The Effects of Ocean Acidification on the Chemosensory Behavior of the Deep-Sea Urchin *Strongylocentrotus fragilis*

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ABSTRACT

Rising atmospheric carbon dioxide (CO2) is a climate changing gas, increasing ocean temperatures and acidic levels in ocean systems. Ocean Acidification (OA) is known to have profound affects on various energy flows within ocean ecosystems, individual species and consequently affecting human society. The oceans play a major role in providing human resources that we use daily. It is important society understands how OA can affect our oceans in different ways, such as the physiology of marine species. The tolerance for OA in deep-sea species is not well known. A short-term analysis explored effects of OA on the deep-sea urchin *Strongylocentrotus fragilis (S. fragilis)*, specifically looking at the chemosensory behavioral response when exposed to food. Three Y-maze treatments using three pH levels, including 7.2, 7.5 and 7.8, and *Macrocsytis pyrifera* (Giant Kelp) as a chemosensory cue were performed on *S. fragilis*. Our null hypothesis predicted *S. fragilis* will not present significant behavioral difference in response to increased acidic conditions. Results revealed the pH levels used to insignificantly affect *S. fragilis* chemosensory behavior.

INTRODUCTION

Rising atmospheric CO2 emissions, caused by recent anthropological activity, is causing a rise in ocean acidification (OA). Ocean acidification is the absorption of atmospheric CO2 into the ocean. OA decreases calcium carbonate (CaCO3) saturation levels and pH in the oceans' chemical composition (Natural Research Council). Ocean CO2 absorption is reaching depths over 1000 m in the Eastern Pacific, significantly affecting marine ecosystems and life histories (Taylor et al., 2013). Analyzing Echinoderms, most commonly referring to urchins and sand dollars, they are keystone ecosystem engineers in all types of marine environments. Studies have shown species to have tolerance to OA. Recent research preformed on shallow-water echinoderms present a physiological compensation for lower pH levels by consumption of bicarbonate from their surroundings (Pane, 2007).

Multicellular marine organisms, like the deep-sea urchin, generally have lower pH levels than seawater. Internal pH is even lower (~0.4 pH units) than their extracellular fluids. They are able to passively regulate a buffering system by secreting or eliminating acid or base through specific organs (Natural, p. 50). Acid-base exchange allows external pH/pCO2 acclimation. However, tradeoffs can occur, such as decreased growth or fitness in their daily activity. Acclimation associates with an organism's metabolic rate. This is the species ability to transport oxygen and CO2 (National. p. 50). Where an organism lives, whether shallow, mid, or deep depths, they will respond differently and accordingly to their biological limitations to OA effects. Effects of OA on the physiology of individual species is important to understand in order to consider the more broad question to how OA will affect ecosystems important to society (Taylor et al., 2013). Society depends on the function and health of deep-sea ecosystems for regulation of fisheries, providing multiple resources and their role in biogeochemical cycles (Taylor et al., 2013).

MATERIALS AND METHODS

Collection of *S. fragilis*

Using the suction sampler along the Ventana remotely operated vehicle (ROV), two collections of *S. fragilis* were collected on June 17, 2013 and July 17, 2013 at a depth of 220 m on the benthic surface in the Monterey Bay Canyon. *S. fragilis* were immediately stored in coolers with seawater during ship transportation back to the seawater lab at the Monterey Bay Aquarium Research Institute (MBARI). Arrival to the seawater lab, *S. fragilis* were transferred and divided into three aquaria set at pH levels of 7.2, 7.5 and 7.8. Using a garden/pooling technique, *S. fragilis* were acclimated to the later pH levels for a minimum of eight days before used for experimentation. *S. fragilis* were allowed to continue to acclimate as experimental trials progressed. The June collection obtained 220 urchins, and the July collection 11 urchins. The collections were kept in separate aquaria and not feed.

Collection of *M. pyrifera*

Collection of kelp was obtained directly from the beach. The kelp was stored in 2.5 gallon buckets with a constant flow of ambient seawater. To ensure freshness and limit prolonged decomposition, fresh kelp was collected weekly prior to the weeks experimental trials.

Y-maze Design

Construction of three Y-mazes implemented three treatments of OA with a constant water flow. A flow of ambient seawater (14 º C and 8.0 º C) was maintained for three set pH levels, including 7.2, 2.5 and 7.8. Seawater was supplied via a seawater system within MBARI campus. Three buckets were filled with equal amounts of kelp (*M. pyrifera*) and supplied with a flow of seawater that included three set pH levels. The buckets were hung above the Y-mazes enabling a flow of water through the left or right arms of the Y-mazes. Kelp was used as a sensory cue. Two inflows of seawater were regulated for each Y-maze. One inflow contained a sense of kelp and the other without. Both inflows contained a set pH level. Determination of which arm the two distinct flows were assigned to, randomization was succeeded via a randomization method using excel.

Using the same excel randomization method, the three pH levels were randomly assigned to each Y-maze for each trial.

Flow rate through the Y-mazes were set at 280-290 mL/min (± 0.2) or 260-270 mL/min (± 0.2). Prior to each trail, the randomized pH flows were allowed a minimum of 5 minutes to flow through the Y-mazes. This method was used to limit contamination from the previous trial.

Behavioral Measurements

Prior to each trial, a flip-test was performed on each urchin. A flip-test involved placing an urchin on its backside and timing how long it took for the urchin to flip right-side up. The longer the flip took, the lower their health condition.

The chemosensory behavior of *S. fragilis* was monitored using over-head cameras. A camera was set-up above each Y- maze, enabling a full view of all three Y-maze bodies. A time-flux of two minutes captured images, as the urchin moved its way throughout the Y-maze. Snapshots were downloaded to a computer for further analysis. Snapshots enabled me to record and track the movement of the urchins within a maximum time response of 120 minutes. A green piece of tape was placed on the mid-way point of each Y-arm to indicate a 'final-line' of choice made by the urchin. The final-line symbolized the 'choice' the urchin made in response to either the kelp, no kelp or were indecisive. Height and width measurements were taken for each urchin to consider data analysis.

RESULTS

The chemosensory behavioral response of *S. fragilis* exposed to three scenarios of OA, using kelp as a chemosensory cue for feeding, revealed insignificant results. A Fisher Exact Test of P=0.89 revealed an insignificant probability (Fig. 1).

Analyzing the response time versus the three pH levels, the average response time between the urchins that chose kelp and no kelp were statistically insignificant (Fig. 2 and 3). Running an Analysis of Variance Test (ANOVA), p-values of 0.128 and 0.150 were calculated for Urchins that responded to kelp or no kelp. These values imply to not reject the null hypothesis; *S. fragilis* will not present significant behavioral differences in response to increased acidic conditions.

However, analyzing results specifically for urchins responding to kelp, a potential trend for higher pH levels to cause a faster response time can be observed (Fig. 2).

A comparison of the two urchin collections in July and June, the total response time versus pH levels for *S. fragilis* revealed pH levels to not have a significant effect on the total urchin response frequencies. However, urchins collected earlier in June (Fig. 5) choice kelp more often than the urchins collected later in July (Fig. 4). These results imply the "old" urchins were hungrier. Since, urchins were not feed once collected these results may be of significance.

Figures:

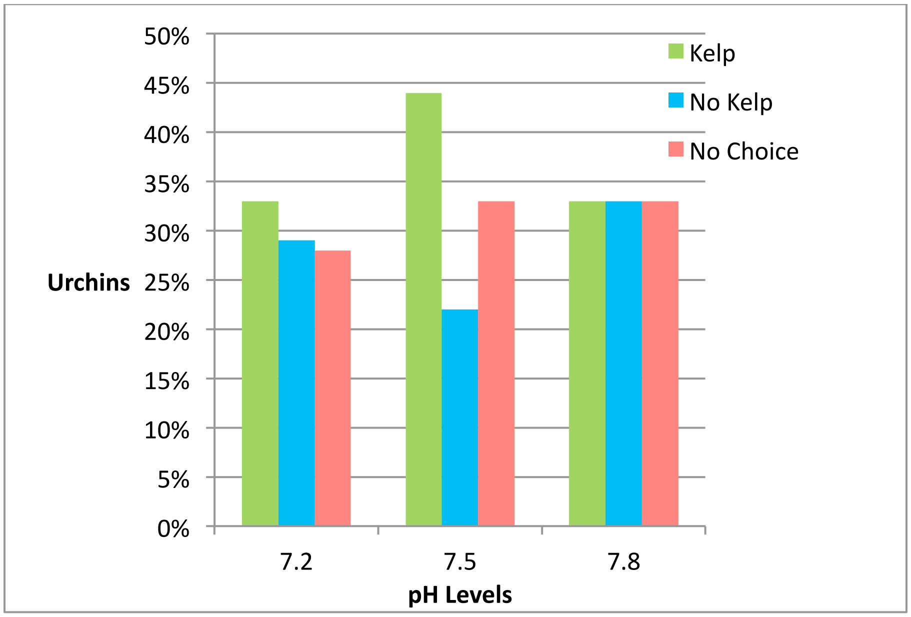

Fig. 1 - No significant pattern_between differing pH levels and Urchin response frequency towards kelp, no kelp or were Indecisive (Fisher Exact Test value: P = 0.89)

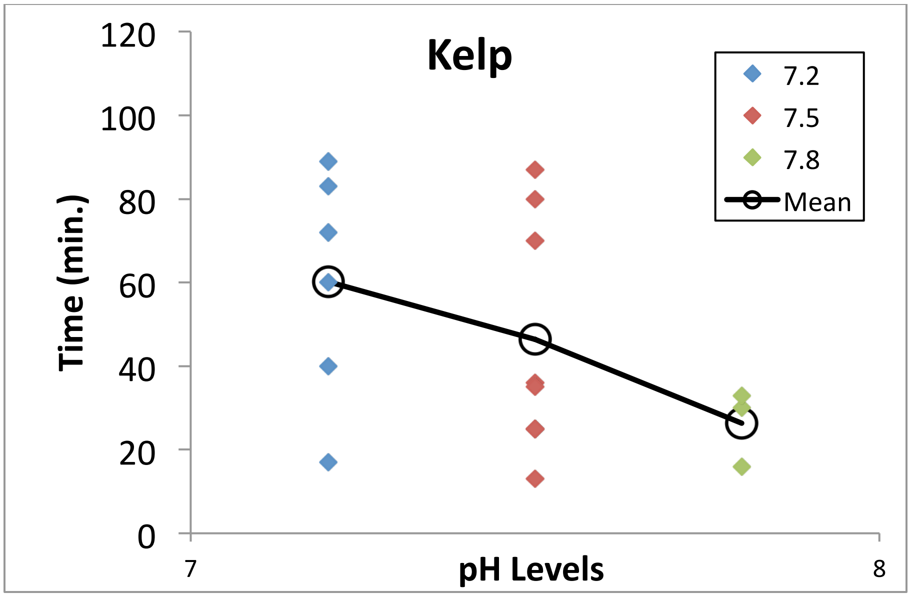

Fig. 2 – *S. fragilis* response time towards kelp when exposed to pH levels of 7.2, 7.5 and 7.8. ANOVA reveals a p-value = 0.218, indicating insignificant behavioral response differences between the pH levels. Green marks represent a pH of 7.8. A potential trend for urchins acclimated to higher pH levels to respond faster can be observed.

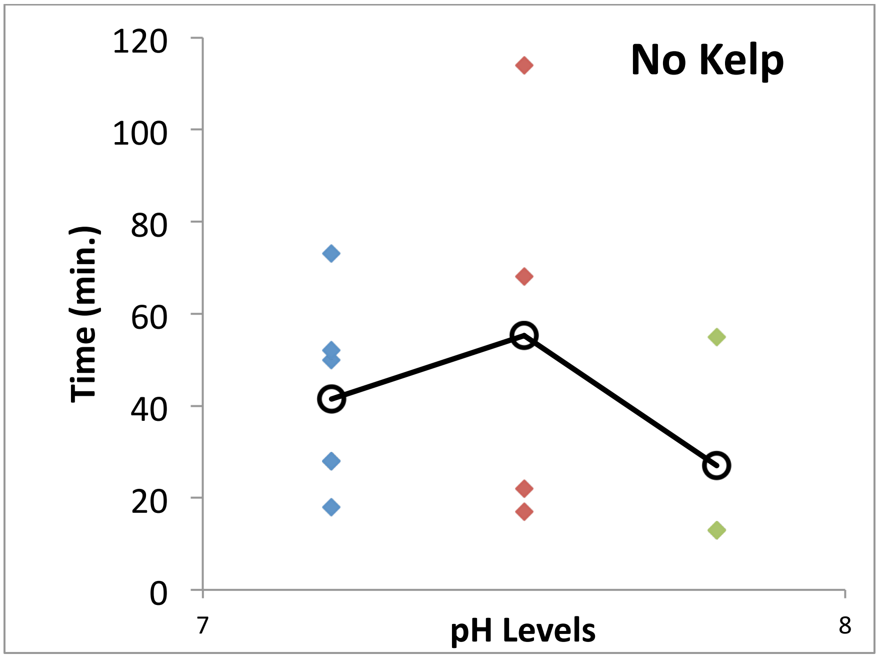

Fig. 3 – S. fragilis response time towards no kelp when exposed to pH levels 7.2, 7.5 and 7.8. ANOVA reveals a p-value = 0.510, indicating insignificant behavioral difference between the pH levels.

"New" June 17, 2013	7.8	7.5	7.2
K	3	8	6
N	3	4	7
U	3	6	5
PA = 0.904, PB = 0.890			

Fig. 4 – Collection of *S. fragilis* on June 17, 2013. Urchins were collected at 220m depth, 8 degrees Celsius.

"Old" July 17, 2013	7.8	7.5	7.2
K	3	5	4
N	1	0	1
U	1	0	0
PA = 0.725, PB = 0.359			

Fig. 5 - Collection of *S. fragilis* on July 17, 2013. Urchins were collected at 220m depth, 8 degrees Celsius.

DISCUSSION

Urchins inhabit a wide variety of environments, ranging from shallow shorelines (0-10m depths) to deep-sea canyons, such as the Monterey Bay Canyon, Deep-sea urchins have slower metabolic rates in relation to depth and pressure; they will acclimate to OA effects differently compared to shallow-water urchins. They are also able to tolerate a wide range of hydrodynamic flows, but limited research has been pursued on the chemosensory response of urchins when subjected to OA effects (Pisut, 2004). OA affects may alter their ability to sense prey, predators, and other foods. It is known deep-sea urchins consume a variety of foods including decomposing organic material, seaweeds, diatoms, forminifera, and shell remnants (Jackson, 1958). Whether deep-sea urchins will be able to sense certain cues, such as food, under certain hypoxic conditions as well, under ambient conditions, is yet to be determined.

Previous Research

With decreased pH levels, bicarbonate is not able to form as easily at deep depths, thus altering habitual patterns of benthic animal activity and compensation. Research performed on *Strongylocentrotus franciscanus,* the shallow-water red sea urchin, Michael O'Donnell was able to analyze a reduced physiological response when subjected to elevated CO2 conditions and increased temperature (O'Donnell, 2009). The chemosensory behavior towards food and the ability to sense with increased OA effects were analyzed within this experiment. Chemosensory guidance is often dependent on fluid flow. Low water flow causes chemicals within a system to more likely spread diffusively, thus causing chemical concentration to decline when approaching an organism (Pisut, 2004). Marine organisms are able to detect odors at a magnitude dependent on their movement (speed). Slow moving organisms, like urchins, may sense information at a lower rate, where faster moving organisms, like crabs, sense odor more erratically (Pisut, 2004). Sea urchin species are unique for their radial symmetrical shape. Their tube feet, likely acting as chemoreceptors, cover their entire exterior test. Their unique chemosensory functions may allow them to detect odor at certain concentration profiles within a flume (Pisut, 2004).

Although limited knowledge exists for an urchin's chemosensory response towards food, there have been multiple studies performed analyzing an urchin's response towards predatory cues. The tropical sea urchin *Diadema antillarum*, the long-spine black sea urchin, showed an avoidance response to crushed con- and heterospecifics, involving moving away from the odor of the crushed source (Pisut, 2004). This study supports the potential for chemosensory response by *S. fragilis*, when stimulated by a food kelp-sensed gradient flow.

CONCLUSIONS/RECOMMENDATIONS

The results for this short-term experiment testing effects of OA on the chemosensory behavior of the deep-sea urchin, *S. fragilis*, concluded further research needs to be performed for significant results to occur. The behavioral responses that were collected through the experiment signifies the July collection for *S. fragilis* were may have been influenced by multiple factors. Factors in include the urchins were simply not hungry, had not been given enough time to acclimate to their pH levels , thus causing abnormal responses, as well variable temperature and/or flow fluctuations within the seawater lab system at MBARI. Further research and trials on this experiment would effectively help support or reject the null hypothesis, that OA pH levels will not have any significant effect on the chemosensory behavior of the deep-sea urchin, *S. fragilis.*

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