Effects of ocean acidification on growth rate, calcified tissue, and behavior of the juvenile ochre sea star, *Pisaster ochraceus.*

by
Melissa Linn Britsch

A THESIS

submitted to

Oregon State University

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in partial fulfillment of
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degree of

Honors Baccalaureate of Science in Biology
(Honors Scholar)

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Melissa Linn Britsch for the degree of Honors Baccalaureate of Science in Biology presented on April 26, 2017. Title: Effects of ocean acidification on growth rate, calcified tissue, and behavior of the juvenile ochre sea star, *Pisaster ochraceus.*

Abstract approved:_____

Bruce Menge

Anthropogenically-induced increases in the acidity of the ocean have the potential to seriously harm marine calcifying organisms by decreasing the availability of carbonate (CO_3^{2-}) used to make shells. I tested the effects of lowered pH on juvenile *Pisaster ochraceus,* an intertidal sea star and keystone predator in the eastern Pacific Ocean. Populations of *P. ochraceus* were greatly reduced by outbreaks of sea star wasting disease, which has the potential to alter community structure and lower biodiversity in the intertidal region. However, large numbers of juvenile *P. ochraceus* have recruited to the rocky intertidal and their ability to persist will be important for the recovery of *P. ochraceus* populations. To test the effects of pH, I studied the growth rate, calcification, righting time, and movement and prey-sensing ability in the PISCO laboratory mesocosm at Hatfield Marine Science Center. The results of the experiments showed non-significant trends towards a negative effect of pH on growth rate and righting time. Few studies have been done on the effects of pH on sea stars and the results are highly species-specific. Additional research is needed clarify and make accurate predictions about the effects of pH on juvenile *P. ochraceus.*

Key Words: ocean acidification, *Pisaster ochraceus,* calcification, behavior, physiology

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

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Melissa Britsch, Author

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Introduction

Increasing anthropogenically-generated CO_2 in the atmosphere is a major driver of global climate change, with effects including higher average air and surface water temperatures and increased acidification of the ocean (Kleypas et al. 1999, IPCC 2014). The ocean absorbs about one third of the added CO_2, which undergoes a series of chemical reactions that change ocean water chemistry in a process known as ocean acidification (OA). In the past 250 years, OA has resulted in a decrease in ocean pH from approximately 8.2 to 8.1 pH units, and it is predicted to decrease an additional 0.7 pH units by 2300 (Zeebe 2012). This might not seem like a large change, but pH is expressed on a logarithmic scale and a 0.1 unit change is equivalent to a ten-fold change in pH, and this has occurred incredibly quickly relative to the historical rate of change of the environment (Zeebe 2012).

The chemical reactions involved in ocean acidification are as follows (aqueous forms of chemical compounds dissolved in water are represented by (aq)):

$$CO_2(g) + H_2O\ (l) \leftrightarrow H_2CO_3\ (aq) \leftrightarrow\ HCO_3^-\ (aq) + H^+(aq) \leftrightarrow CO_3^{2-}\ (aq) + 2H^+\ (aq)$$

Carbon dioxide is in dynamic equilibrium with carbonic acid (H_2CO_3), bicarbonate ion (HCO_3^-), and carbonate ion (CO_3^{2-}). The species that are dissolved in water are denoted as (aq). Due to the effects of Le Chatelier's principle, if the concentration of one species changes then the concentrations of the other species must change as well to maintain

equilibrium (Torres 2007). Adding CO_2 to the ocean increases the concentration of H_2CO_3, which dissociates to form H^+ and HCO_3^- and drives the above reaction to the right. Some of the additional H^+ ions react with CO_3^{2-} ions in the ocean and form HCO_3^-, with a net result of that the concentration of HCO_3^- increases the concentration of CO_3^{2-} decreases. If atmospheric CO_2 concentrations exceed 840 ppm, which is possible by the end of this century (Feely et al. 2008), the concentration of HCO_3^- is predicted to increase approximately 15% and the concentration of CO_3^{2-} is predicted to decrease by approximately 48% from pre-industrial concentrations (Fabry et al. 2008).

OA presents a serious problem to marine organisms, especially those that have skeletal structures made up of calcium carbonate ($CaCO_3$) because the amount of the carbonate ion (CO_3^{2-}) available in the water for use building skeletons decreases as the ocean becomes more acidic (Fabry et al. 2008). The availability of Ca^{2+} and CO_3^{2-}, known as the saturation state and symbolized as Ω, varies with water depth and ocean conditions and is changing as the ocean acidifies (Feely et al. 2008). One example of high, naturally varying ocean conditions is in upwelling-dominated ecosystems. Upwelling brings water with lower pH and lower than average $CaCO_3$ concentrations to the surface. The Pacific Ocean along the west coast of the United States experiences upwelling during the summer and this process is predicted to become stronger as levels of CO_2 in the atmosphere increase (Bakun 1990, Bakun et al. 2010, Iles et al. 2012, Wang et al. 2016). Organisms living in these environments are often well-adapted to pH fluctuations (Appelhans et al. 2014), but such fluctuations will be amplified by climate change. Hence, coastal ecosystems may be affected by OA sooner than other ecosystems (Thomsen and Melzner 2010, Gruber et al. 2012).

Recent research has shown that marine organisms are experiencing reduced calcification, elevated levels of stress, and reduced growth rates due to OA (Manzello 2012, Kroeker et al. 2013, Appelhans et al. 2014, Keppel et al. 2015, Jellison et al. 2016). However, OA affects more than the calcification processes. Organisms have to spend additional energy to maintain cellular homeostasis in the presence of increased H^+ ions, resulting in changes in energy allocation, growth, shell formation, and reproduction (Thomsen and Melzner, 2010, Keppel et al. 2015). Some marine organisms also show behavioral changes, such as disrupted prey-avoidance behavior in the black turban snail, *Tegula funebralis* (Jellison et al. 2016), and disrupted olfactory sensing and reduced settlement of the larval clownfish *Amphiprion percula* (Munday et al. 2009). The number of studies on OA has increased dramatically in recent years, and a meta-analysis (Kroeker et al. 2013) found trends of negative effects from OA across many marine taxa that are both calcifiers and non-calcifiers. Although there is wide variability in response to OA among species, mobile animals seem to be better protected than sessile ones.

These studies show that responses to OA can be highly variable and may be species-specific, complicating efforts to predict the effects of OA. This is exemplified by echinoderms, a diverse group of marine organisms that often have planktonic larvae and benthic adults (Dupont et al. 2010). Sea urchins and brittle stars (ophiuroids) have been studied extensively (Dubois 2014) but fewer studies have been done on sea stars (Table 1). The planktonic larvae of sea stars are often non-calcifying and do not resemble juvenile and adult sea stars until they settle onto benthic substrates and metamorphose into their adult body plan, which contains calcified structures called ossicles. The different morphology, physiology, and ecology at different life history stages suggests

that the response of sea stars to acidification may vary greatly. Recent studies indicate that sea star larvae tend to respond negatively to OA, although increased temperatures may be more damaging (Dupont et al. 2010, Nguyen et al. 2012, Kamya et al. 2014). Few studies have been done on adult sea stars and the effects of OA on these life stages are not clear at this time.

OA is only one of the stressors that marine organisms have to cope with as the oceans change. Another serious concern is the increase in sea surface temperatures, which will continue to rise as atmospheric concentrations of CO_2 increase (IPCC 2014). Numerous studies (Gooding et al. 2009, Nguyen et al. 2012, Kamya et al. 2014, Keppel et al. 2015) tested the effects of temperature on sea stars. All except Gooding et al. (2009) found temperature to negatively affect growth rates, especially when it interacts with the effects of pH (Table 1).

This study examines the effects of OA on the growth, calcification, and behavior of the ochre sea star, *Pisaster ochraceus. Pisaster ochraceus* is a keystone predator that is essential for maintaining species diversity in the intertidal zone of the Pacific Northwest coast (Paine 1966, Paine 1974). Keystone species have disproportionately large impacts on ecosystems relative to their total biomass (Power, et al. 1996, Menge et al. 2013). In 2014, populations of *P. ochraceus* were greatly reduced due to the effects of sea star wasting disease (SSWD; Menge et al. 2016). The loss of *P. ochraceus* is predicted to cause changes in the community structure of the low intertidal, including increased mussel density and biomass and reduced species diversity (Paine 1966, Menge et al. 2016). One interesting observation is that the loss of adult sea stars due to SSWD was followed by an increase in *P. ochraceus* recruits resulting in a change in the size structure

of the population. Menge et al. (2016) hypothesized that this was due to decreased competition with adults rather than increased settlement of recruits, but the high number of surviving recruits provides an excellent research opportunity.

While it is generally hypothesized that all marine calcifying organisms will show negative responses to OA, two recent papers (Keppel et al. 2015, Gooding et al. 2009) studying the effects of OA and temperature on adult sea stars showed conflicting results. Gooding et al. (2009) found decreased calcification in *P. ochraceus* only at low temperatures but increased growth rates at low pH and high temperatures, while Keppel et al. (2015) found decreased calcification and growth in *Asterias rubens* at low pH levels and at increased temperatures (Table 1). Additionally, Appelhans et al. (2014) showed that the righting time of sea stars, which can be used to estimate their ability to re-attach to rocks after being disrupted, was not affected by lowered pH. However, all these studies investigated adult life stages. More studies are needed to determine the effects of OA on sea stars at juvenile life history stages.

I tested the effects of pH on juvenile *P. ochraceus* because sea stars exposed to high-stress environments could show behavioral and physiological changes that have both direct and indirect effects on the intertidal ecosystem. I hypothesized that with decreasing pH, in juvenile *P. ochraceus* 1) growth and calcification will decrease, 2) the righting time will increase, and 3) the rate and direction of movement will slow and become less random. Because the survival of the *P. ochraceus* juveniles will be very important for the recovery of the species after SSWD, my results will inform the potential for recovery and in turn how SSWD might impact structure and biodiversity of the low rocky intertidal community.

Materials and methods

Experimental Design

One method to experimentally determine the effects of OA on marine organisms employs a mesocosm, a system that allows researchers to manipulate specific aspects of the environment in a controlled manner (here, the pH of sea water). The PISCO-Menge Lab at Oregon State University maintains an OA mesocosm at Hatfield Marine Science Center (HMSC). It has flow-through tanks that use water from Yaquina Bay, near Newport, Oregon. The water from Yaquina Bay experiences fluctuations in temperature and salinity (Kentula and DeWitt, 2003), which provides a realistic setting for the laboratory experiments. Relative to field settings, mesocosm experiments allow researchers greater control by enabling isolation and testing of specific variables.

The experiment was performed in the OA mesocosm at HMSC from May 8-23, 2016, using juvenile (3-7g) *Pisaster ochraceus* collected from Yachats Beach, Oregon, where personal observations have shown *P. ochraceus* to be abundant. Juveniles are useful study organisms because they haven't started producing gametes, which removes the confounding effect of energy allocation to reproduction. The sea stars were collected on April 11, 2016, and allowed to acclimate to the sea water tables at HMSC while being fed small mussels (*Mytilus* spp.) for 28 days until the start of the experiment. They were individually housed in 1 pint containers with mesh-covered holes on two sides to allow water to flow through the cages (Fig. 1). The sea stars were randomly assigned to each container and pH treatment and were placed in one of two pH treatments, 30 per treatment for a total of 60 sea stars.

The two pH treatments were low pH (7.57 ± 0.11) and ambient pH (7.91 ± 0.16), one per sea water table. The low pH represented projected pH values by the end of the century (Fabry et al. 2008) while the ambient treatment had a similar pH to the ocean near Newport, Oregon in the spring. Both treatments were maintained at elevated temperatures (14°C) by paddle aquarium heaters. Incoming water was supplied from the upper water tables in the mesocosm where Ph. D. student Jenna Sullivan was running a simultaneous experiment on the effects of temperature and pH on *P. ochraceus* recruits. Sea stars were kept underwater near the inflow from the upper table to ensure they got fresh seawater by placing plastic plates over groups of four or five containers to weigh them down. The original average mass of sea stars in the low pH treatment (3.55g ± 1.50g) did not differ from the ambient pH treatment (3.60g ± 1.35g) (Welch's two sample t-test, p= 0.8924).

Water Parameters

The pH, temperature, and salinity of the water were checked every four hours using a YSI probe. pH was adjusted by changing the amount of CO_2 flowing through the mass flow controller of the mesocosm. The inflow water was held in a storage tank at the head of each water table and chilled, and CO_2 was added to the water through a Venturi injector as it flowed out of the holding tank to adjust the individual pH treatments. We also took bottle samples of the water in Ms. Sullivan's experiment once per day to be analyzed for pH and alkalinity later in the laboratory. My experiment used the same water so I used those data for my analysis. The pH from the bottle samples was measured using a SAMI ocean pH sensor and the alkalinity was measured using titration and analysis with an Ocean Optics DH-2000 spectrophotometer.

Calcified Mass

To measure calcification, 14 sea stars from the low pH treatment and 16 from the high pH treatment were randomly selected at the end of the experiment and sacrificed to measure calcification. The sea stars were placed in individual plastic bags and frozen. They were later removed from the freezer and placed into an 80 °C oven and dried to a constant weight. After they were dry, the sea stars were weighed, and then heated to 500 °C in a muffle furnace. They were left for five hours after the oven reached temperature in order to burn off the organic tissue. They were re-weighed after cooling to determine the weight of calcified tissue and the ratio of calcified to organic material.

Behavior: Righting time, movement, and prey detection

Sixty sea stars were used for these experiments: 30 from the low pH treatment and 30 from the ambient pH treatment. Sea stars were checked daily for signs of disease and dead individuals were removed. Live sea stars were blotted dry on a paper towel and weighed on the first and last days of the experiment (i.e. day 1 and day 16). Righting time was measured by placing each sea star onto its dorsal side and recording the time in seconds required for the sea star to right itself and begin to move away from the flipping site. The ability of sea stars to detect prey was measured by placing the sea stars in a square 10 L container over the center of a paper with 10 concentric 1 cm circles drawn onto it (Figs. 2, 3). This experiment was done twice, once with 10-15 small mussels in a corner of the container and once with no mussels, to control for the effect of prey in the water and to quantify the difference in movement if prey were present. Timing was started when the sea star began to move and the time when the junction of the arm with the central disc leading edge of the sea star crossed into a new circle was recorded.

Movement within each 1 cm circle was recorded as outward or diagonal/changing direction. Outward velocity was estimated as cm/s. The percent time spent moving in a straight line versus diagonal/changing directions, the average velocity when moving straight, and the escape time, or the total time necessary to leave the outermost circle on the ring, were all analyzed to determine if prey were detected and new space explored. Estimates of the rate of movement in cm/s were based only on movements perpendicular to the 1 cm circles.

Data Analysis

Data analysis was done using the R statistical program (version 3.2.0) and the ggplot2 graphing package. Six sea stars died during the experiment and were removed from the analysis. A Welch's t-test was used to determine initial mass differences between the treatments and a two sample t-test assuming equal variance was used to analyze the change in mass, the difference in righting time, and the difference in dry mass to calcified mass. Movement and prey detection was tested using three one-way ANOVA tests and the explanatory variables were the pH treatment and the presence/absence of food. The dependent variables were the percent time moving in a straight line, the average velocity in a straight line, and the escape time. One sea star had an extremely long righting time and another had an abnormally large change in growth. These were considered outliers and were removed from the analysis because they had a substantial effect on the results of the t-tests. Only 40 sea stars that were completely healthy at the end of the experiment were tested in the prey-detection and movement experiments.

Results

Mass

The average change in mass of the low pH treatment was smaller (0.13 g ± 0.15g) than the average change in mass of the ambient pH (0.18 g ± 0.16g), but the magnitude of the differences in these changes was not statistically significant (two sample t-test, $p = 0.23$, Fig. 4).

Righting time

Although righting time was slower in the low pH treatment, the average righting time of the sea stars did not differ between the low pH (233.7s ± 129.3s) and ambient pH (181.8 s ± 116.2s) treatments (two sample t-test, $p=0.1317$, Fig. 5). As indicated by the large standard errors, high variability in the data obscured any potential meaningful difference in righting speed.

Calcified Weight

There was no evidence for a difference between the ratio of dry mass to calcified mass between the low pH (1.425g ± 0.0352g) and the ambient pH (1.430g ± 0.0320g) (two sample t-test, $p = 0.6779$, Fig. 6). This is not surprising, given the short (2 week) span of the experiment.

Movement and Prey Detection

Analysis of the percent time moving straight showed that the presence/absence of prey had a significant effect on the movement of the sea stars ($p < 0.01$, Fig. 7), while the pH treatment and the interaction between prey and pH treatment were not significant. The analysis of the average velocity in a straight line did not reveal an effect of either food or

pH ($p > 0.2$, Fig. 8) on the velocity of the sea stars. The escape time of sea stars in the presence of food, however, was longer (269.83 ± 113.90 seconds) then the escape time of sea stars without food present (221.58 ± 81.50 seconds), ($p = 0.0302$, Fig. 9). The pH treatment and the interaction between food and pH were not significant predictors of change in the behavior of the sea stars (Table 2).

Discussion

The results of this experiment show a weak negative effect of lowered pH on the growth rate and righting time of *P. ochraceus* after 16 days of exposure, and no effect on calcification or direction of movement. The slight difference in righting time between the experimental treatments suggests that sea stars exposed to stressful conditions may experience changes in behavior, but larger sample sizes will be necessary to determine clear trends. The prey detection and movement experiments show that sea stars spend more time meandering and moving in random directions in the presence of prey compared to sea stars not exposed to prey, although the rate of movement remains the same. These results suggest that potentially, the sea stars sensed their prey and moving randomly in an attempt to find it. pH had no effect on the direction of movement or the average velocity of sea stars, which demonstrating that while they modified their behavior in response to prey, stressful OA conditions did not change this response.

Several confounding factors could be implicated in the low statistical significance of this study. The sea stars were not fed after the start of the experiment in order to prepare them for the prey detection tests because their response to the presence of prey might be weaker if they were full, masking the effect of food on their behavior. Starving the sea stars could have reduced their soft tissue growth and the rate of calcification because the sea stars would have devoted energy to maintaining basic metabolism instead of growth. *P. ochraceus* has a high thermal tolerance (Gooding et al. 2009) and the average temperature of this experiment (14 °C) might not have been high enough to cause stress. Additionally, the two week duration of the experiment may not have been long enough for changes in growth, calcification, and behavior to become evident. Many

experiments on the effects of pH and growth of juvenile and adult sea stars were much longer [e.g. 10 weeks for Keppel et al. (2015) and 6 months for Appelhans et al. (2014)]. Long experiments give organisms time to grow and to experience the physiological effects of ocean acidification and long-term stress, but shorter experiments can provide insights as well, particularly for intertidal organisms such as *P. ochraceus* that experience large, daily fluctuations in environmental conditions.

P. ochraceus may have an advantage for coping with OA because it is adapted to the environmentally stressful conditions of the rocky intertidal, but stronger upwelling events are predicted with climate change (Bakun 1990, Bakun et al. 2010, Wang et al. 2016). Upwelling brings cool, acidic water to the surface of the ocean, thereby lowering the pH and the sea surface temperature for short periods of time (Iles et al. 2012). While cooler water temperatures may relieve the temperature stress on organisms, it is unclear if the effect will remediate the negative stress caused by low pH. A short-term pH experiment might not capture long-term physiological adaptations to stressful conditions, but it is valuable to provide insight on the effects of OA on intertidal organisms such as *P. ochraceus,* which experiences frequent changes in environmental conditions. Unfortunately, the highly species-specific results of these studies makes it challenging to use *P. ochraceus* as a model organism to predict the effects of OA on sea stars beyond the intertidal.

Although I didn't find a difference in the calcification of the sea stars in my experiment, Gooding et al. (2009), Appelhans et al. (2012), and Keppel et al. (2015), thought that sea stars experienced changes in their metabolic energy budgets in response to the effects of OA, which affected growth and calcification. This may have happened in

my experiment but the sample sizes were not large enough to capture it. However, the sea stars in each of the previously published experiments responded differently to OA. *P. ochraceus* experienced lowered calcification but an increase in total wet tissue mass (Gooding et al. 2009), while *A. rubens* maintained similar rates of calcification when exposed to high-stress conditions, but it produced less wet tissue mass (Appelhans et al. 2012, Keppel et al. 2015). Unfortunately, these studies used different experimental methods to determine the ratio of dry tissue to calcified tissue, which may have affected the final results. Appelhans, et al. (2012) dried the sea stars and then burned away the organic material in a muffle furnace, using similar methods to my experiment, while Keppel et al. (2015) and Gooding et al. (2009) dried the sea stars and obtained the calcified tissue by soaking the sea stars in bleach until the organic tissue was removed, filtering the calcified tissue from the solution, and then re-drying the calcified tissue. The variation in results demonstrates the need for more studies to obtain a clear understanding of the effects of OA on calcification in sea stars.

The previous researchers suggested that the respective sea stars studied had different physiological strategies in responding to OA and temperature. Gooding et al (2009) thought that *P. ochraceus* took advantage of warmer water temperatures to increase growth in the short term, while Appelhans et al. (2012) and Keppel et al. (2015) thought that *A. rubens* maintained calcification in the stressful conditions but consequently did not have additional energy to devote to soft tissue growth. These researchers attributed some of the observed differences to the species and the habitats to which they are adapted. Intertidal ecosystems are frequently exposed to air and can experience large changes in temperature, especially during daytime summer low tides,

while subtidal systems are constantly submerged and have relatively stable environmental conditions.

Other marine organisms also experience metabolic changes due to OA. Stumpp et al. (2012) found that larval green sea urchins (*Strongylocentrotus droebachiensis*) maintain calcification rates at the expense of lowered total body mass, and Munday et al. (2009) noticed changes in sensing ability of the larval clownfish *Amphiprion percula.* Adjusting metabolic energy budgets to cope with stress may help calcifying organisms in the short-term, but it is not clear if they will be able to adapt to OA in the long-term to compensate for the rapid changes that are occurring in the ocean.

OA also affects sea stars during their larval life stage. Dupont et al. (2010) tested the effect of pH on the larval development of the sea star *Crossaster papposus* and found that pH had a positive effect on larval growth rates. Gonzalez-Bernat et al. (2013) tested the effect of pH on larvae of *Odontaster validus,* and found negative effects of reduced pH, although in several studies increased temperature had a strong negative effect that may have been more damaging than low pH (Kamya et al. 2014, Nguyen et al. 2012). This demonstrates the importance of multiple-factor experiments to understand complex environmental variation.

Several studies (e.g. Gooding et al. 2009, Nguyen et al. 2012, Byrne et al. 2013, Kamya et al. 2014) have tested multiple, interactive effects in their OA experiments. They often found that the effects resulted from interactions between pH and temperature, although the strength of the interactions could vary. My experiment was limited to a single environmental factor, which may allow a better understanding of the specific

reactions of sea stars to lowered pH levels. Further studies should examine pH variations in conjunction with other environmental factors such as temperature and food. This will help to capture potential synergistic or antagonistic physiological responses to stressful conditions. While there is still variability in the results of studies on the effects of OA, additional multiple factor experiments accounting for temperature and pH will help us understand the complex changes occurring in the ocean (Kroeker et al. 2013).

I reviewed all literature testing the effects of low pH, increased temperature, or a combination of both factors on sea star predation rate, growth, calcification, survival, and righting time. Only eleven studies are published and the first took place in 2009. Several studies focused exclusively on larval development while others focused on adult growth and behavior, and they varied in length from less than a week to over 9 months long. The results were highly variable and seem to change between life stage and species, although there was a general trend towards negative effects of OA. My experiment was only the third to test the physiology of juvenile sea stars and the high variation in results from these studies makes it difficult to accurately predict the effects of OA on sea stars at any life stage.

My study helps clarify the response of *P. ochraceus* to anthropogenically-induced changes in the ocean, and demonstrates areas where more research is needed to understand the effects of OA on juvenile sea stars. The ocean is changing extremely rapidly (Zeebe 2012) and while organisms like *P. ochraceus* may be able to acclimatize in the short-term, the effects of rapid OA may occur too quickly for species to adapt. Additionally, individuals interact with many other organisms within their species and with organisms in other species and these interactions are very important to ecosystems.

Changes at the species level could cause changes in species interactions which could have cascading effects on the entire ecosystem.

More research is needed to clarify the effects of OA on marine organisms so that accurate predictions can be made at the levels of species and larger taxonomic groups. It is especially important to determine the effects of OA on *P. ochraceus* because of the large reduction in the population size due to recent outbreaks of sea star wasting disease. *P. ochraceus* is an important intertidal predator and its growth and survival could have cascading effects on entire ecosystems. Removal of *P. ochraceus* is predicted to cause an increase in mussel bed size and a net loss of biodiversity in the intertidal (Paine 1966). However, recent observations suggest that these effects vary by site (S. Gravem, personal communication). Even if adult *P. ochraceus* can adapt to the warmer temperatures and increased acidity that are predicted for future ocean conditions, if larval and juvenile *P. ochraceus* cannot adapt then the species may have difficulty recovering from SSWD. The long-term effects of SSWD on intertidal ecosystems are not yet clear, but changes in the ocean due to climate change could impact the recovery of *P. ochraceus* and that will affect the community structure of the entire ecosystem.

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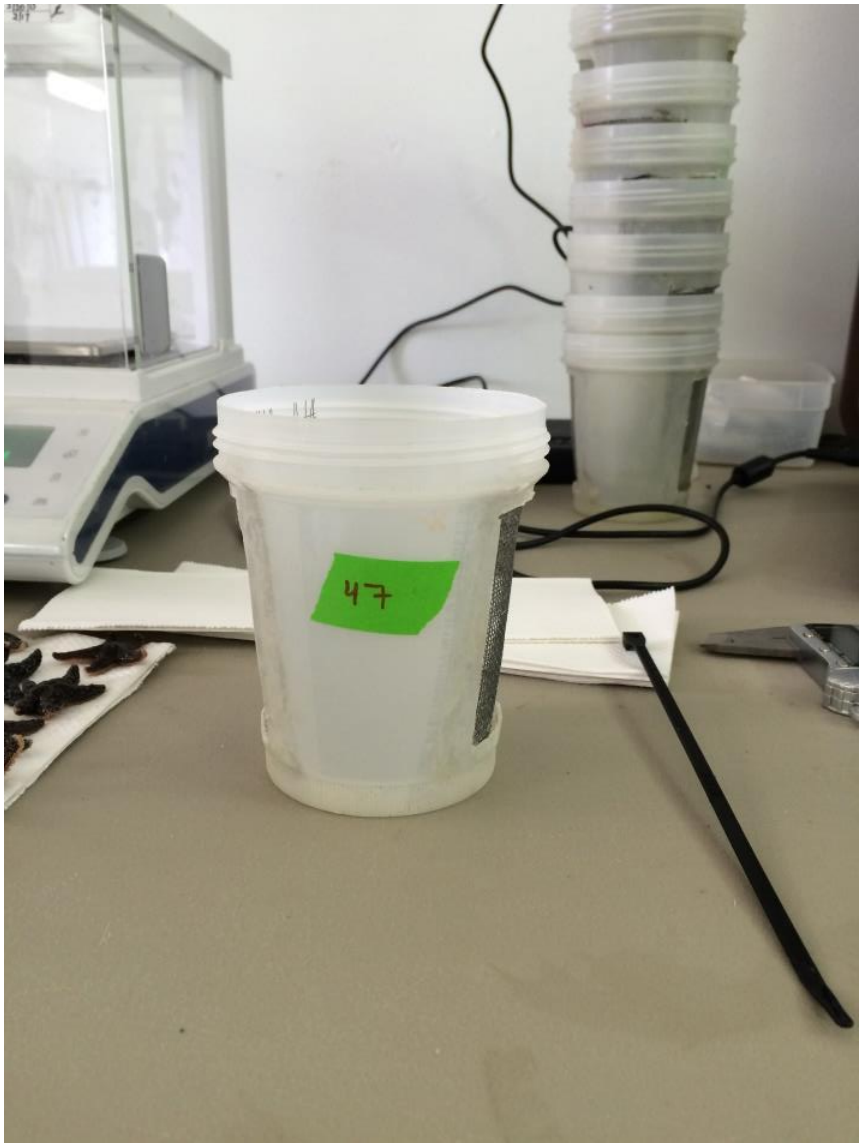

Figure 1: labeled 1-pint container for a sea star, with mesh on two sides to allow water flow.

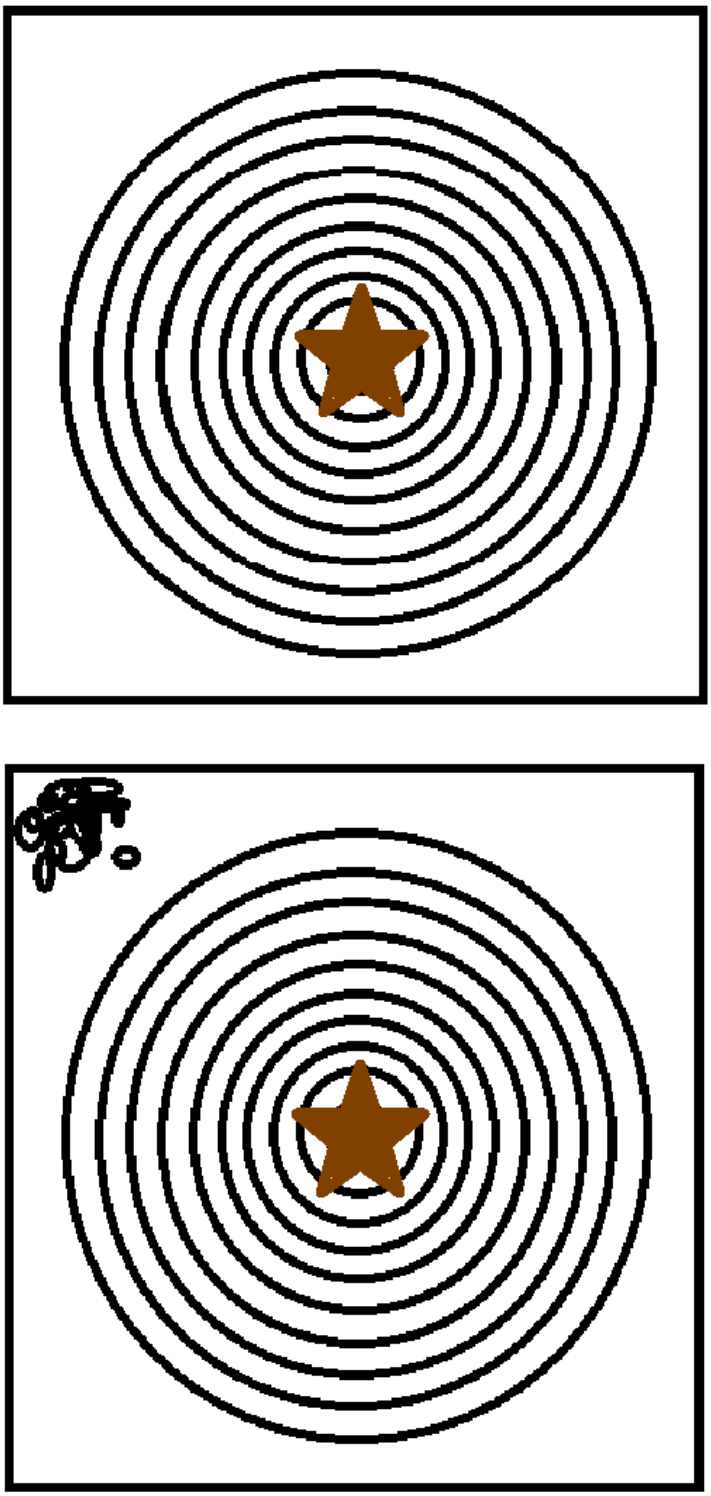

Figure 2: diagram of sea star in prey-finding experiment without (top) or with prey present (bottom).

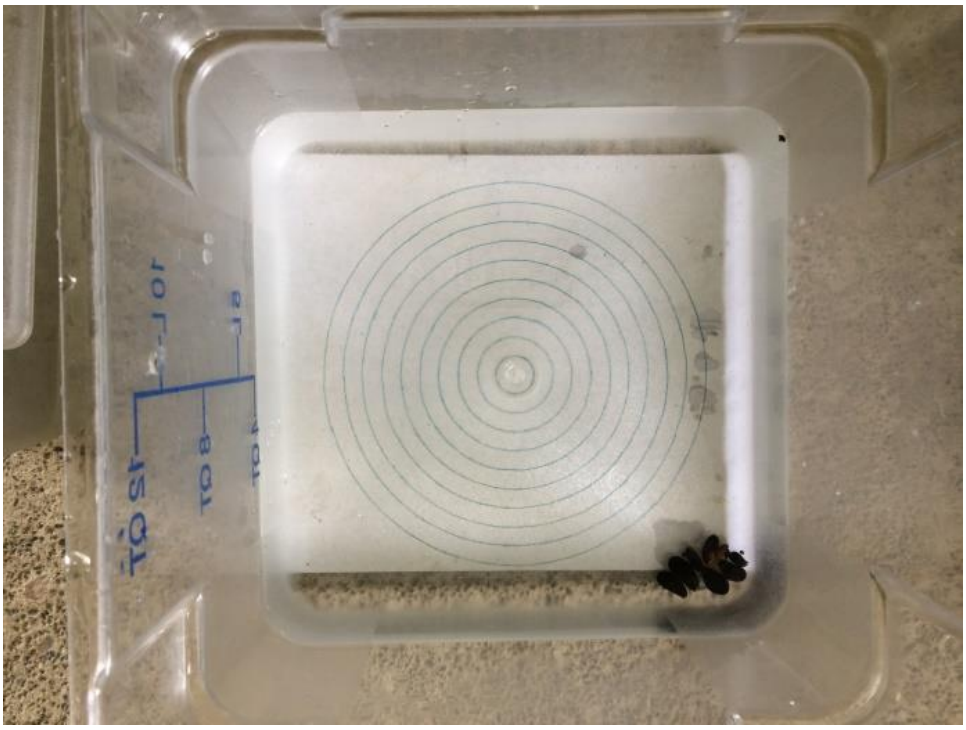

Figure 3: image of prey-finding experiment with prey (*Mytilus* spp.) present in lower right corner.

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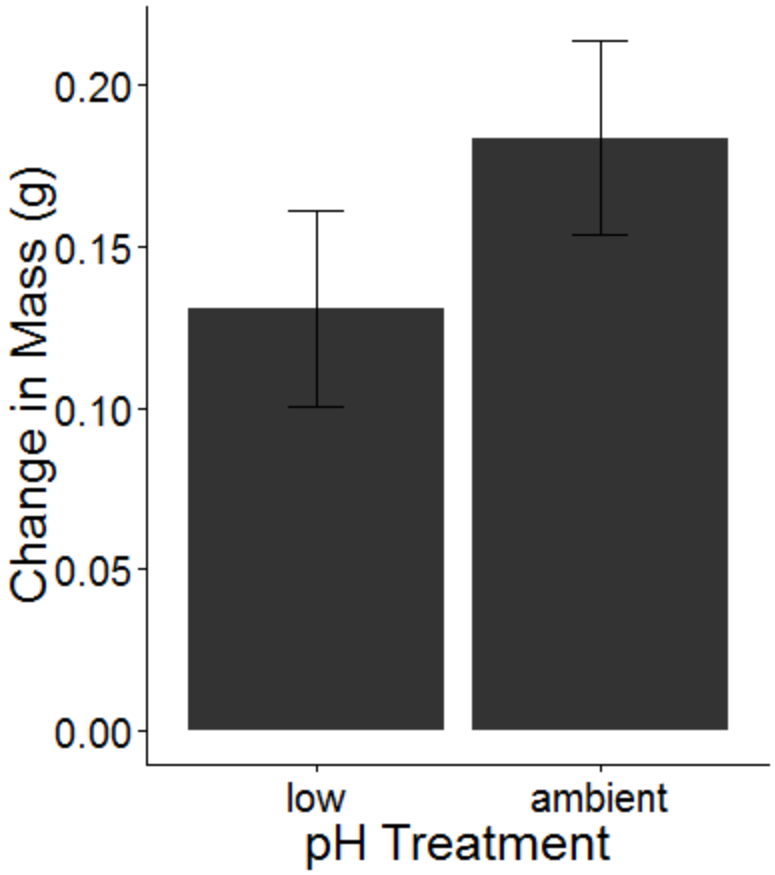

Figure 4: Change in mass between low pH (7.57 ± 0.11) and ambient pH (7.92 ± 0.16) treatments, with standard error bars.

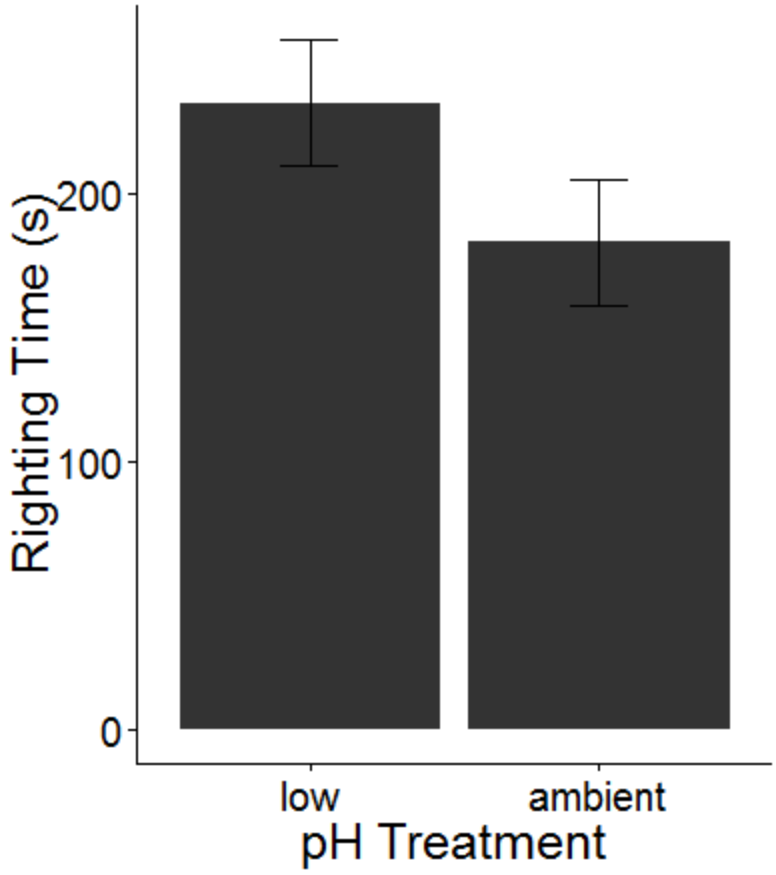

Figure 5: Difference in average righting time in seconds between low pH (7.57 ± 0.11) and ambient pH (7.92 ± 0.16) treatments,with standard error bars.

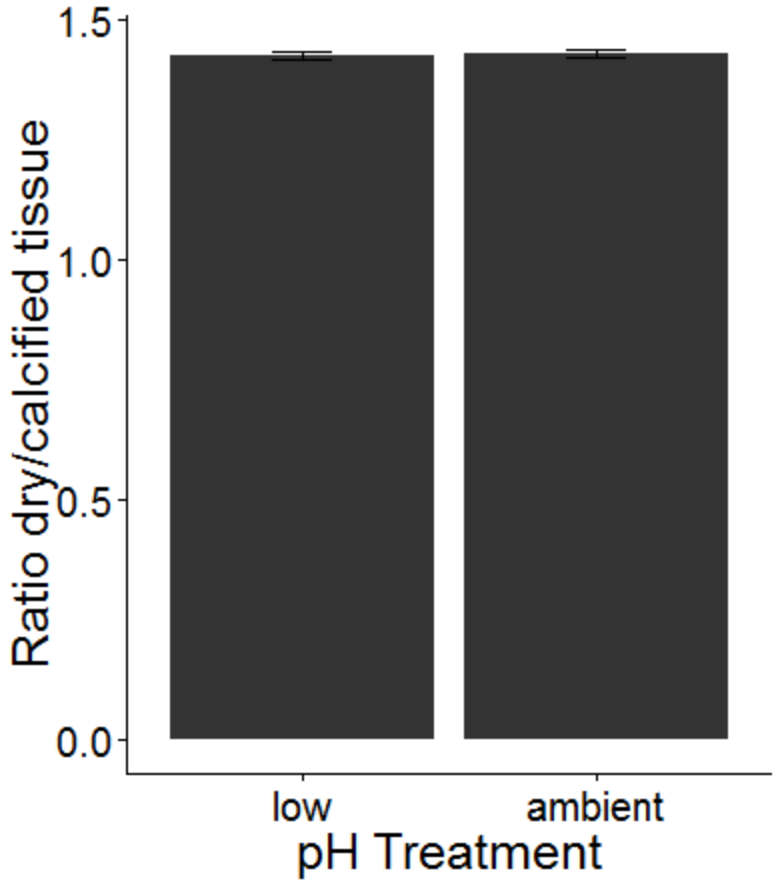

Figure 6: Difference in average ratio of dry/calcified tissue between low pH (7.57 ± 0.11) and ambient pH (7.92 ± 0.16) treatments, with standard error bars.

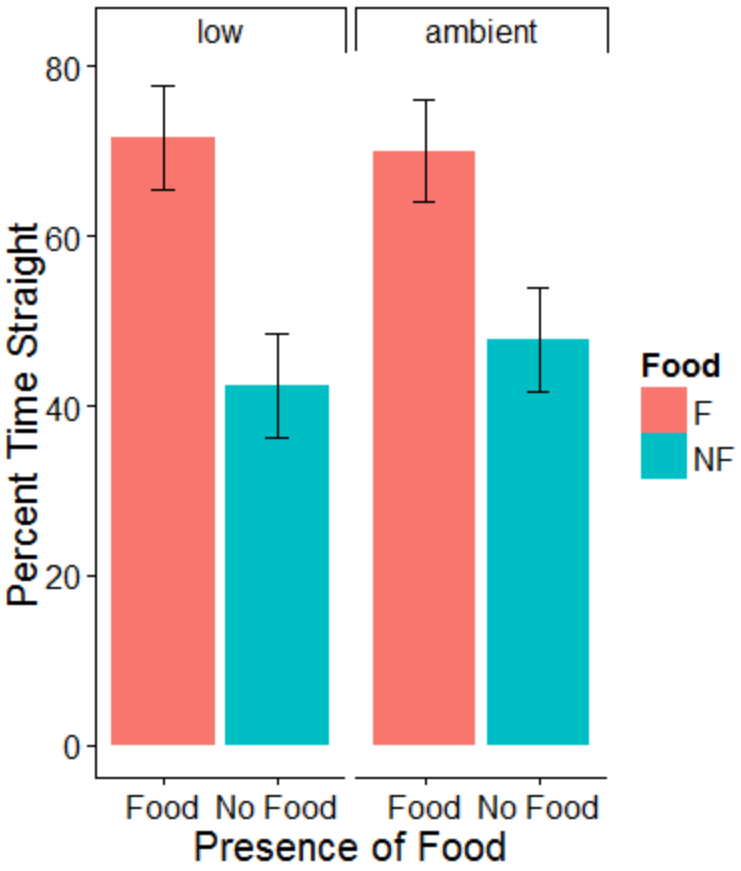

Figure 7: Average percent time moving in a straight line between low pH (7.57 ± 0.11) and ambient pH (7.92 ± 0.16) treatments, and the presence or absence of food, with standard error bars.

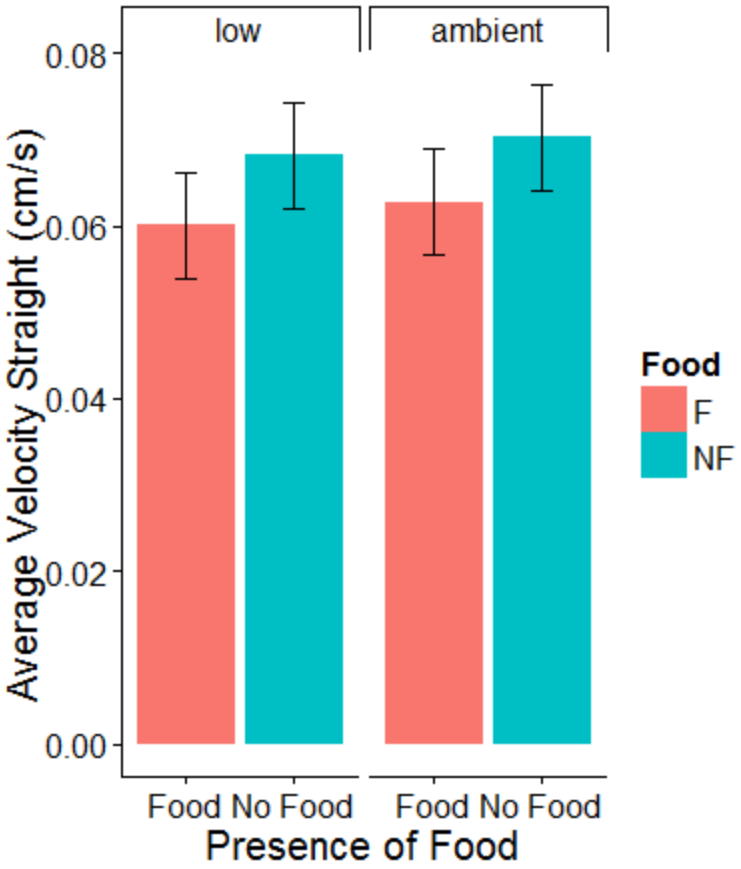

Figure 8: Average velocity in a straight line between low pH (7.57 ± 0.11) and ambient pH (7.92 ± 0.16) treatments, and the presence or absence of food, with standard error bars.

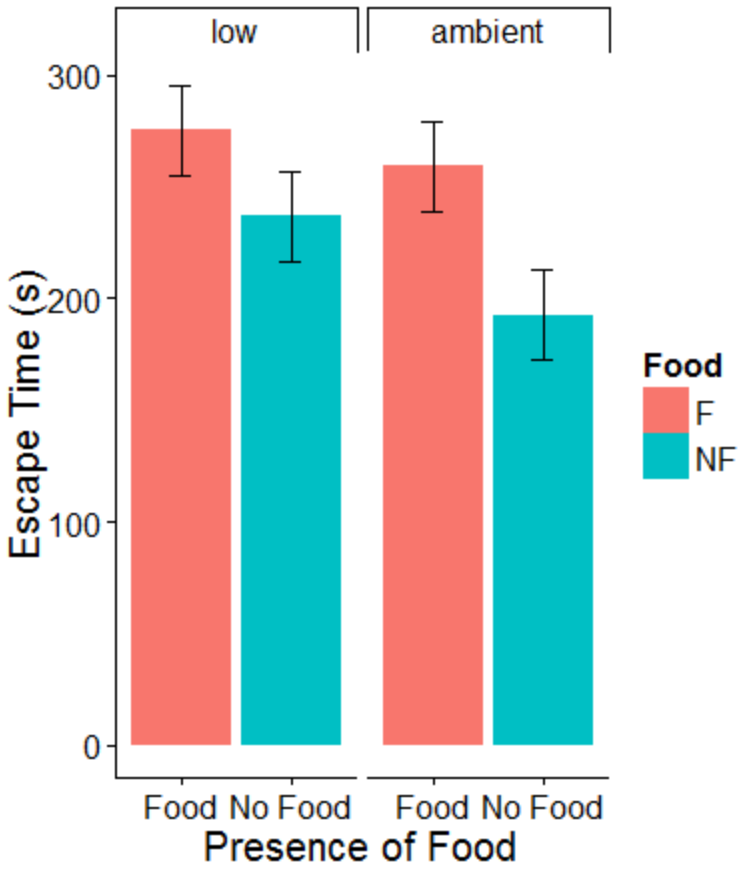

Figure 9: Difference in average escape time between low pH (7.57 ± 0.11) and ambient pH (7.92 ± 0.16) treatments, and the presence or absence of food, with standard error bars.

Table 1: summary of literature reviewing effects of OA on sea stars

Species	Lifestage	response to low pH					Temperature	Experiment Duration	Other Effects	Reference
		Growth Rate	Feeding	Calcification	Ratio soft:calcified tissue	Survival				
Pisaster ochraceus	Juvenile	Positive	Positive	Negative	0/+	No effect	Positive	10 wk	Multiple temperature treatments, interactive effect of temperature and pH, net positive effect	Gooding et al, 2009
Asterias rubens	Adult	Negative	Negative	NA	NA	NA	NA	10 wk	medium acidification may enhance growth	Appelhans et al, 2012
Asterias rubens	Juvenile	Negative	Negative	0	NA	No effect	NA	39 wk	Righting time not affected	Appelhans et al, 2014
Crossaster papposus	larval/juvenile	Positive	NA	noncalcifying larvae	NA	No effect	NA	38 days	OA has a positive effect; faster growth at low pH	Dupont et al., 2010
Odontaster validus	larval	Negative	NA	noncalcifying larvae	NA	Negative	NA	58 days	Strongest effect at lowest pH	Gonzales-Bernat et al., 2013
Patiriella regularis	larval	Negative	NA	noncalcifying larvae	NA	Negative	Negative	4 wk	Interaction of temperature and pH more negative	Bryne et al., 2013
Luidia clathra	Adult	No effect	NA	No effect	No effect	NA	NA	14 wk	Arm regeneration and righting time not affected	Schram et al., 2011
Asterias rubens	Adult	Negative	Negative	Negative	Negative	NA	Negative	10 wk	pH and temperature interaction, largest negative effect at lowest pH	Keppel et al., 2015
Acanthaster planci	larvae	Negative	NA	noncalcifying larvae	NA	Negative	Negative	10 days	Interactive effect of high temperature and low pH on growth and survival	Kamya et al., 2014
Meridiastra calcar	larvae	Negative	NA	noncalcifying larvae	NA	Negative	Negative	5 days	Interaction of temperature and pH, but temp more significant than pH.	Nguyen et al., 2012
Acanthaster planci	Larvae	Negative	NA	noncalcifying larvae	NA	Negative	NA	17 days	Settlement reduced at low pH	Uthicke et al., 2013

Table 2: summary of one-way ANOVA testing effects of prey and pH on sea star behavior.

Summary Table one-way ANOVA					
	df	**Sum of Square**	**Mean Square**	**F Value**	**Pr (>F)**
Percent Time Straight					
Presence/Absence Prey	1	14871	1481	19.798	2.82E-05
pH Treatment	1	73	73	0.097	0.756
Interaction	1	236	236	0.315	0.500
Residuals	78	58587	751		
Average Velocity Straight					
Presence/Absence Prey	1	0.00127	0.00127	1.522	0.221
pH Treatment	1	0.00010	0.00010	0.124	0.726
Interaction	1	0.00000	0.00000	0.001	0.970
Residuals	78	0.06520	0.00084		
Escape Time					
Presence/Absence Prey	1	47721	4721	4.874	0.0302
pH Treatment	1	16940	16940	1.730	0.1923
Interaction	1	3635	3635	0.371	0.5441
Residuals	78	763726	9791		