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Investigation of the Effects of Ocean Acidification on Juvenile *Homarus americanus* Feeding Behavior

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Abstract

Climate change has led to increased CO\(_2\) absorption by ocean surface waters, resulting in decreased pH levels, a phenomenon known as ocean acidification (OA). A primary class of marine organism behavior is impacted by OA, in particular, olfactory-mediated behaviors related to foraging and feeding success. However, the biological mechanisms responsible for the documented impairment of foraging and feeding-related behaviors remains in question. We find that juvenile American lobsters (*H. americanus*) exhibit significantly faster rates of total feeding times in response to short-term low pH exposure. However, total search time and total handling time were not affected by pH when examined separately, indicating that an additional variable not considered in this study may be responsible for the increase in total feeding rates. We hypothesize that increases in total feeding rates are a short-term exposure response to low pH levels due to increased energetic demands and a higher biological importance on acquiring food energy resources. We suggest that slower rates of feeding and impaired behaviors seen in past literature are likely a result of longer-term exposure effects. We also review proposed physiological mechanisms for the impairment of marine organism chemoreception. We suggest that environmental interactions with odorants and receptors, specifically protonation events, alter the odorant affinity for respective receptors as an immediate cause for chemoreception impairments. It is possible a reversal of ion flow through the GABA\(_A\) receptor results in behavioral alterations seen in more long-term exposures via the “GABA\(_A\)R Model.” The review portion of this paper also suggests that future OA research efforts should be aimed at considering understudied factors such as exposure length, food availability, and ontogenetic implications.
Introduction

Climate change has become an increasingly prominent global phenomenon in all aspects of life. Anthropogenic activities have greatly increased carbon dioxide (CO$_2$) concentrations in the atmosphere (Tresguerres & Hamilton, 2017). Rapid increases in CO$_2$ levels in the atmosphere have led to an increased uptake of CO$_2$ by ocean surface waters. The absorbed CO$_2$ becomes hydrated in water, forming carbonic acid (H$_2$CO$_3$), which then produces a surplus of H$^+$ as it dissociates to form H$^+$ and bicarbonate ions (HCO$_3^-$; Fabry et al., 2008). This results in more acidic waters as the increased prevalence of H$^+$ lowers pH levels. This is a process known as ocean acidification (OA) and it is affecting marine ecosystems across the globe (Caldeira & Wickett, 2003; Porteus et al., 2021). These changes in ocean chemistry present many critical physiological and ecological consequences in marine environments (Durant et al., 2023; Klymasz-Swartz et al., 2019).

Early OA research efforts were focused on impacts on calcification. Researchers hypothesized that altered ocean chemistry would reduce carbonate concentration in the ocean which is a building block for hard structures of marine organisms, subsequently impairing calcification (Clements & Comeau, 2019; Clements & Darrow, 2018; Kleypas & Langdon, 2006). More recent efforts have broadened the scope of what aspects of marine life may be impacted by OA. Beyond calcification, acidified waters have been found to impact development of embryos and larvae of many marine organisms (Frommel et al., 2012, 2016; Rossi et al., 2015), reproduction (Miller et al., 2013), metabolic rate (Couturier et al., 2013; Enzor et al., 2013; Rummer et al., 2013), and behavior (Allan et al., 2013; Dixson et al., 2010; Dodd et al., 2015; Domenici et al., 2012; Hamilton et al., 2013; Munday et al., 2009, 2010, 2016; Richardson
et al., 2021; Rossi et al., 2015). However, results are widely variable, and many findings are likely species specific, leaving many of the effects of OA and their physiological mechanisms unknown.

**Behavioral Implications of Ocean Acidification**

Behavioral implications of OA are of particular concern in recent literature. OA has the potential to dampen species interactions through the impairment of numerous behaviors. This is because the biological basis that mediates many of these behaviors is chemical communication via chemoreception. Chemoreception, specifically olfaction, is crucial to marine organisms’ ability to feed, reproduce, and communicate (Porteus et al., 2021). Marine organisms exposed to elevated levels of CO$_2$ have demonstrated impaired responses to olfactory cues related to foraging and feeding, resource assessment, and decision making (Draper & Weissburg, 2019; Durant et al., 2023; Porteus et al., 2018, 2021; Wang & Wang, 2020).

Of particular importance is marine invertebrates, since calcifying organisms are considered to be more sensitive to elevated levels of CO$_2$ than other marine organisms, such as fish. This is because the altered states of ocean chemistry are likely to introduce changes to the internal physiology of many organisms in terms of their acid-base balance (Raven et al., 2005). Fish are generally more efficient at regulating their internal acid-base balance than calcifying invertebrates, making them less sensitive to acidification (Dissanayake & Ishimatsu, 2011; Grosell, 2006; Marshall & Grosell, 2005).

**Chemoreception in Marine Organisms**

A large portion of behaviors observed to be impacted by OA are mediated by chemoreception. Marine organisms have particularly well-developed chemosensory systems
(Clements & Comeau, 2019; Klymasz-Swartz et al., 2019). This is because a majority of communication completed in marine ecosystems is through chemical cues released into the water (Ache, 1982). Marine organisms employ various categories of chemoreception to interact with their environment. Among these categories is olfaction, or the sense of smell through chemical cues called odorants (Derby, 2011). Olfaction is vital as it is used for many important behaviors, including mate selection and reproduction, social communication, and the detection of alarm cues for predator presence, habitat selection and navigation (Derby, 2021). Moreover, chemoreception allows for organisms to discriminate between odorant mixtures, allowing for efficient assessments of the environment, such as food quality, and subsequent decision-making (Derby, 2021). Lastly, olfaction is a useful sensory modality for marine organisms as the aquatic environment may experience fluctuations in many factors, including visibility. Since odorants can propagate over long distances remaining unchanged, they serve as a useful and reliable communicative tool for marine animals (Atema, 1995).

Crustaceans are model organisms for aquatic studies on olfaction due to their well-developed olfactory systems (Ache & Derby, 1985; Derby, 2021; Durant et al., 2023; Derby & Schmidt, 2017; Schmitt & Ache, 1979). Since crustaceans have poor vision, they rely heavily on olfaction as a dominant sensory system for survival and fitness (Schmidt & Mellon, et al., 2010). The olfactory organs in crustaceans that house olfactory receptors, specifically ionotropic receptors, are the antennules (Harzsch & Krieger, 2018; Schmitt & Ache, 1979; Solari et al., 2017; Waldrop et al., 2016). Routine movements, referred to as “flicking,” allow odorants in the environment to be sensed at a greater frequency since it exposes olfactory sensilla (Schmitt & Ache, 1979). Overall, the ability of marine organisms to engage in olfaction is critically important to their overall fitness, particularly in crustaceans which rely heavily on olfaction for
communication and decision making. With the increasing threat of OA to marine ecosystems across the globe, ocean chemistry has the potential to greatly impact the effectiveness of chemoreception, posing as a massive threat to entire populations of marine organisms that rely on this form of communication (Porteus et al., 2021).

**Foraging Behaviors: Prey Detection and Search Time**

There is evidence that suggests the ability of animals to use chemoreception is impaired by increased CO$_2$ and low pH levels. Studies have shown that hermit crabs exhibited lower rates of antennule flicking (de la Haye, et al., 2012; Durant et al., 2023; Kim et al., 2016) and decreased locomotion (de la Haye, et al., 2012) under low pH exposure. Moreover, there is evidence that elevated CO$_2$ levels decrease nerve sensitivity in response to prey odorants, indicating that OA effects may raise the threshold of detection for some odorants (Durant et al., 2023). Additional studies on various crab species show that the ability to successfully locate food items was significantly impaired by low pH (Richardson et al., 2021; Roggatz et al., 2019), indicating that their chemoreceptive functions for food cue detection was likely affected by pH conditions. Overall, the impairment of olfactory-mediated foraging behaviors indicate that acidified ocean conditions impair food detection via chemoreception and reduce search success rates in crustaceans.

**Foraging Behaviors: Feeding and Handling**

Other factors related to foraging behavior that appear to be impacted by OA include the ability of marine organisms to successfully and efficiently handle prey items once captured. One study showed that hermit crabs demonstrated impaired resource assessment in low pH conditions, such that individuals were less likely to select optimal shells for shelter and
individuals that did switch to a more optimal choice did so at slower rates (de la Haye et al., 2011). The reduced rates of decision-making while seeking shelter may indicate that organisms could experience impaired decision-making while foraging since both behaviors are mediated by chemoreception. This would lead to decreased efficiency in successful feeding events under ocean acidified conditions. Other studies have shown crustaceans to have reduced locomotion in response to elevated CO$_2$ levels in terms of distance traveled and average speed during entire feeding periods (Alenius & Munguia, 2012; de la Haye et al., 2012; Dissanayake & Ishimatsu, 2011). Additionally, total feeding rates have been found to be significantly reduced in acidified environments, including variables such as consumption rates, search time, prey handling, and the time to break open prey items (Appelhans et al., 2012; Dodd et al., 2015; Richardson et al., 2021; Saba et al., 2012; Wang et al., 2018; Wu et al., 2017). However, many results are inconclusive or variable, with some studies finding no significant change in prey handling times and feeding rates (Appelhans et al., 2012; Glaspie et al., 2017; Landes & Zimmer, 2012) and others showing increased rates of successful feeding events in acidified environments (Li & Gao, 2012). With so many variables involved in feeding, such as resource assessment, search time, and handling time, it is of interest to determine which components of total feed time of marine organisms are the key variables impacted by OA so that we can better understand what is causing behavioral changes.

**Primary Investigations**

While there is a growing body of literature describing various effects of acidification on the foraging behavior of marine organisms, results remain highly variable with a lack of tested physiological mechanisms. This present study seeks to provide further evidence for the effects of OA on foraging behavior through the investigation of the effects of varying pH levels on foraging rates of juvenile American lobsters (*Homarus americanus*). This paper also reviews
proposed mechanisms regarding the physiological mechanisms of impaired chemoreception in marine organisms and seeks to be utilized as a recommendation for future research avenues.

The primary research component of this paper utilizes juvenile *H. americanus*, a long-lived species found along the North American coast of the Atlantic that not only holds a crucial role in marine ecosystems but is a key fishery across coastal North American regions, making them a critically important species (Klymasz-Swartz et al., 2019; Lawton & Lavalli, 1995). We exposed lobsters to assigned pH levels and ran behavioral assays to observe the impacts of pH on olfactory-mediated foraging behaviors. Observed behaviors included: (1) total feed time, (2) total search time, and (3) total handling time (Table 1). We predicted that juvenile *H. americanus* would exhibit decreased total feed rates, total search rates, and total handling rates in response to low pH levels. We hypothesize that acidified environments would impair the ability of juvenile lobsters to successfully use olfaction to forage.

**Methodology**

**Specimen Collection and Maintenance**

Juvenile *H. americanus* (n = 32) were collected via a mix of tide pooling at Odiorne Point, NH (n = 21) and trapping and diving off the coast of New Castle Island, NH (n = 11). Specimen collection took place between the months of June and October, 2023. Carapace length was measured for all lobsters collected. Those with a carapace length greater than 50 mm were released. The lobsters used in this study ranged in carapace length from 23 mm to 50 mm.

Individuals were held at the UNH Coastal Marine Laboratory in Rye, NH. Lobsters were housed in a large tank with seawater containing air stones and flow through water. They were
isolated in separate Tupperware containers with holes for water flow to prevent the occurrence of physical interactions. Individuals were monitored every 3 days and Tupperware containers were rinsed to maintain adequate health and habitat conditions. Additionally, the lobsters were fed every 4 days with shucked blue mussels. Feeding took place in their individual Tupperware containers and empty shells were removed during routine Tupperware rinsing.

**Experimental System**

20 aquarium tanks (91.5 L) were utilized for this experiment. Each tank was equipped with a plastic shelter in one corner and a plastic divider spanning approximately half of the tank width and clipped to the side so that the tank was split into a third and a two-thirds section (refer to Figure 1). The divider was utilized as a visual barrier between the lobsters and prey items (blue mussels) during behavioral assays.

Tanks used a flow-through system with automated monitoring and adjustments of pH. pH was manipulated via the bubbling of CO₂ into the tank and injections were mediated via a solenoid valve and Apex Fusion software. Temperatures were kept at ambient levels. Electrodes in each tank monitored pH levels and temperature. If the pH rose above set target levels, CO₂ was bubbled into the tanks to lower the pH. Likewise, if the pH dropped below set target levels, air passed through a soda ash (sodium carbonate) filter (CO₂ free air) was injected into the tank to raise the pH. Target pH levels were individually set at a distinct pH ranging between 7.3 to 7.8. Electrodes were calibrated on a weekly basis using NBS calibration solutions (7 pH and 10 pH).

Throughout the duration of pH exposure periods, weekly water samples were taken to measure pH and total alkalinity levels. Samples were taken once a week for 4 weeks. pH was
measured via electrodes with calibration standards on the NBS scale. pH was converted to the total scale using pH measurements via spectrophotometry using cresol purple, a pH indicator dye. Total scale calibration and absorbance readings via spectrophotometry allowed for the calculation of the total pH. Additional samples were frozen for later analysis, during which the samples were thawed out overnight and measured via an automatic titrator.

![Behavioral assay tank set-up](image)

**Figure 1.** Behavioral assay tank set-up

**Exposures**

A total of 2 trials with fifteen behavioral assays each (n = 15) were conducted in November and December, 2023 Lobsters were initially weighed and measured before being assigned to a tank and given 24 hours to acclimate to the experimental system at ambient conditions. During the acclimation period, individuals were fed shucked blue mussels and empty shells were removed via siphoning. Next, the lobsters were exposed for 10-days to a pH level ranging from 7.3- 7.8. Lobsters were monitored daily for survival. During trial 1, one lobster died (n = 1) and another lobster molted (n = 1) during the exposure period. During the exposure period of trial 2, a CO₂ tank replacement was necessary. All lobsters were returned to ambient conditions.
conditions for 48 hours until the target pH levels could be reestablished. Lastly, all tanks were drained and cleaned between trials 1 and 2.

**Behavioral assays**

Behavioral assays were performed within the exposure tanks. Behavioral assays were conducted via time lapse video recordings and were performed across a 40-hour period that ran overnight to allow the lobsters to feed regularly as nocturnal foragers. Additionally, overnight assays allowed for reduced lab activity disruptions to lobster behavior. Red lights allowed for video recordings and previous research suggests that red light is outside of the visual spectrum for crustaceans (Goldsmith & Fernandez, 1986). Video recordings were taken via a time lapse camera set at five-second intervals.

The 40-hour behavioral assay was split into three periods: (1) without prey, (2) intermission, and (3) with prey. The without prey period began in the evening when a blank rock was placed in the tank so that it was behind the visual barrier. The without prey period lasted for a total of 16 hours, after which the blank rock was removed for an intermission period of 8 hours. Finally, the with prey period (which is the focal assay analyzed for this work) consisted of the final 16 hours and began with the addition of a rock containing blue mussel into the tank behind the visual barrier (Figure 1).

Following the completion of the behavioral assays, we analyzed the videos to calculate three data metrics: (1) total feed time, (2) total search time, and (3) total handling time (refer to Table 1 for metric definitions).

**Table 1.** Behavioral metrics observed during video analysis of behavioral assays.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Definition</th>
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...
Total feed time | The time it took from the addition of prey items (rock with attached blue mussels) to completion of the first successful feed, measured by signs of completion (e.g. crushed mussel shells, abandonment of the mussel after significant handling, foraging for new mussels after significant handling).
---|---
Total search time | The time from the addition of prey items (rock with attached blue mussels) to the first success at locating prey items, measured by the first contact made with prey items.
Total handling time | The time from the first observable manipulation of a prey item (blue mussel) to the last observable manipulation during the first successful feeding event.

**Data Analysis**

pH calculations from water sampling spectrophotometry were compared to pH electrode measurements transformed to the total scale to calculate gaps between the two values. Value gap calculations from the 4 sampling periods were averaged to determine the total tank pH.

Behavioral data were analyzed in Microsoft Excel and R. To investigate the effects of pH on lobster foraging behavior we used a linear model and log transformed our behavioral response metric. We inspected diagnostic plots to determine if the data met the assumptions of the model. This was completed for each of the 3 behavioral metrics.

**Results:**

Reduced pH decreased juvenile *H. americanus* total feed time (p = 0.05). Individuals were observed to complete their first successful feed at significantly faster rates at lower tank pH levels (Figure 2). However, there was no significant effect of pH on total search time (p = 0.78; Figure 3) or total handling time and (p = 0.30; Figure 4).
Figure 2. Total feed time of juvenile *H. americanus* across varying pH levels. There was a significant correlation between total feed time and pH level ($p = 0.05$). The rate of the completion of the first successful feeding event, including the time it took to locate, handle, and consume prey items (blue mussels), was significantly lower at lower tank pH levels.

Figure 3. Total search time of juvenile *H. americanus* across varying pH levels. There was/was not a significant correlation between total search time and pH level ($p = 0.78$). Tank pH did not significantly impact the time it took individuals to successfully locate prey items (blue mussels).
Figure 4. Total handling time of juvenile *H. americanus* across varying pH levels. There was not a significant correlation between total handling time and pH level (p = 0.30). Tank pH did not significantly impact the time it took individuals to successfully locate prey items (blue mussels).

**Discussion**

Successful feeding occurred at faster rates under more acidic conditions (p = 0.05). However, search time and handling time were not significantly impacted by seawater pH. We only found pH effects when factors were taken all together for a total feed time. These findings do not support our predictions that low pH levels would significantly impair olfactory-mediated behaviors. Further, this result is inconsistent with much of the current existing literature that found reduced feeding rates in response to low pH. This unexpected outcome sheds light on numerous hypotheses concerning understudied underlying variables and proposed physiological mechanisms.

We hypothesize that faster rates of feeding at lower pH levels are a result of short-term exposure to OA conditions. It is likely that increased energetic demands activate a short-term “pH shock” response (Porteus et al., 2021) that places an increased importance on foraging
behaviors. Additionally, since reduced pH was found to impact total feed time but not the isolated metrics of search time and handling time, we hypothesize that a separate component is impacted by OA, resulting in altered total feeding rates. Such components not investigated in this present study are physiological mechanisms responsible for chemoreceptive functioning that may be directly impacted by reduced pH levels. It is also of important consideration that ocean acidification effects may be lifecycle stage specific. Below we review these various components in more depth.

**Effects of Short-term vs Long-term OA Exposure**

We hypothesize that our findings reflect differences in short-term and long-term exposures to OA. The observed increase in feeding rates may be a result of an initial response from short-term exposure to OA conditions. Acidified environments are thought to cause physiological stress on organisms as the energetic costs to maintain homeostasis and continue normal processes, such as growth, reproduction, and calcification, are increased (Hofmann et al., 2010; Howes et al., 2015; Sokolova et al., 2012; Wood et al., 2008). Food serves as the primary supplier of energy that drives biological and behavioral processes. If an individual fails to acquire and consume food, they will experience limited energy reserves, resulting in the necessary allocation of stored energy resources (Brown et al., 2020; Sokolova et al., 2012). Certain biological activities that are deemed less important will be downregulated so that energy resources may be used for vital processes (Brown et al., 2020; Dissanayake & Ishimatsu, 2011). For example, several coastal marine species have shown the downregulation of less important energy consuming processes and an upregulation in crucial internal processes, such as osmotic regulation (Freire et al., 2008; Lucu & Towle, 2003).
The increased energetic demands placed on organisms under ocean acidification conditions may place higher importance on foraging and feeding to meet the high energy demands, resulting in increased rates of foraging (Klymasz-Swartz et al., 2019). Recent findings suggest that if sufficient food is available, marine organisms will engage in foraging, a high-energy consuming behavior, despite internal energetic constraints imposed by elevated CO$_2$ levels in the environment (Hernán et al., 2016; Pan et al., 2015; Ramajo et al., 2016; Thomsen et al., 2013; Towle et al., 2015). However, this ability to increase feeding rates in compensation for increased energetic demands is likely to be highly variable between different species (Dissanayake & Ishimatsu, 2011). More research should be dedicated to species-specific abilities to tolerate short-term exposure to acidic environments, allowing for increased food acquisition. Moreover, increased consideration should be given to investigating how food availability affects observed behaviors under OA conditions, a variable that has been widely ignored in past literature (Kroeker et al., 2014).

It is likely that increased foraging in compensation for high energetic demands is an initial response to short-term exposure to acidified conditions. In other words, increased feeding rates may be an early response to “pH-shock” (Porteus et al., 2021). It is likely that such behaviors have been sustained in the wild since pH levels in the ocean, although experiencing a net decline, experience common fluctuations (Porteus et al., 2021). Past literature methodology has predominantly involved short-term exposure periods (Alenius & Munguia, 2012; Appelhans et al., 2012; de la Haye, et al., 2011, 2012; Dissanayake & Ishimatsu, 2011; Dodd et al., 2015; Durant et al., 2023; Kim et al., 2016; Landes & Zimmer, 2012; Richardson et al., 2021; Roggatz et al., 2019; Saba et al., 2012; Wang et al., 2018), with exposure periods only lasting a few hours to a few months. Additionally, an investigation of antennule “flicking” rates in hermit crabs
showed that although some behavioral trends seemed apparent after immediate exposure, statistically significant effects were not observed until 7 days of exposure (Kim et al., 2016), indicating that more long-term exposure is necessary to observe the full extent to which decreased pH levels can impact foraging behavior. Therefore, it is important that more long-term exposures are investigated in future studies. Overall, further research is required to investigate the different effects of short-term and long-term exposure of marine organisms to low pH levels.

**Physiological Mechanisms as a Missing Link**

Another way in which the results deviated from our predicted outcomes was that significance was only found when all factors were taken together (total feed time) and compared to pH levels in the tanks. Moreover, pH did not influence the independent components of feeding events (total search time and total handling time) during short-term exposures, indicating that search time and handling time could not be the reason that total feeding rates increased. We hypothesize that a separate condition is responsible for the change in total feeding rates. It is possible that direct effects of reduced pH on physiological mechanisms related to chemoreception are causing many of the behavioral altercations observed. A growing body of literature has been accumulating evidence for two primary mechanistic proposals for the physiological effects of elevated CO₂ levels in the marine environment. These two mechanisms involve the “GABAₐ receptor (GABAₐR) model” and the protonation of odorants and receptors exposed to the environment. Both mechanisms are described in further detail below.

**The GABAₐR Model Hypothesis**

The “GABAₐR model” is the most widely proposed mechanism for dampened chemoreception as a result of ocean acidification (Tresguerres & Hamilton, 2017). The model
suggests a reversal of ion flow through the GABA_A receptor. The biological prerequisite for this mechanism is the development of hypercapnia. Hypercapnia occurs when there are elevated levels of CO_2 in an organism’s internal fluids (e.g. blood) because of an increase in environmental CO_2. The increased concentration of CO_2 in the marine environment shifts the concentration gradient, such that the amount of CO_2 diffused into the blood is elevated. To restore internal acid-base balance and maintain homeostatic pH, marine organisms typically upregulate the secretion of H^+ and accumulate HCO_3^- in the blood (Larsen et al., 2014; Tresguerres & Hamilton, 2017). While the physiological mechanism remains unknown, marine organisms that become hypercapnic with increased HCO_3^- fluid levels have shown to have decreased Cl^- concentrations in their blood plasma as well, possibly to maintain internal net ionic charge balances (Heisler, 1988; Larsen et al., 1997; Toews et al., 1983). The change in extracellular Cl^- concentration disrupts the equilibrium of the electrochemical gradient. This instigates a reversal of ion flow through cell receptors, specifically the GABA_A receptor. Under normal conditions, Cl^- flows into the cell via the GABA_A receptor with some additional permeability for other negatively charged ions such as HCO_3^- (Bormann et al., 1987; Inomata et al., 1986; Tresguerres & Hamilton, 2017). When concentrations of Cl^- decreases outside of the cell and HCO_3^- concentrations increases, the flux of ion flow through the GABA_A receptor is reversed. The reversal of net ionic movement through the cell, specifically the influx of Cl^- out of the cell, ultimately results in a functional switch of the neuron from inhibitory activity to excitatory. This alters neuron signaling and behavioral responses to olfactory cues in the environment (Nilsson et al., 2012; Staley et al., 1995; Stein & Nicoll, 2003; Tresguerres & Hamilton, 2017). It is hypothesized that OA conditions in marine environments will result in mild hypercapnia in marine organisms across the globe, resulting in this ion flow reversal of the
GABA_A receptor, explaining, in part, some of the physiological bases for behavioral alterations in response to acidification.

Multiple studies sought to gather evidence to test this hypothesis that a functional switch of the GABA_A receptor is the mechanism responsible for behavioral impairments after exposure to elevated CO_2 levels (Chivers et al., 2014; Chung et al., 2014; Lai et al., 2015; Munday et al., 2016; Nilsson et al., 2012; Ou et al., 2015; Regan et al., 2016; Watson et al., 2014). Gabazine, a known antagonist for the GABA_A receptor, was used to treat marine organisms that had been exposed to waters with high CO_2 levels. It was predicted that gabazine would block the excitatory action of the GABA_A receptor, switching its function back to inhibitory, thus restoring normal behavioral functioning. This prediction was found to be true in numerous studies (Chivers et al., 2014; Chung et al., 2014; Lai et al., 2015; Munday et al., 2016; Nilsson et al., 2012; Ou et al., 2015; Regan et al., 2016; Watson et al., 2014), building evidence in support of the hypothesis of the GABA_AR model. Several studies found that the ability of gabazine to restore behavioral outcomes depended on environmental conditions, such that the activation of GABA_A receptors provides different behavioral outcomes in OA and normal conditions (Hamilton et al., 2013). However, the GABA_A receptor is biologically complex, suggesting that more research is required to support the GABA_AR model and understand the specific mechanisms occurring (Porteus et al., 2021).

Since maintaining functional acid-base levels is of crucial importance to marine organisms, it is likely that there is redundancy in the physiology of the animals such that additional or back-up mechanisms exist to regulate and restore disruptions to homeostatic levels (Noble et al., 2014). For example, another mechanism to consider involves potassium (K^+) ion channels, specifically the Na^+/K^+/2Cl^- cotransporter 1 (NKCC1) and the K^+/2Cl^- cotransporter 2
(KCC2). NKCC1 is known to transport Cl\(^-\) into the cell while KCC2 transports Cl\(^-\) out of the cell. The interplay of these two ion channels indicates a regulatory function for the intracellular and extracellular concentration of Cl\(^-\) (Hartmann & Nothwang, 2022; Sung et al., 2000). Therefore, under hypercapnic conditions, it may be likely that the downregulation of NKCC1 and the upregulation of KCC2 moves Cl\(^-\) out of the cell to counteract the reversal of ion flow through the GABA\(_A\) receptor, allowing for normal receptor functioning to continue (Tresguerres & Hamilton, 2017). This is just one of numerous ionic mechanisms that may explain inconclusive results in studies investigating the GABA\(_A\)R model or alternative mechanisms for physiological responses to OA altogether.

Moreover, there may be differences in mechanisms employed in short-term and longer-term responses. For example, one study found evidence of altered behavior as a result of impaired olfaction in fish at relatively low levels of elevated CO\(_2\) and a complete lack of larval fish olfaction at higher levels of CO\(_2\) (Munday et al., 2010). This suggests that a different short-term mechanism may be at play since low levels would be unlikely to result in significant acid-base changes in the organism to impact GABA\(_A\) receptor function (Tresguerres & Hamilton, 2017). If the GABA\(_A\) receptor model is a mechanism at play in response to more long-term exposure to low pH levels, then it is possible that this study did not allow sufficient time for the effects of this chemical mechanism to be observed.

Lastly, the restoration effect observed by gabazine treatments may not be directly a consequence of binding to the GABA\(_A\) receptor, as other ion channels may be involved. It will be important to test other substances associated with the binding of the GABA\(_A\) receptor to ensure that other gabazine receptor binding sites on other membrane proteins are not responsible for the changes observed. More research is required to understand the exact biology that occurs within
the organism to produce such behavioral outcomes (refer to Tresguerres & Hamilton, 2017 for a review of many possible neurobiological considerations). Overall, more evidence must be generated from future studies in order to support, refute, or expand knowledge of the GABA_A model.

The Protonation Hypothesis

Another recently proposed mechanism for physiological mechanisms responsible for impaired chemoreception under OA is the protonation hypothesis. The protonation hypothesis suggests that low pH levels alter the protonation state of odorants in the environment, subsequently changing their 3D conformation and reducing the affinity for binding to chemoreceptors in olfactory organs or marine organisms (Porteus et al., 2021). Electrophysical evidence exists demonstrating a rapid impairment in neuronal firing at olfactory neurons due to a decreased sensitivity to odorants (Durant et al., 2023). This suggests that there is another mechanism at play that shows a more immediate effect than the GABA_A receptor. The protonation hypothesis is a noteworthy consideration as an explanation for these rapid responses to OA environmental conditions.

Changes in water chemistry through ocean acidification may have more direct effects on the impairment of chemoreception by marine organisms. It is hypothesized that chemical alteration to chemical odorants may interfere with the link between the organism and the environment, preventing communication via olfaction from occurring. An addition proposed mechanism describes that the increased concentration of H^+ in acidified waters can react with specific chemical groups that are common to odorant compounds (Porteus et al., 2021). The protonation of these compounds changes bond angles as differential electron pair distribution results in changes in repulsion between chemical groups, ultimately altering bond angles from
the central atom of the molecule (Gillespie & Hargittai, 2013). The alteration of bond angles changes the overall structure and 3D conformation of the odorant molecule altogether. Moreover, protonation can change the net ionic charge of the molecule since it adds a proton that can neutralize negative charge or introduce positive charge to the substance. Protonation can also alter charge distribution and polarity as a result of conformational changes (Roggatz et al., 2016; Porteus et al., 2021; Velez et al., 2019). These properties can alter odorant affinity for receptor binding sites on the organism’s chemoreceptors (Porteus et al., 2021; Schirrmacher et al., 2021).

Similarly, low pH levels as a result of OA may also result in protonation of receptor proteins involved in chemoreception (Tierney & Atema, 1988). Receptor proteins often rely on the presence of specific residues of subunits to allow for an active conformation that exposes and readies the binding site at the binding domain. It is hypothesized that if components of the receptor are altered via protonation, the receptor may undergo a conformation change to an inactive form that prevents binding from environmental odorants or to a state with reduced affinity for odorant binding (D’Souza & Strange, 1995; Gillard & Chatelain, 2006; Porteus et al., 2021; Tombaugh & Sapolsky, 1990; Traynelis & Cull-Candy, 1991).

The protonation and deprotonation of chemical compounds such as odorants and their corresponding receptors is a rapid and reversible process that can occur even as a result of minor changes in pH levels (Porteus et al., 2021; Roggatz et al., 2016). This suggests that immediate chemical alteration to substances involved in chemoreception may be responsible for short-term exposure effects observed in marine organisms to OA conditions. However, recent literature describes a retention of impaired chemoreception at a consistent magnitude over longer-term exposure. This indicates that the protonation hypothesis as a physiological mechanism for impaired olfactory-mediated behaviors functions in conjunction with other, more long-term
processes. Thus, the protonation model and GABA\(_A\)R model may both be active in mediating observed pH-induced behavioral changes. However, which mechanism is at play and when, and whether either exists as a complete explanation remains unclear and requires additional research.

Of additional consideration are the implications of food availability on these proposed physiological mechanisms. Olfactory nerve recordings have demonstrated that an increased concentration of odorants at the respective chemoreceptor results in a higher frequency of action potentials fired, such that the neuron responds at a greater frequency (Hubbard & Velez, 2020). Moreover, a higher concentration of odorants is necessary to meet the detection threshold and fire off action potentials in environments with elevated CO\(_2\) levels compared to normal levels (Porteus et al., 2018; Velez et al., 2019). These findings suggest that increased food availability, in terms of food abundance and distance, impacts the ability of marine organisms to detect and perceive olfactory cues in the environment. It is possible the present study in this paper placed food items at too close distances to fully observe the effects of OA on foraging behavior and chemoreception. Due to the relatively small tank dimensions, the food items were placed at shorter distances to the organisms. The ability of odorants to maintain potency as it propagated over such a short distance may have compensated for and overridden any physiological impairments occurring. This may explain the failure to see a significant correlation between total search time and tank pH levels. Our findings indicate a need for further research to incorporate investigations of food concentration on physiological mechanisms of chemoreception in OA conditions.

**Ontogenetic Considerations**

Many crustaceans are known to undergo drastic changes throughout their life cycle. These changes are often apparent through distinct morphological and behavioral characteristics
defined at each life cycle stage that impact how the individual interacts with its environment (Haug, 2020). Many of these observed phenotypic changes are the result of differential gene expression between juvenile and adult stages of marine organisms resulting in differences in resiliency and sensitivity to environmental factors (Frommel et al., 2020). It is of extreme importance that life cycle stage-specific vulnerabilities be considered in ocean acidification research. This is because different ontogenetic stages often respond to the environment differently based on different needs at that specific stage (Frommel et al., 2020). Additionally, organisms exposed to OA conditions early in life may show effects at later life stages, a possibility that has been scarcely investigated thus far and requires more research. (Frommel et al., 2020). The whole picture of how OA impacts ecosystems cannot be understood until life cycle stage differences are unveiled.

Importantly, differences in ontogenetic stages inform how projected ocean pH levels will impact ecological populations, since different stages will show varying sensitivities. For example, the early benthic juvenile stage of many marine invertebrates is a transitional stage towards adult that is characterized by a high sensitivity to abiotic factors such as OA (Gosselin & Qian, 1997; Small et al., 2016; Tankersley & Wieber, 2000), indicating a high likelihood that early benthic juveniles will suffer from increased impairments in acidified environments and therefore exhibit lower fitness than their adult counterparts. Additional documented evidence for ontogenetic implications includes studies on crustaceans demonstrating changes in ionoregulation throughout developmental stages (Brown & Terwilliger, 1992; Spicer, 1995) and evidence that the Dungeness crab (Metacarcinus magister) exhibits differential vulnerability to OA (Berger et al., 2021). Additionally, one study that tested OA conditions on the consumption rates and feeding behavior of juvenile and adult Orchestoidea tuberculata found that juvenile and
adults showed significantly different responses to the OA (Benitez et al., 2016), indicating stage-specific OA behavioral impacts. In this paper’s present study, juvenile *H. americanus* were investigated. If this research is expanded, it may be useful to include adults to investigate differences between the two life stages. Overall, future research is required to fully understand the behavioral implications of ocean acidification particularly the mechanistic basis and stage specific effects.

**Conclusion**

As OA becomes an increasingly common issue across the globe, research efforts have been targeted at unveiling how OA impacts the behaviors of marine organisms and the mechanisms behind impairments so that we can better understand the ways in which the ocean’s ecosystem are being threatened by elevated levels on CO₂. Much of the normal functioning of marine ecosystems relies on food web interactions between predators and prey. Since predator-prey interactions are largely based on behaviors (Clements & Comeau, 2019), the health of marine ecosystems is at risk. Understanding the effects of environmental changes, such as ocean acidification, on the behavior of marine organisms is largely important to understanding the ecology of the globe’s oceans and future implications of these changes.

Assessing the effects of OA on marine organisms has been a relatively recent effort. Of specific interest are the impacts of OA on behaviors mediated by chemoreception since chemoreception plays a vital role in an organism’s ability to interact with its environment. Chemoreception allows for marine organisms to forage, mate, detect predators, find shelter, interact with conspecifics, and navigate their environment (de la Haye et al., 2012; Kim et al.,
2016; Richardson et al., 2021; Wang & Wang, 2020), making chemoreception a crucial skill for
an organism’s overall fitness. Our study investigated the effects of pH on foraging behaviors
related to chemoreceptive functioning. We only found an effect of pH on total search feeding
time, but not for the individual components (search time and handling time). We hypothesize that
organisms experiencing short-term exposures to OA environments exhibit two levels of
behavioral responses. The first is a short-term “pH-shock” response characterized by impaired
odorant detection as a result of odorant and receptor protonation in the environment. A longer-
term mechanism is proposed to involve a reversal of ion influx into the GABA_A receptor, altering
neuronal activity that subsequently changes behavioral responses to the environment. We also
argue that future research should consider factors such as exposure length, food availability, and
ontogenetic implications in future investigations of OA on behavior.

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