) across the gills, and with increases in H⁺ concentration in seawater, HCO₃⁻ uptake may be slowed and the period of post-moult calcification may be extended (Cameron 1985; Whiteley 2011). Delay in post-moult calcification can leave crustaceans vulnerable to predation; thus, ocean acidification has the potential to increase crustacean mortality rates (Whiteley 2011).

1.6.2 *Physiology*

When submerged, crustaceans are constantly in contact with seawater through the gills, which exchange gases and ions with the surrounding environment (Taylor and Taylor 1992). Aquatic organisms are more likely to be affected by changes in CO₂ concentration than marine organisms since metabolic CO₂ and HCO₃⁻ levels are generally much lower in marine organisms than those in terrestrial organisms, leaving a smaller buffer for changes in the concentrations of CO₂ and HCO₃⁻ (Nilsson et al. 2012). Changes in carbonate chemistry in the marine environment lead to a decrease in pH in the seawater, which then decreases the pH in the extracellular compartment, or the hemolymph (Whiteley 2011). Acid-base homeostasis is the process by which organisms

maintain a pH that supports the functioning of processes such as respiration, protein synthesis, and metabolism (Taylor and Whiteley 1989; Wheatly and Henry 1992). This is accomplished through compensatory mechanisms, the foremost of which are the carbonate buffering system and iono-regulation in crustaceans (Whiteley 2011). The carbonate buffering system is a process in which organisms buffer increases in H^+ by incorporating excess H^+ into HCO_3^- ions (Wheatly and Henry 1992). Iono-regulation, which is the process by which carbonate buffering occurs, is the ion exchange of HCO_3^- for Cl^- and H^+ for Na^+ across the gill epithelia (Taylor and Taylor 1992; Whiteley 2011). A slow metabolism is generally correlated with inefficient iono-regulation (Whiteley 2011). Exposure to pH decreases could affect crustacean growth, reproduction, and behavior by channeling energy away from these functions and towards physiological compensation for low pH (Whiteley 2011).

Adult *M. magister* specimens exhibit the ability to compensate for acid-base disturbance in the haemolymph by efficiently iono-regulating during short-term (24 h) exposure to extremely low-pH (7.08) seawater (Pane and Barry 2007). This study suggests that iono-regulation of *M. magister* adults may not be a limiting factor to survival. It is possible that *M. magister* larvae may be more susceptible to changes in carbonate chemistry. A study on the effect of high- CO_2 (1000 ppm) seawater on *M. magister* larvae at Day 1 and Day 5 of development shows that these organisms may exhibit increased swimming speed in CO_2 -acidified seawater, indicating a possible metabolic effect (Christmas 2013). Feeding rates and gross growth efficiency were not impacted by high- CO_2 treatment (Christmas 2013). Red king crab *Paralithodes*

camtschaticus larvae exhibited decreased yolk size in acidic conditions, longer zoeal length at hatch, increased eye size, increased calcification, and decreased survival with greater acidity (Long et al. 2013). In accordance with the suggestion of Doney et al. (2009b) to administer longer-term laboratory experiments at realistic pH conditions, additional physiological research is necessary to determine if and what detrimental effects ocean acidification will bear on *M. magister* and other crustacean populations.

1.6.3 Behavior

Looking at behavior such as risk assessment and response to visual, olfactory, and auditory cues is one way of assessing physiological impacts on crustaceans. Through testing hypotheses about mechanisms for behavioral changes under pH stress, researchers can elucidate how changes in pH can alter physiological responses, which in turn may cause shifts in behavior.

One way to study crustacean behavior is through observing antennular flicking rates, which correspond to olfactory perception. Antennular flicking rates in adult hermit crabs are reduced at pH 6.8, which could be indicative of decreased metabolic rate or a disruption to information gathering and processing (De la Haye et al. 2011). Hermit crabs exposed to this high-CO₂ treatment were also less likely to switch into suitable shells, a behavior that relies on olfactory and visual cues, and to detect prey olfactory cues.

These adverse impacts of high-CO₂ seawater indicate a disruption to olfactory function. Further research indicates that this behavior may be mediated by an inability to detect odor due to a direct effect of low pH on chemo-receptive function (De la Haye et al. 2011, De la Haye et al. 2012), which could include neural disruption (Ferrari et al.

2012). Since the olfactory sensory organs are congruent across the subphylum Crustacea, similar responses and mechanisms can be tested in other crustacean species (Hallberg et al. 1992). No studies as of December 2013 have addressed the response of larval crustaceans to sensory cues.

Crustacean behavior, physiology, and calcification may be affected by ocean acidification. An examination of the possible behavioral responses to low pH will elucidate the possible impacts on these organisms. These include avoidance, metabolic stress, and info-disruption, which is a general term for impairment of perception and cognition by anthropogenic interference (Briffa et al. 2012, Lurling and Scheffer 2007). I will demonstrate how these three different responses to low pH could manifest in crustacean behavior.

One pathway by which organisms may alter their behavior due to increased CO₂ concentrations in seawater is avoidance. In the case of a localized low-pH marine habitat, which may occur naturally or anthropogenically, animals have the option of responding to this low pH environment by avoiding the area through locomotion (Pörtner and Peck 2010). An example of a possible localized anthropogenic increase in CO₂ concentration is the proposed underwater CO₂ storage sites that have the potential to leak and acidify a pocket of seawater (Hawkins 2004). Intertidal organisms may detect high-CO₂ conditions and leave the water, which can be a costly energetic trade-off (Bibby et al. 2007, Briffa et al. 2012, Murray et al. 2013; Amaral et al. 2013). On a long-term scale, as habitats change, organisms adapted to those habitats may leave that region as conditions become less favorable (Pörtner and Peck 2010), resulting in a restructuring of ecosystems.

Organisms exposed to high-CO₂ conditions have to cope with physiological pressure, which can lead to changes in behavior. As described above, in high-CO₂ conditions, marine organisms exert more energy maintaining acid-base homeostasis through compensatory mechanisms involving the carbonate buffer system and ionoregulation, thus increasing the metabolic load (*Page 10*) (Whiteley 2011; Briffa et al. 2012). When energy is consumed by these processes, it is diverted away from vital behavioral functions like reproduction, feeding, and aggressive behaviors that require a high circulation rate (Briffa et al. 2012). This decrease in metabolic scope could have population-level impacts.

A third proposed mechanism for behavioral impact of ocean acidification is through the disruption of perception and cognition. Info-disruption can be associated with the processing of visual (Ferrari et al. 2010), auditory (Simpson et al. 2011), and olfactory (Dixson et al. 2010) cues. Olfactory cues are part of the process known as chemoreception, in which odor molecules in seawater bind with receptor sites on the olfactory sensory organs of marine organisms (Tierney and Atema 1988). Disruption to perception due to ocean acidification may be explained by a change in the charge distribution at odor molecule receptor sites, a change in the ionic state of odor molecules, or physical damage to sensory organs (Briffa et al. 2012; De la Haye et al. 2012). Disruption to cognition due to low pH could occur through damage to neural mechanisms. The present study focuses on the potential impacts of low pH on the neural processes of zoeae.

1.7 Zoal phototaxis

In the field and in the laboratory, swimming behavior of zoeae is influenced in part by light. Larvae can respond to light through phototaxis and through photokinesis. Phototaxis is the active change of the direction of an organism along the axis of a beam of light (Fraenkel and Gunn 1940). Movement toward the light is known as positive phototaxis, while movement away from the light is known as negative phototaxis (Diehn et al. 1977). Photokinesis is a change in velocity of an organism when it is stimulated by a change in light intensity regardless of direction (Diehn et al. 1977; Fraenkel and Gunn 1940; Crisp and Ghobashy 1971). The present study uses photokinesis to measure phototaxis.

M. magister larvae, along with the majority of zooplankton, typically migrate vertically towards the surface of the water column at night and farther down in the water column during the day in order to avoid predation (Iwasa 1982; Reilly 1983). The depth and timing of vertical migration varies with larval stage and environmental conditions (Jacoby 1982; Sulkin 1984). Zoeae are negatively buoyant, meaning they sink when they are not swimming (Foxon 1934; Spaargaren 1979). In order to regulate their vertical position in the water column, they orient themselves using light, pressure, and gravity (Sulkin 1984). The lower threshold for phototaxis, meaning the lowest level at which a zoea senses light, acts as a barrier to upward migration during the day (Forward et al. 1984). *M. magister* zoeae phototax negatively at sunrise, and maintain depth during daylight hours (Forward 1986; Hobbs and Botsford 1992). Although phototaxis likely contributes to diel vertical migration, the primary influence on vertical migration may not be phototaxis, but rather geotaxis, which is directional movement along the axis of

gravitational pull (Sulkin 1984). In order to avoid the confounding factors of gravity and pressure in the laboratory, researchers use horizontal aquaria with a horizontal beam of light to isolate phototaxis (e. g. Adams and Paul 1999). Horizontal manipulative photostimulation, which is the method employed in the present study, may result in positive phototaxis, but not negative phototaxis (Forward et al. 1984). Observing phototaxis in the laboratory can be useful for studying photophysiology, which is the study of how light interacts with physiology, but is not analogous to vertical migration in the field (Forward et al. 1984). In conditions simulating natural angular light distribution, zoeae exhibit negative phototaxis but not positive phototaxis (Forward 1986). Positive phototaxis in zoeae is a product of unnatural laboratory lighting conditions (Forward 1986). I will describe the utility of chronic bioassay of zoeal phototaxis in the laboratory setting by describing zoeal phototransduction.

1.8 Phototransduction

When a zoea receives a visual cue, light hits the cornea and lens of the compound eye and travels through nerve cells to photoreceptors known as rhabdomes, where the image forms. A series of neural fibers links the rhabdomes to the brain (Litzinger and Del Rio-Tsonis 2002). Within the brain, synapses serve as information bridges between neurons. Each neuron has one axon, which outputs information, and each axon has several axon terminals, which send chemical signals called neurotransmitters through the synapse from one neuron to the next (Stufflebeam 2008). The process of phototransduction, or the conversion of a visual stimulus to a neural signal, differs between invertebrates and vertebrates. Glutamate (Stuart 1999; Kolb et al. 2013), GABA-

A (Førsgren et al. 2013) and dopamine (Burgess and Fero 2012) are implicated as neurotransmitters in phototaxis in vertebrate brains, while histamine (Stuart 1999) and possibly serotonin (Kain et al. 2012; Perrot-Minot et al. 2013) fill this role in arthropod invertebrate brains. However, little is known about neurotransmitters involved in the vision of invertebrates (Warrant and Nilsson 2006). Histamine has been identified as the neurotransmitter associated with photoreception in many arthropod taxa (Stuart 1999). Histamine is the neurotransmitter associated with photo reception in at least four arthropod species: juvenile barnacle *Balanus amphitrite* (Stuart et al. 2007; Stuart et al. 2002), planktonic crustaceans *Daphnia magna* and *Daphnia pulex* (McCoole et al. 2011), and the brown blowfly *Calliphora stygia* (Hardie 1989). However, some arthropods do not employ histamine in photoreception, such as the copepod *Calanus finmarchus* (Hartline and Christie 2010). Experimental evidence supports the function of serotonin working in conjunction with histamine as the neurotransmitter involved in phototaxis in *Gammarus pulex*, a freshwater amphipod (Perrot-Minot et al. 2013). Thus, the neurotransmitter associated with photoreception and phototaxis in larval crab has not been ascertained, but histamine is a likely candidate.

High seawater CO₂ levels predicted to occur by the year 2100 clearly detrimentally impact the behavioral responses of larval fish to olfactory cues as well as lateralization, which is a direct measurement of brain function, due to the reversal of the function of neurotransmitter GABA-A (Nilsson et al. 2012, Domenici et al. 2012). GABA-A plays an important role in both vertebrate and invertebrate behavior (Tsang et al. 2007) and could be a mechanism for general changes in zoeal behavior. The only

invertebrate to my knowledge analyzed for the role of GABA-A in phototaxis is *Drosophila*, the common fruit fly. It appears that *Drosophila* does not rely on GABA-A for phototaxis (Leal et al. 2004), and fellow arthropod *M. magister* could have homologous neurology. The proposed mechanism for impairment of vertebrate phototaxis is the through the GABA-A receptors; however, this may not be applicable in invertebrate photophysiology. Hence, further research on phototransduction in larval crabs would benefit an understanding of the mechanism by which phototaxis in *M. magister* could be affected by CO₂-induced acidification. More information about the type of receptor associated with photoreception in *M. magister* would provide researchers with the ability to determine the mechanism of any effect of high-CO₂ seawater on larval phototaxis.

Although no studies to my knowledge have been completed to date examining the impact of ocean acidification on phototaxis in any invertebrate, studies on neural disruption due to low pH indicate the possibility that ocean acidification could lead to an altered behavioral response to light (Ferrari et al. 2012; Førsgren et al. 2013; Nilsson et al. 2012). A bioassay on patterns of locomotion and phototaxis is a common and useful manner in which to determine the impact of a physiologically challenging environment on zooplankton (Whitman and Miller 1982). The bioassay employed in this study will be chronic, or sublethal (Sprague 1969), as it is intended to investigate behavioral effects of heightened levels of seawater pCO₂ on larval crab phototaxis.

2. INTRODUCTION

2.1 Identification of the problem

Since the industrial revolution, anthropogenic carbon dioxide (CO₂) emissions have elevated the atmospheric CO₂ concentration from a global average of 287 ppm (Etheridge et al. 1996, Meehl et al. 2007) to an average in October 2013 of 394 ppm (NOAA 2013, unpublished data). The ocean and the atmosphere exchange gases and equilibrate, and as atmospheric CO₂ concentration increases, oceanic CO₂ concentration increases at the same rate (Doney et al. 2009b). Accordingly, analyses of global measurements of inorganic carbon show that the world's oceans absorb approximately half of anthropogenic carbon emissions (Sabine et al 2004). When CO₂ enters seawater, it reacts chemically with H₂O to create carbonic acid. Carbonic acid dissociates, increasing the concentration of hydrogen ions in seawater (Doney et al. 2009b). This lowered pH, which is defined as the negative logarithm of the hydrogen ion activity (Covington et al. 1985), can impact the physiology of marine organisms due to dependence on the chemistry of the seawater in which they are immersed. This process of anthropogenic reduction of global seawater pH is known as ocean acidification (OA).

Changes in pH may have broad effects on the physiology of marine organisms (Fabry et al. 2008, Doney et al. 2009b). Numerous physiological indices in marine organisms show a relationship with tolerance of pH changes, including metabolic rate (Lannig et al. 2010), calcification (Smith and Buddemeier 1992, Kleypas et al. 1999), survival (Chambers et al. 2013), reproduction (Miller et al. 2013), fertilization rate (Barros et al. 2013), growth (Byrne et al. 2013), acid-base homeostasis (Stumpp et al.

2012), and behavior (Dixson et al. 2010). As evidence from manipulative ocean acidification simulation experiments is mounting, it is becoming clear that the effects of OA will show variation within genera (Ferrari et al. 2011) and even among individual organisms (Kelly et al. 2013). Research initiatives are predicting the ecological and subsequent socioeconomic effects that ocean acidification will incur on the planet (Harrould-Kolieb and Herr 2012). In order to make these predictions, researchers are quantifying species-specific ocean acidification impacts and applying them to whole-ecosystem models. For instance, the National Oceanic and Atmospheric Administration (NOAA) is implementing the Atlantis Ecosystem Model (Kaplan et al. 2010) in order to better understand the potential consequences of OA.

2.2 Behavioral impacts of OA

Acidified conditions can induce three pathways of behavioral response: info-disruption, avoidance, and metabolic stress (Briffa et al. 2012). Changes in metabolism due to uptake of hydrogen ions into the interstitial fluid may lead to impaired behavioral response in marine organisms (Briffa and Sneddon 2007). Acid-base homeostasis, which maintains pH conditions in the interstitial fluid of invertebrates, demands more metabolic energy as pH levels become stressful (Whiteley 2011). As metabolic load increases, the energy available for other activities such as feeding and locomotion decreases, hence altering behavior (Briffa et al. 2012). A second pathway for changes in behavior due to shifts in CO₂ concentrations is avoidance, since animals exposed to localized pH changes may move away from these areas (Bibby et al. 2007, Pörtner and Peck 2010).

Info-disruption, the anthropogenic impairment of sensory perception and cognition (Lurling and Scheffer 2007), can be associated with the processing of visual (Ferrari et al. 2012), auditory (Simpson et al. 2011), and olfactory (Dixson et al. 2010) cues. Perception may be disrupted through physical damage to sensory organs (Spicer et al. 2006, Munday et al. 2009), and cognition may be disrupted through a variety of neural mechanisms, reviewed below (Briffa et al. 2012).

2.3 Vulnerability of the study organism to OA

The Dungeness crab *Metacarcinus magister* (Decapoda: Brachyura) is a benthic invertebrate that inhabits the rocky sandy-mud intertidal zone (Karpov 1983) along the west coast of North America from the Santa Barbara Channel in California to the Aleutian Islands in Alaska, including Puget Sound (Pauley et al. 1989). *M. magister* egg clutches, which are held in the pleopods of the female crab, hatch between January and April in Washington State (Clever 1949). The larvae, known as zoeae, join the zooplankton, where they progress through five zoeal instars with a molt between each stage until they become megalopae and finally settle as juvenile crabs (Poole 1966). The Dungeness crab zoea is an ideal study organism for a chronic bioassay on phototaxis since it is vulnerable to ocean acidification as a larval zooplankter, as a crustacean, as an organism that demonstrates phototaxis, and as an ecologically and economically important species. I will describe the vulnerability of the study organism to ocean acidification by examining crustaceans, marine larvae, phototaxis, habitat, and ecosystem.

2.3.1 Crustaceans

The impact of ocean acidification on brachyuran crabs is poorly understood. Acidified water breaks down calcium carbonate molecules like calcite and aragonite, making these minerals less bioavailable for construction of body parts such as shells (Orr et al. 2005). The carapace of brachyuran crabs is composed more heavily of calcite than aragonite, which is a softer mineral than calcite (Boßelmann et al. 2007). Blue crab *Callinectes sapidus* shows increased calcification with increased acidity (Ries et al. 2009). Red king crab *Paralithodes camtschaticus* larvae exhibited decreased yolk size in acidic conditions, longer zoeal length at hatch, increased eye size, increased calcification, and decreased survival with greater acidity (Long et al. 2013). There may be shifts in calcium uptake and thermal tolerance of spider crab *Hyas araneus* with combined effects of temperature and low pH (Walther et al. 2009; Walther et al. 2011). The vulnerability of crustaceans to ocean acidification may lie not in calcification, but in other physiological and behavioral impacts (Whiteley 2011). For instance, hermit crab *Pagurus bernhardus* shows reduced antennular flicking rates in high-CO₂ water, which could indicate impacts on metabolism or info-disruption (De la Haye et al. 2011).

2.3.2 Marine larvae

Biological ocean acidification impacts can vary with life stage (Fabry et al. 2008). Larval marine organisms are particularly vulnerable to changes in seawater chemistry, including pH (Kurihara 2008, Findlay et al. 2008, Chan et al. 2011). Reef fish larvae swim towards olfactory and visual predator cues instead of away from them at high levels of CO₂ (Dixson et al. 2010; Ferrari et al. 2012). The feeding behavior and food selection of larvae of the Chilean abalone are detrimentally affected by decreases in pH (Vargas et

al. 2013). These studies indicate that the larval stage of development is vulnerable to behavioral impacts of ocean acidification in marine organisms.

2.3.3 *Phototaxis*

Larval zooplankton subsist at a low trophic level in the food web and are at high risk of predation (Nelson 1925). Many species have evolved behavioral adaptations to avoid this risk (Singarajah 1969, Forward 1977). Diel vertical migration is a behavioral mechanism by which marine zooplankton avoid predation (Iwasa 1982). *M. magister* zoeae migrate up in the water column as the sun sets and sink as the sun rises (Forward 1986). Predators can see the shadow from sunlight cast by these animals, so zoeae exclude predation by other animals that occupy the surface waters by increasing depth in the water column (Iwasa 1982). Zoeae sink in the water column in response to shadow cues, which is thought to be a predator avoidance behavior (Forward 1977; Morgan 1987). Zooplankton respond differently to angular diffused light, which occurs in the field, than they do to directional light in a laboratory setting (Veirhagen 1958; Forward 1986).

Phototaxis is the directional movement of an organism along the axis of a beam of light (Fraenkel and Gunn 1940). Movement toward the light is known as positive phototaxis, while movement away from the light is known as negative phototaxis (Diehn et al. 1977). The biological function of the phototactic behavior of zoeae demonstrated in a laboratory setting is unexplained, since zoeae generally demonstrate negative phototaxis in the field and positive phototaxis in the laboratory (Forward 1986). It is common to assess the sublethal impacts of a substance on larval crustacean behavior by

studying phototaxis (Bartolomé and Sánchez-Fortún 2005; Kolkmeier and Brooks 2013; Wu et al. 1997), and the behavior of *M. magister* zoeae in a horizontal light chamber is well understood (Jacoby 1982), thus providing a reliable index for behavioral change. Visual cues are important in mediating the behavior of zoeae, in activities such as predator avoidance, and any alteration in the ways in which zoeae process these cues has the potential to bear fitness and survival effects. An understanding of zoeal phototaxis under acidic conditions could gauge the impact of high-CO₂ seawater on behavior of *M. magister* zoeae and provide information about overall brain function and metabolic rate through assessments of phototaxis and activity level. Impairment of behavior could lead to population-level impacts in *M. magister* populations, which could have ecological and socioeconomic repercussions.

2.3.4 Habitat

The chemical conditions of the habitat of the study organism, coastal northeast Pacific and tidal estuaries such as Puget Sound, may have lower average pH than global average oceanic pH (Feely et al. 2008). This is due to seasonal coastal upwelling, natural biotic activity, and anthropogenic atmospheric and terrestrial emissions, as outlined below (Doney et al. 2009a; MacFadyen et al. 2008; Feely et al. 2010). Upwelling of cold, nutrient-rich, high-CO₂ ocean water occurs as the global oceanic conveyor belt, which downwells in the North Atlantic, accumulates CO₂ from organic matter and upwells in the Northeast Pacific (Broecker and Peng 1992). Based on models, the average CCS pH decreased from 8.12 to 8.04 between the years 1750 and 2005, and by the year 2050 under current emission rates, could decrease to a pH of 7.92 (Gruber et al. 2012). Natural

biotic activity in the form of algal blooms is common in Puget Sound, and when the phytoplankton die off, their biomass is consumed by bacteria, which consume oxygen and give off CO₂, contributing to the acidity of the seawater (Feely et al. 2010). Since Puget Sound is an estuary, anthropogenic nutrients such as nitrogen flow into the Sound and often accumulate due to sluggish flow (Feely et al. 2010). Nutrient accumulation can lead to increases in biotic activity and thereby decrease pH, as described above (Feely et al. 2010; Khangaonkar et al. 2012). Anthropogenic terrestrial combustion of fossil fuels near the coast can emit nitric and sulfuric acid, which are absorbed into the seawater and further acidify the Puget Sound (Feely et al. 2010).

The pH experienced by zoeae may vary temporally during daily vertical migrations and larval development (Long et al. 2013). The pH range of the seawater surrounding the substrates in which *M. magister* broods is currently unknown, but it is likely that this habitat is low in pH (Mathis et al. 2011). The vulnerability of the study organism's habitat to rapid changes in pH creates a need to understand the potential impacts of ocean acidification on marine ecosystems in this region.

2.3.5 Ecosystem

The Dungeness crab is an ecologically important species as well as a socioeconomic staple in western North America. *M. magister* zoeae are important prey sources of Chinook salmon, pink salmon, and coho salmon, as well as rockfish and herring, hence this species' valuable role in the pelagic ecosystem (Orcutt et al. 1976; Reilly 1983; Prince and Gotshall 1976). A change in the behavior of *M. magister* zoeae

could shift population dynamics and have ecosystem-level and socioeconomic implications.

2.4 Proposed mechanism of behavioral impairment

The neurotransmitter GABA-A, the main inhibitory transmitter in the vertebrate brain, is implicated in CO₂-induced aberrations in larval fish behavior such as excessive risk-taking (Munday et al. 2010), boldness (Munday et al. 2010), hypersensitivity to light (Førsgren et al. 2013), and inability to discriminate between ecologically sensitive olfactory (Dixon et al. 2010), visual (Ferrari et al. 2012), and auditory (Simpson et al. 2011) cues. When seawater pH decreases, marine fish (Brauner and Baker 2009) and crustaceans (Truchot 1975; Whiteley 2011) maintain acid-base homeostasis by increasing bicarbonate uptake and releasing chloride into the seawater. An opening of the GABA-gated chloride channel causes an influx of chloride ions, leading to a hyperpolarization and inhibition of the neuron. An outflux of chloride, which may be caused by a decrease in seawater pH, changes the ion gradient across the membrane of the receptor cell and leads to depolarization and excitation, sending a neural signal to the brain (Nilsson et al. 2012). Førsgren et al. (2012) propose neural overexcitation as the mechanism for increased speed to light in goby larvae under high-CO₂ treatment. The neurotransmitter responsible for phototaxis the brain of many amphipods, and is histamine (Stuart 1999). Thus, overexcitation of the histamine-gated chloride channel in the zoeal brain could lead to increased phototactic response in zoeae reared in high-CO₂ seawater. This hypersensitivity to light could be maladaptive and lead to expenditure of energy on weak

or absent light cues (Bradbury and Vehrencamp 2011), with fitness and survival consequences.

In order to determine the potential impacts of ocean acidification on larval Dungeness crab phototaxis, I conducted a manipulative experiment simulating potential future oceanic conditions. This chronic behavioral bioassay examined the response of *M. magister* zoeae to directional light in a laboratory setting when reared in three levels of CO₂-manipulated seawater. The present study represents the second study addressing the impact of high-CO₂ seawater on the behavior of a larval crustacean (*see* Christmas 2013), the second study to address this impact on larval phototaxis (*see* Førsgren et al. 2013), and the first to assess this impact on the phototactic behavior of a larval crustacean.

3. METHODS

3.1 Experimental system and carbon chemistry measurements

Zoeae were reared in an aquarium system at the Northwest Fisheries Science Center ocean acidification laboratory (Seattle, WA), managed by the National Oceanic and Atmospheric Administration. Seawater was collected from Elliott Bay in January of 2013 and housed in a reservoir at the laboratory. The reservoir is filtered to 1 µm to remove organic particles, exposed to a UV filter which kills microbes and viruses, and degassed using Liqui-Cel membrane contactors (Membrana, Weppertal, Germany), so that the seawater re-enters the system free of CO₂. A protein skimmer removes metabolic waste compounds such as nitrates and nitrites. To prevent tank isolation, each treatment

system continuously exchanges water with the system reservoir, with 100% turnover of each treatment every day, essentially resetting seawater chemistry.

All materials used in the aquarium system were pre-soaked in seawater to remove trace chemicals or impurities. Seawater flows from the reservoir through PVC pipes to a holding tank, and then into a header tank containing the equipment necessary to control seawater chemistry (*see below*). From each header tank, seawater flows into a series of large boxes which are fitted with flow meters and water outputs to which rearing jars are attached, floating in each box. The boxes act as water baths to keep the jars at constant temperature. Seawater flows through the output of each rearing jar, into each box, and back to the system reservoir. A 1- μm mesh bag filter was placed in front of the seawater outflow of each rearing jar to prevent larval escape.

Seawater chemistry, specifically pH, is controlled at the header tanks by a program built using LabView Software (National Instruments, Austin, Texas) and maintained by bubbling one of five gas solutions (air, CO₂-free air, pure nitrogen, pure carbon dioxide, or pure oxygen) through air stones. CO₂-free air is generated with CO₂ adsorbers, which capture the gas with a semi-permeable membrane (Twin Towers Engineering, Broomfield, Colorado). The pH is controlled to a precision of 0.05 pH units.

Seawater salinity is monitored within header tanks with a Honeywell conductivity probe and verified with discrete samples. Temperature, pH and dissolved oxygen in each treatment are continuously measured with temperature probes, a pH probe (Durafet), and a dissolved oxygen transmitter, respectively (Honeywell Process Solutions). The Durafet pH probe in each experimental treatment continuously recorded pH and was calibrated

with a pH-certified Tris buffer (Dickson Laboratory, Scripps Institution of Oceanography) that is able to measure pH to a precision of 0.01 pH units. We verified pH, alkalinity, and dissolved organic carbon conditions once daily with a spectrophotometer (Ocean Optics USB 2000+ Fiber Optic Spectrometer) and m-cresol purple dye (Sigma Aldrich) in all treatments tanks and 250 mL rearing jars. Verification of pH conditions in 4 L rearing jars occurred only once and values were found to be accurate.

For this study, tanks were held at three pCO₂ levels: control (pCO₂ = 400 µatm; pH = 8.2); mid-level (pCO₂ = 1600 µatm; pH = 7.6); and high-level (pCO₂ = 3200 µatm; pH = 7.17). The temperature was held at 12° C in all treatments. Oxygen was maintained at a 90% saturation level. Nutrients, bacteria, and phytoplankton were not added to the system.

3.2 Specimen collection and larval rearing

Saratoga Passage, the site of specimen collection, is located in the Puget Sound, a fjord in northwest Washington State, USA. The passage is situated between mainland Washington and Camano Island. The NOAA dive team collected a gravid *M. magister* individual on February 13, 2013 and held her at the NOAA Mukilteo Research Station (Edmonds, WA) in a system consisting of a series of 1-m² lidless aquaria with flow-through seawater held at ambient conditions from Saratoga Passage. The crab was fed intertidal bivalves from Saratoga Passage and light conditions were ambient.

Using forceps, egg strands were extracted from the female on March 28, 2013, at the NWFSC, after 43 days in captivity, using forceps (Wickham 1979). Egg strands were extracted from haphazardly selected locations within the egg clutch. Eggs were placed

haphazardly in flow-through jars (250 mL CO₂-impermeable-PET plastic) on PVC manifolds in the aquarium system and incubated at the three levels of pCO₂ described above. Zoeae selected for the behavioral experiment hatched on April 4 and April 5, after a period of 8 to 9 days of incubation. No handling of eggs occurred during incubation aside from removal of jar lids to determine hatch state. To control for photoperiod exposure, tanks were covered in black plastic at the end of each work day and uncovered at the beginning of each work day.

Upon hatching, zoeae were moved to larger plastic flow-through jars (4 L) with a flow rate of approximately 6 L/hr. A total of 150 newly hatched zoeae were placed in each jar. There were 2 jars in each CO₂ treatment; one for each hatch day. Zoeae that hatched on April 4 were held separately from zoeae that hatched on April 5. Every third day, water in jars was changed and zoeae were fed *Artemia salina* nauplii (1 nauplius / mL). Behavioral tests were conducted 21 days after hatch date. Animals that hatched on April 4 were tested on April 25, and animals that hatched on April 5 were tested on April 26. Molt data during rearing was not recorded, so zoeal instar at time of behavioral test cannot be ascertained. However, it is likely that the zoeae were in the second instar since in *M. magister*, the first zoeal molt occurs at approximately day 18, and the second molt occurs at approximately day 29 (Poole 1966).

Photograph of behavioral test setup.

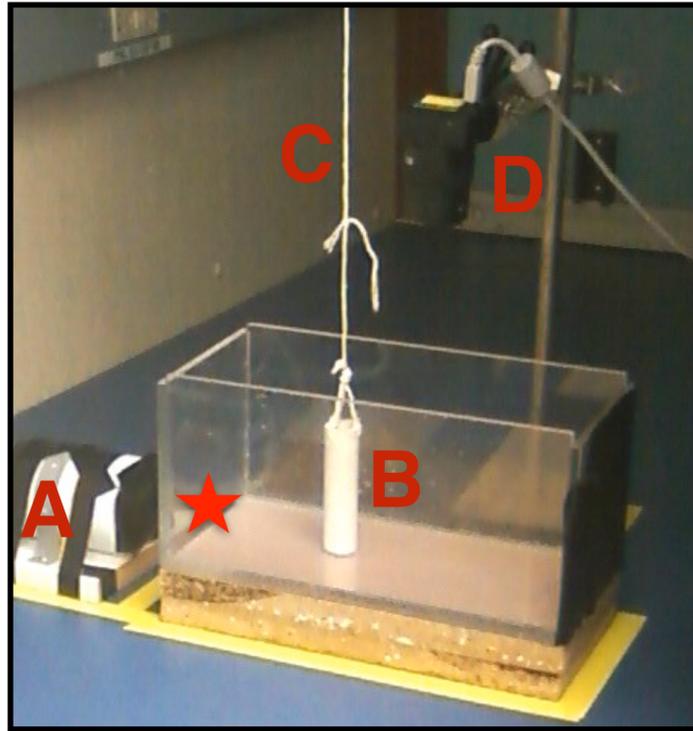


Figure 1. Lateral view of experimental aquarium in behavioral test setup. (A) LED bulb in PVC housing. (B) PVC pipe used to ensure uniform release of zoeae. (C) String used to lift PVC and release zoeae. (D) Digital video camera for image recording. Star indicates position of light.

3.3 Behavioral test

To understand how elevated CO₂ conditions impact zoeal phototaxis, larvae were exposed to light and control treatments. A total of sixty larvae were tested. Twenty larvae from each CO₂ treatment were tested, ten of which were exposed to a light treatment and ten of which were exposed to a control treatment. Each zoea was tested exactly once. In order to ensure that the researcher remained unbiased during behavioral tests and analyses, colleagues haphazardly rearranged the lids of the 450 mL jars containing each individual zoea before each time trial. In addition, a colleague recoded each video file at

random to ensure the researcher was blind to CO₂ treatment. Larval behavior was recorded for each 3 minute time trial by using a video camera (see below). Zoeae were transferred from 4 L cultures to 450 mL plastic jars by selecting zoeae that swam to the top of the 4 L jar upon removal of the bag filter. This selection criterion increased the likelihood that the swimming behavior of zoeae was uniform. A single zoea was placed in each of six jars. Zoeae were tested individually but pulled from the flow-through system in batches of six. Each batch constituted a time trial. Jars were held in a water bath between removal from flow-through system and behavioral test at 12° C for no more than 90 minutes during each time trial. The time elapsed between removal and testing ranged from 6 minutes to 77 minutes. To determine any effect on behavior based on time away from flow-through system, the time at which each individual zoea was removed from the flow-through system was recorded and subtracted from the time at which it was tested in order to calculate time elapsed.

The study took place in a darkened room. To minimize disturbance, the researcher remotely filmed the trials at a distance of 1.5 m. Video data was collected using a Chameleon digital camera (Point Grey Research Inc.) and recorded using FlyCapture2 (Point Grey Research Inc.). One LED lightbulb, housed within PVC fittings, was placed 2.3 cm from the edge of the Plexiglass box. Neutral density photo filters were layered to manipulate brightness. Luminosity was measured at 3.7 lumens using HOBOWare Data Logger software with a PAR sensor placed before the photo filters.

The behavioral test consisted of the exposure of each zoea either to the LED light treatment or to a dark control treatment. To ensure that each zoea was initially placed at

an equal distance from the light source, each zoea was placed in a PVC pipe (9 cm long, 1.5 cm diameter) within a Plexiglass box (*Figure 1*) containing 450 mL of seawater (26.5 cm long, 11.5 cm wide, 1.4 cm deep) from one of the three CO₂ treatments. The LED light was activated remotely, and the PVC pipe was lifted remotely to release zoea 11.2 cm from the light, slightly offset from the center of the box. Larvae were filmed for 3 minutes after an acclimation period of 30 seconds. The film captured the movement of each zoea during each test. Each zoea was tested exactly once. Larval preference of side of aquarium (right side vs. left side) was tested during preliminary trials and found to have no effect on whether the zoeae swam to the light.

3.4 Video analysis

A total of 59 videos were analyzed. Twenty individuals from each CO₂ treatment were tested with ten larvae exposed to light treatment and ten larvae exposed to a dark control treatment. One video (3200 ppm pCO₂ in the light treatment) was lost.

Video analysis was conducted using ImageJ (NIH Image) software. Video files were decimated by 30 frames to condense files using the program VirtualDub (VirtualDub.org) yielding a total of 90 frames for each 3-minute video, which were then analyzed. As a result of this treatment, the temporal difference between consecutive frames was 1.9987 seconds.

The location of the zoea in each frame was recorded in pixel coordinates using ROI Manager (ImageJ, NIH Image). Due to glare from the infrared lighting system, there were blind spots in each video. When zoeae swam into the blind spots, pixel coordinates were not recorded.

Using ImageJ and Adobe Photoshop (Adobe Systems Incorporated), a rectangle was superimposed on top of each frame 1.39 cm left of the wall of the aquarium adjacent to the LED bulb (*Figure 2*). The absence or presence of the zoeae in the rectangle in each frame was recorded to denote proximity to light.

Demarcation of rectangular area in aquarium closest to light.

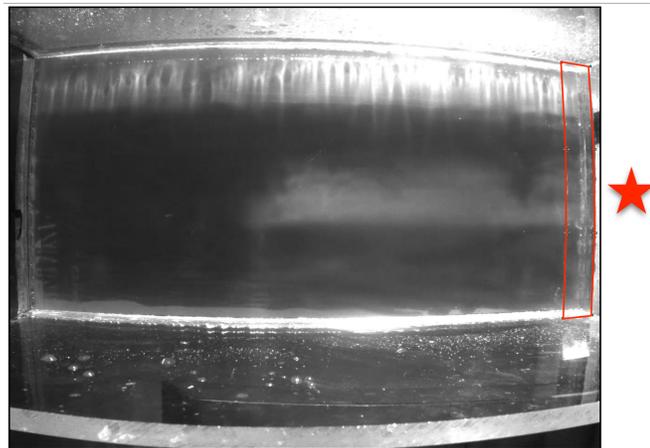


Figure 2. Overhead view of experimental aquarium. Fisheye lens in video camera caused image bowing. Rectangle outlined in red was used to record zoeal position, providing index of approach of light. Star indicates position of light.

3.5 Statistical analysis

Pixel coordinate data were processed to determine the overall speed and speed to light. This study will use the term “light” in reference to “the space closest to the light,” as a shorthand. Grid cell data were processed for approach of light, speed to light, time to light, proportion of time at the light, and proportion of time away from the light once the light was reached.

Overall swimming speed

Overall swimming speed (cm/s) is defined as the centimeters per second traveled by each zoea over the course of the 3-minute video.

Control group: A one-way ANOVA was conducted across all three CO₂ treatments within the control group to isolate the overall activity level outside the context of photokinesis.

Light treatment group: A one-way ANOVA was conducted exclusively among zoeae exposed to the light treatment across CO₂ treatments.

Approach of light

Approach of light is defined as a yes or no (0, 1) response to the question of whether the zoea reached the area closest to the light.

Control vs. light treatment: A one-way ANOVA analyzing entrance of area closest to the light was run across all zoeae to compare the control treatment against the light treatment.

CO₂ treatments: A one-way ANOVA was run across all zoeae exposed to the light treatment to compare CO₂ treatments.

Speed to light

Speed to light is defined as the centimeters per second traveled from the pixel coordinates marked on the first frame of each video to those marked on the first frame in which the zoea entered the area closest to the light. A one-way ANOVA was run to determine variance across CO₂ treatments among zoeae that reached the area closest to the light.

Time to light

Time to light is defined as the time (in seconds) that passed between the first frame of each video and the first frame in which the zoea entered the area closest to the light. A

one-way ANOVA was run among all zoeae that were exposed to light treatment and reached the light.

Proportion of time at light

Proportion of time at light is defined as the ratio of time (in seconds) each zoea spent at the light to the time each zoea spent elsewhere. A one-way ANOVA was run across CO₂ treatments among all zoeae that were exposed to light treatment and reached the light.

Proportion of time spent away from light once light was reached

Proportion of time spent away from light once light was reached is defined as the ratio of time (in seconds) each zoea spent away from the light after the light was reached to the time each zoea spent at the light. This metric shows how the tendency to stay at the light varies across CO₂ treatments. A one-way ANOVA was run across CO₂ treatments among all zoeae that were exposed to light treatment and reached the light.

4. RESULTS

4.1 Overall swimming speed

4.1.1 Control group

There was no significant difference in overall swimming speed among control CO₂ treatments ($n = 30$, $F(30, 3) = 0.4777$, $p = 0.6253$). However, a linear pattern is exhibited in mean overall swimming speed across CO₂ treatments, in which mean overall swimming speed decreases as CO₂ levels increase (*Figure 3*). The mean overall swimming speed for the 400, 1600, and 3200 ppm pCO₂ treatments in the control group was 0.23 ± 0.14 cm/s, 0.20 ± 0.09 cm/s, and 0.179 ± 0.12 cm/s, respectively.

Effect of pCO₂ level on overall swimming speed in the control group.

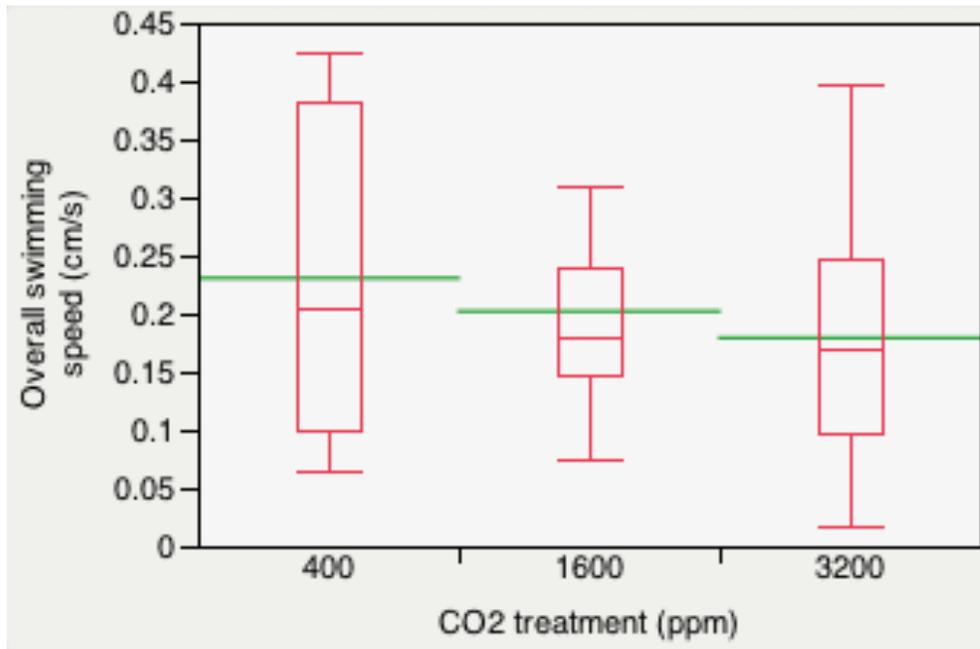


Figure 3. A one-way ANOVA shows no significant impact of CO₂ treatment on overall swimming speed in the control group ($n = 30$, $F(30, 3) = 0.4777$, $p = 0.6253$). A very weak, non-significant trend suggests a decrease in overall mean swimming speed with increased CO₂ level. Green lines indicate mean. Red lines within box plots indicate median.

4.1.2 Light treatment group

There was no significant difference in overall swimming speed among CO₂ treatments for zoeae exposed to light ($n = 29$). However, a linear pattern in mean overall swimming speed is exhibited across CO₂ treatments, in which mean overall swimming speed decreases as CO₂ level rises, similar to the control group (*Figure 3*). The mean overall swimming speed for the 400, 1600, and 3200 ppm pCO₂ treatments was 0.19 ± 0.08 cm/s, 0.18 ± 0.12 cm/s, and 0.17 ± 0.11 cm/s, respectively. This corresponds to a 5%

and 14% greater mean overall swimming speed for zoeae in the 400 ppm pCO₂ treatment relative to animals from the 1600 and 3200 ppm pCO₂ treatments, respectively.

4.2 Approach of light

4.2.1 Control vs. light treatment

Zoeae exposed to a control treatment (n = 30) were significantly less likely to swim to the light than those exposed to the light treatment (n = 29) (χ^2 (1, n = 59) = 26.8, $p < 0.0001$) (Figure 4). In 2 out of 30 trials, zoeae exposed to a control treatment swam to the light. In 24 out of 29 trials, zoeae exposed to the light treatment swam to the light. Accordingly, zoeae exposed to a control treatment swam to the light $7 \pm 25\%$ of the time and zoeae exposed to the light treatment swam to the light $83 \pm 38\%$ of the time.

Effect of light treatment on proportion of zoeae that reached light.

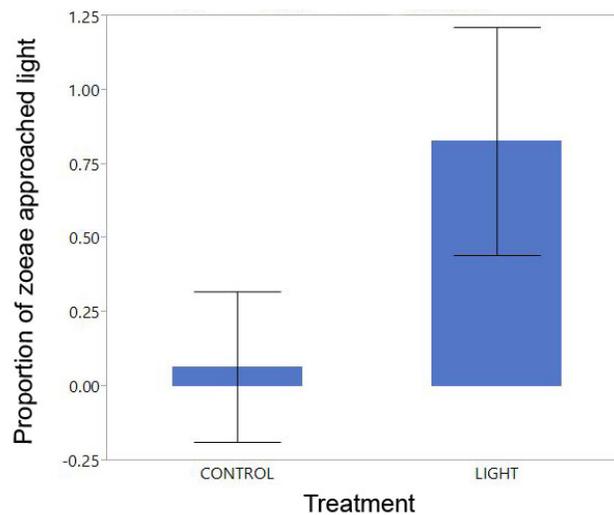


Figure 4. A one-way ANOVA shows a significant effect of light treatment on proportion of zoeae that approached the light (χ^2 (1, n = 59) = 26.8, $p < 0.0001$). Each error bar was constructed using 1 standard deviation from the mean.

4.2.2 CO₂ treatment

CO₂ treatment was not a significant effect on whether the zoea exposed to the light treatment swam to the light ($\chi^2 (2, N = 29) = 0.5518, p = 0.5601$) (Figure 5). Zoeae reared in 400 ppm pCO₂ seawater swam to the light 8 out of 10 times, whereas those in the control treatment swam to the light 1 out of 10 times. Zoeae reared in 1600 ppm pCO₂ seawater swam to the light 9 out of 10 times, whereas animals in the control treatment entered swam to the light 0 out of 10 times. Zoeae reared in 3200 ppm pCO₂ seawater swam to the light 7 out of 9 times, whereas those in the control treatment swam to the light 1 out of 10 times (Table 1). Accordingly, zoeae reared in 400 ppm pCO₂ seawater swam to the light $80 \pm 12\%$ of the time. Zoeae reared in 1600 ppm pCO₂ seawater swam to the light $90 \pm 12\%$ of the time. Zoeae reared in 3200 ppm pCO₂ seawater swam to the light $78 \pm 13\%$ of the time.

Effect of pCO₂ level on proportion of zoeae that reached light.

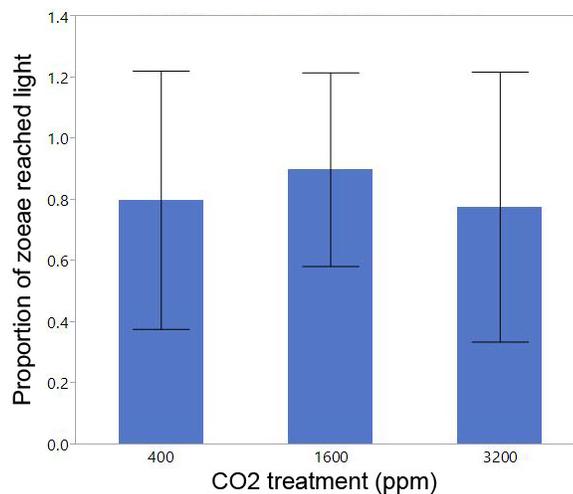


Figure 5. A one-way ANOVA shows no significant impact of CO₂ treatment on whether zoeae reached the light ($\chi^2 (2, N = 29) = 0.5518, p = 0.5601$). Each error bar was constructed using 1 standard deviation from the mean.

Number of zoeae that reached area closest to the light
by CO₂ treatment and light treatment.

pCO ₂ treatment (ppm)	# individuals reached light out of # videos analyzed (light)	# individuals reached light out of # videos analyzed (control)
400	8 out of 10	1 out of 10
1600	6 out of 10	0 out of 10
3200	7 out of 9	1 out of 10

Table 1. Number of individual zoeae that reached the area closest to the light out of number of videos analyzed, in light and control (dark) treatments.

4.3 Speed to light

A one-way ANOVA was conducted among CO₂ treatments to examine the speed to light. The individuals analyzed were from the non-control group that reached the light (n = 24). There was no statistically significant difference in speed to light among CO₂ treatments ($F(2, 2) = 0.8347, p = 0.4479$). The mean speed to light for zoeae reared in 400, 1600, and 3200 ppm pCO₂ seawater were 0.32 ± 0.24 cm/s, 0.33 ± 0.34 cm/s, and 0.58 ± 0.64 cm/s, respectively. The mean speed to light for zoeae reared in the 3200 ppm pCO₂ treatment was 44% and 42% greater than that of the animals reared in the 400 and 1600 pCO₂ treatments, respectively (*Figure 6*).

Effect of pCO₂ level on speed to light.

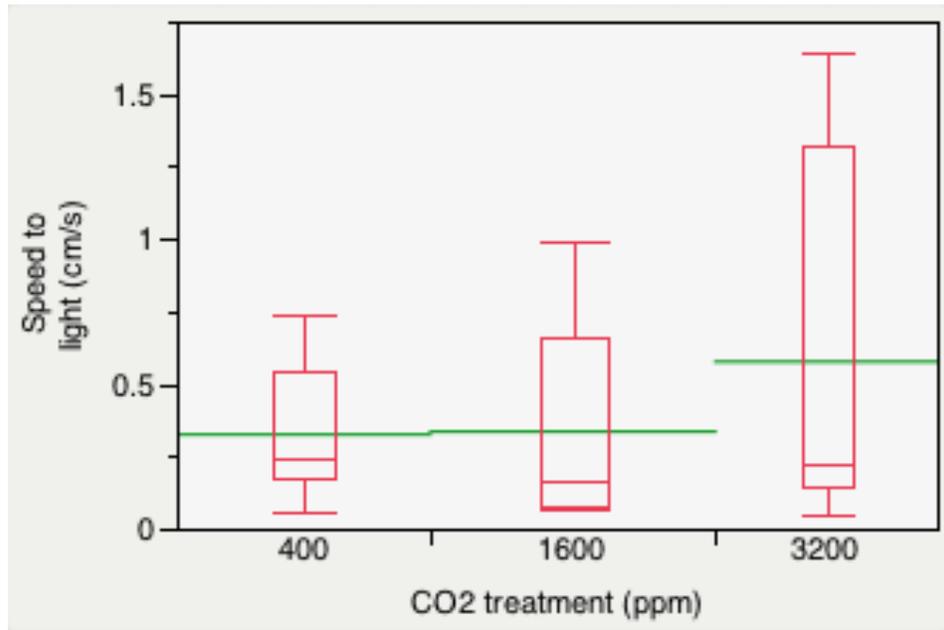


Figure 6. A one-way ANOVA shows no effect of CO₂ level on speed to light ($F(24, 2) = 0.8347$, $p = 0.4479$). A weak, non-significant trend indicates an increase in speed to light with increased CO₂ level. Green lines indicate mean. Red lines in box plots indicate median.

4.4 Time to light

For zoeae that reached the light ($n = 24$), pCO₂ treatment was not a statistically significant effect on the time taken for the zoea to reach the light ($F(24, 3) = 0.1989$, $p = 0.8211$) (*Figure 7*). The mean amount of time (in seconds) for zoeae to reach the light for animals reared in 400, 1600, and 3200 ppm pCO₂ seawater was 55 ± 52 s, 68 ± 53 s, and 51 ± 60 s, respectively.

Effect of pCO₂ level on time to light.

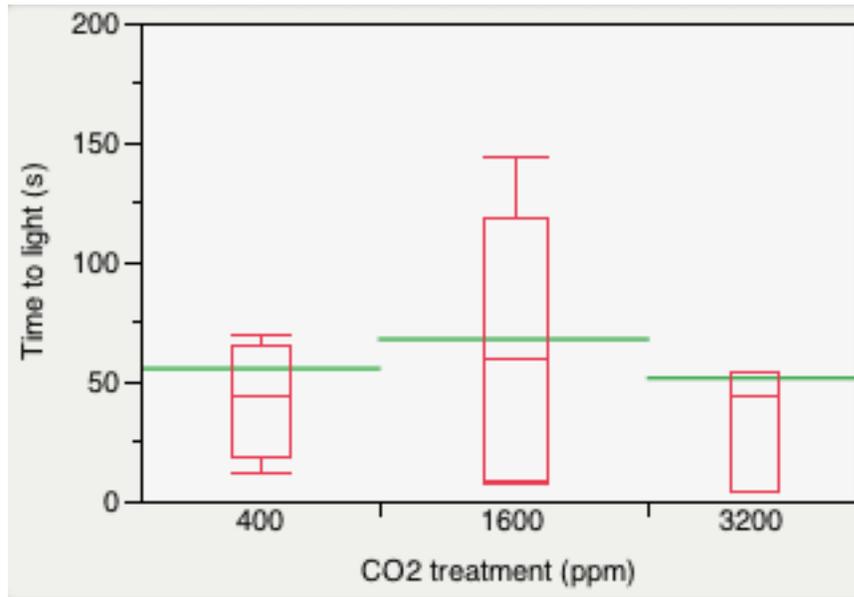


Figure 7. A one-way ANOVA shows no effect of CO₂ level on time to light ($F(24, 3) = 0.1989$, $p = 0.8211$). Green lines indicate mean. Red lines in box plots indicate median.

4.5 Proportion of time at light

For all zoeae exposed to light ($n=29$), there was no significant effect of CO₂ treatment on proportion of time spent at light ($F(29, 2) = 0.1429$, $p = 0.8675$). The mean proportion of time spent at light for zoeae reared in 400, 1600, and 3200 ppm pCO₂ seawater was 0.57 ± 0.37 , 0.53 ± 0.35 , and 0.56 ± 0.37 , respectively.

4.6 Proportion of time away from light once light was reached

For all zoeae that reached the light ($n=24$), there was no significant effect of CO₂ treatment on proportion of time spent away from the light once the light was reached ($F(24, 2) = 0.6159$, $p = 0.5496$). However, a relationship is shown in which proportion of time spent away from the light once the light was reached increases as CO₂ treatment decreases (*Figure 8*). The mean proportion of time spent away from the light once the

light was reached for animals reared in 400, 1600, and 3200 ppm pCO₂ seawater was 0.15 ± 0.06 , 0.07 ± 0.05 , and 0.08 ± 0.06 , respectively. The mean proportion of time spent away from the light once the light was reached for zoeae reared in 400 ppm pCO₂ seawater was 55% and 50% greater than those reared in 1600 and 3200 ppm pCO₂ seawater, respectively.

Effect of pCO₂ level on proportion of time away from light once light was reached.

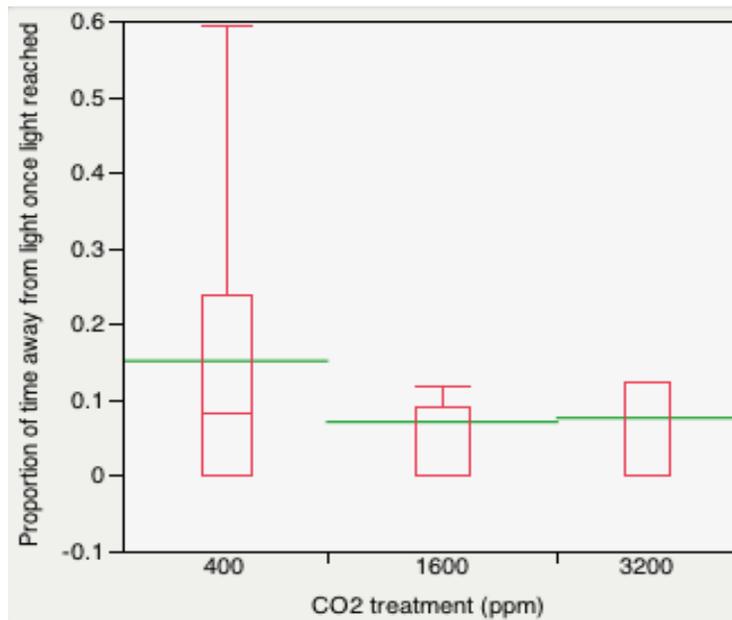


Figure 8. A one-way ANOVA shows no effect of CO₂ level on proportion of time away from light once light was reached ($F(2, 24) = 0.6159$, $p = 0.5496$). However, very weak, non-significant variance indicates that proportion of time away from light once the light was reached may be greatest in the 400 ppm pCO₂ treatment. Green lines indicate mean. Red lines in box plots indicate median.

5. DISCUSSION

The results of the present study corroborate preceding data (Jacoby 1982) supporting positive phototaxis in the second zoeal instar of *Metacarcinus magister*. No significant results indicate a relationship between CO₂-induced acidification and phototaxis, lightly suggesting that there is no immediate concern for adverse impacts of ocean acidification on phototaxis in second instar *M. magister* zoeae. Non-significant trends suggest that zoeae under high-CO₂ treatment may demonstrate heightened phototaxis, measured by an increase in mean swimming speed to light. Zoeae in this treatment may also be more likely to stay at the light once the light is reached. Another non-significant finding indicates that activity level, as measured by mean overall swimming speed, may fall with increased acidity. First, I will discuss the literature supporting a possible change in phototactic response due to CO₂-induced seawater acidification. Then, I will review my findings in the context of the literature.

5.1 Phototaxis vs. photokinesis

Response to light, which is a complex behavior dependent on a number of factors, can be interpreted as phototaxis and as photokinesis. Phototaxis is the active change of the direction of an animal along the axis of the source of a beam of light (Fraenkel and Gunn 1940, Diehn et al. 1977). Movement towards the light is called positive phototaxis, while movement away from the light is known as negative phototaxis. Phototaxis determines what direction the zoea will take when it swims. Photokinesis, on the other hand, is a change in velocity of an organism when it is stimulated by a change in light intensity regardless of direction (Diehn et al. 1977, Fraenkel and Gunn 1940, Crisp and

Ghobashy 1971). Light is only one of the internal and external factors that may influence phototactic and photokinetic behavior (Sulkin 1984). Both phototaxis and photokinesis may be influenced by light wavelength, intensity, and prior exposure of the study organism to light (Sulkin 1984). The behavior observed in the present study may be deemed positive phototaxis based on the statistically significant tendency of zoeae to swim toward the light more often when the light was activated. This study did not address photokinesis, which measures a change in swimming speed as response to a change in light intensity (Sulkin 1984), since the light intensity was static throughout each behavioral test once the light was activated.

5.2 No effect of CO₂ treatment on behavior

There are several explanations for the observed lack of effect of CO₂ treatment on larval activity and phototaxis. The large variability in natural pH conditions both at the sea floor (Long et al. 2013) and in the water column (Feely et al. 2010) could predispose these animals to tolerance of low pH. However, organisms such as brachyuran crabs (Whiteley 2011) that are well equipped to chemically compensate for acid-base disruption may experience heightened risk of behavioral impacts due to an uptake of bicarbonate ions and a reduction of chloride ions, described below (Nilsson et al. 2012, Munday et al. 2012). My results suggest that there is no effect of heightened CO₂-induced acidity on larval phototaxis; however, this result is non-significant and additional studies with larger sample sizes are necessary to determine this effect.

5.3 Proposed mechanism for increased phototaxis

The expected response to directional light in a horizontal chamber for *M. magister* zoeae is to swim toward the light in demonstration of positive phototaxis (Jacoby 1982). A mechanism for possible CO₂-induced increase in phototaxis in a larval crustacean has not yet been proposed. Two studies, both on larval fish, have been conducted on the effect of high CO₂ on the visual information gathering of marine organisms and may elucidate this mechanism (Ferrari et al. 2012, Førsgren et al. 2013). Larval damselfish *Pomacentrus amboinensis* reared in high CO₂ (850 ppm) seawater that were exposed to predators contained in plastic bags, hence a presumed isolated visual cue, swam toward predators more often than fish larvae reared in control seawater (Ferrari et al. 2012). A study on the effect of high CO₂ on larval temperate goby *Gobiusculus flavescens* demonstrates increased phototactic response, via heightened speed to a directional light source, under increased CO₂ at 1400 ppm (Førsgren et al. 2013). The mechanism for any disruption of response to visual cues could lie in perception or cognition. Disruption of visual perception due to low pH could occur through physical damage to sensory organs (Munday et al. 2009); however, physical damage to visual sensory organs in low-pH seawater has not been assessed. Examination of sensory organs shows that physical damage is not a source of info-disruption in the olfactory (hermit crab *Pagurus bernhardus*, De la Haye et al. 2012) or auditory system (clownfish *Amphiprion percula*, Munday et al. 2009) as a result of increased acidity. Larval fish become more likely to take risk when olfactory, auditory, and visual cues are each isolated, indicating a neural mechanism rather than physical damage (Ferrari et al. 2012). Impairment of cognition through neural disruption is more likely than impairment of perception through physical

damage to sensory organs (Ferrari et al. 2012). Reversal of the function of the neurotransmitter GABA-A, the main inhibitory transmitter in the vertebrate brain, is implicated in CO₂-induced aberrations in larval fish behavior such as excessive risk-taking (Munday et al. 2010), boldness (Munday et al. 2010), hypersensitivity to light (Førsgren et al. 2013), and inability to discriminate between ecologically sensitive olfactory (Dixon et al. 2010), visual (Ferrari et al. 2012), and auditory (Simpson et al. 2011) cues. When seawater pH decreases, marine fish (Brauner and Baker 2009) and crustaceans (Truchot 1975; Whiteley 2011) maintain acid-base homeostasis by increasing bicarbonate uptake and releasing chloride into the seawater. An opening of the GABA-gated chloride channel causes an influx of chloride ions, leading to a hyperpolarization and inhibition of the neuron. An outflux of chloride, which may be caused by a decrease in seawater pH, changes the ion gradient across the membrane of the receptor cell and leads to depolarization and excitation, sending a neural signal to the brain (Nilsson et al. 2012). Førsgren et al. (2012) propose neural overexcitation as the mechanism for increased speed to light in goby larvae under high-CO₂ treatment.

Zoeae reared in high-CO₂ seawater exhibited greater speed to light than the other two treatments. Although this effect was not significant, is reflected in the mean overall speed of zoeae exposed to light, since the animals in the high-CO₂ treatment spent the largest proportion of time stationary at the light than any other group, driving down the mean overall speed. The observed non-significant increases in speed to light and tendency to stay at the light among zoeae in the 3200 ppm pCO₂ treatment may be explained by overexcitation of photoreceptor neurons in the brain of the larval crab. The

neurotransmitter associated with phototaxis in larval crab has not been ascertained, but histamine and serotonin are likely candidates (Page 16). Neuronal depolarization causes a release of histamine, which binds to histamine receptors on the dendrite of the neuron (Stuart et al. 2007). Continuous light stimulation causes continuous histamine release, and an increase in light stimulation causes an increase in histamine release (Stuart et al. 2007). Increased influx of chloride into the histamine-gated chloride channel could lead to overexcitation of the neuron and increased phototactic response. Thus, glutaminergic or GABAergic neurons in vertebrate brains may be depolarized as a result of high CO₂ seawater rather than a light cue, causing overexcitation and behavioral hypersensitivity (Nilsson et al. 2012). In invertebrate brains, an analogous process may occur in histaminergic neurons.

Hypersensitivity to light could affect survival and fitness of zoeae. Maladaptive impulses may lead organisms to exert excessive energy in responding to weak or absent light stimuli (Bradbury and Vehrencamp 2011), thus detracting from processing biologically important visual cues necessary for diel vertical migration and predator avoidance. Failure to avoid predators could lead to increased larval mortality and shifts in populations (Morgan 1995), thus altering ecosystem dynamics and potentially impacting commercially important salmon populations. However, the observed increase in speed to light under high-CO₂ conditions is non-significant and further tests are necessary to elucidate the effect of high-CO₂ seawater on zoeal phototaxis.

5.4 Implications for metabolic rate

A non-significant trend demonstrating reduced overall mean speed with increased acidity in the control group may be indicative of reduced metabolic rate. Adult hermit crabs show a reduction in locomotion when exposed to high CO₂, which could be due to an increased metabolic load as the organism puts more energy into acid-base homeostasis (De la Haye et al. 2012). See Page 10 for a review of the literature on physiological mechanisms of acid-base regulation in crustaceans. Another explanation for the decreased locomotion shown in hermit crabs is the lack of olfactory stimulation, which would be due to info-disruption (De la Haye et al. 2012) (*see* Page 14). However, info-disruption could not be a valid explanation for decreased zoeal locomotion at high CO₂ levels in the control treatment since they were not exposed to a visual cue at all. *M. magister* zoeae incubated in 1000 ppm pCO₂ seawater demonstrated heightened swimming speed in dark conditions than animals incubated in 400 ppm pCO₂ seawater (Christmas 2013), potentially indicating that high-CO₂ seawater may increase larval swimming speed, which could indicate an increase in metabolic rate rather than a decrease. The non-significant finding presented in the present study contradicts the results of Christmas (2013); however, larvae in the present study were tested 21 days after hatching while larvae tested by Christmas (2013) were assessed 1 day after hatching. Thus, divergent trends may be expected due to differential behavior among *M. magister* zoeal instars. For instance, the second zoeal instar of *M. magister* may exhibit reduced phototaxis compared to the first zoeal instar (Jacoby 1982).

Metabolic depression is a potential mechanism for the decreased locomotion exhibited by zoeae in the control group in the present study. An analysis of the respiration

rates of zoeae incubated in varying CO₂ treatments would provide more information about the impact of ocean acidification on zoeal metabolism. The zoeae of northern shrimp show increased metabolism with the combined effect of temperature increases and low pH, but not with the sole factor of low pH. This increase in metabolism could lead to higher maintenance costs and change populations and ecosystems (Arnberg et al. 2013). The effects of low pH on the metabolism of crab zoeae are currently unknown.

5.5 Recommendations for future research

Temporal restraints during the behavioral bioassay prevented me from using a larger sample size. This study assessed the behavior of a singular larva at a time, whereas other studies (Jacoby 1982; Førsgren et al. 2013) on larval phototaxis have observed up to ten larvae in a single test, which is a method that has a greater potential to yield significant results in behavioral bioassays.

Information about the pH range to which zoeae are exposed and adapted in the field, including the benthic zone where crab embryos develop, would inform the pCO₂ levels used in future research. Studies on the effect of CO₂-induced acidification on the respiration rate of crab larvae may provide information about the metabolic rate of these animals and inform future behavioral studies. Examination of the effect of high CO₂ on phototaxis across brachyuran crab species may provide insights as to which species will be affected behaviorally by high CO₂ and predict ecosystem dynamics.

6. CONCLUSION

The findings of this chronic bioassay of high CO₂ seawater on larval phototaxis lightly suggest that there is no dramatic effect of ocean acidification on the phototaxis and activity level of *Metacarcinus magister* zoeae. However, trends that are not statistically significant indicate that *M. magister* zoeae reared in 3200 ppm pCO₂ seawater may show heightened phototaxis when exposed to directional light in a horizontal chamber and decreased overall activity level in the dark. Additional studies are necessary to further assess the effect of high-CO₂ seawater on larval crab.

7. INTERDISCIPLINARY STATEMENT

This thesis research addresses an interdisciplinary problem in which the fields of physiology and environmental change research intersect with implications for ecology and socioeconomics. Specifically, the present study provides information about the potential behavioral impacts of anthropogenic carbon dioxide emissions on the most economically important crab species on the U. S. west coast, the Dungeness crab. Biological restrictions prevent this species from being produced in aquaculture enterprises, so the fishery depends on wild-caught Dungeness crab for revenue. As such, the changes in wild Dungeness crab population size that may result from impacts on larval behavior could impact harvesters and consumers throughout the range of this species. In addition, trophic effects of changes in larval behavior could alter salmon and other finfish populations, leading to ecological, cultural, and economic shifts.

The continuing accumulation of anthropogenic atmospheric carbon dioxide is already drastically altering terrestrial and aquatic ecosystems globally, and models indicate further change is inevitable. Various collaborative organizations among scientists, policy makers, economists, tribes, non-governmental organizations, fishers, and other stakeholders, such as the Blue Ribbon Panel on Ocean Acidification led by former Governor of Washington Christine Gregoire, are identifying and pursuing research, policy, and education goals that will best prepare communities in the Pacific Northwest to adapt to coming marine ecosystem changes. Washington is a progressive state in terms of climate change awareness and is actively working towards a zero emissions goal for 2050. Great obstacles remain in the path to ocean acidification mitigation. Nonetheless, ocean acidification is a growing concern that is gaining awareness both locally in the Pacific Northwest and globally. Research on ocean acidification impacts on marine life will allow those who depend on food from the sea to plan ahead for ecosystem shifts.

The results presented here indicate that there may be no effect of ocean acidification on the phototactic behavior of Dungeness crab larvae, which bodes well for those that depend on this species for livelihood. However, further research is needed in order to assess this impact. This thesis provides information that may be valuable for future research endeavors on the impacts of ocean acidification on larval phototaxis of any marine species that exhibits such behavior. This manipulative experiment may be useful for the Atlantis Ecosystem Model managed by the Ocean Acidification Group at NOAA's Northwest Fisheries Science Center that incorporates information about the

effects of ocean acidification on the Puget Sound food web. The diverse physiological impacts of ocean acidification on organisms may have far-reaching implications and further research across disciplines is necessary to predict these impacts.

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