The effects of ocean acidification on valve strength in chitons (Polyplacophora)

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ABSTRACT

Ocean acidification, the change in ocean chemistry associated with an increase in atmospheric pCO_2, is predicted to have harmful impacts on marine life. In this study, I measured the force required to fracture the protective valves of two chiton species (*Mopalia muscosa* and *Mopalia lignosa*) after ten days of exposure to control conditions and conditions of raised pCO_2. I measured this effect both on valves from freshly sacrificed animals, and on valves that had been dissected prior to treatment. My results show that dissolution of previously dissected valves effects valve strength in *M. muscosa*, but not in *M. lignosa*. Both species may compensate for the effects of dissolution in lowered pH treatments by actively depositing more shell material. *M. musocsa* valves are also, over all treatments, stronger than *M. lignosa* valves. The variation in baseline strength, and in response to ocean acidification, has implications for the future chiton community in the Northeast Pacific. Additionally, my results call for further research on the physiological tradeoffs of increased shell deposition and the ecological impacts of differential responses to ocean acidification.

INTRODUCTION

As ever-increasing amounts of carbon dioxide (CO_2) are absorbed into oceans, water chemistry changes and the resulting ocean acidification (OA) is predicted to significantly impact ocean life (e.g. Langdon *et al.*2000, Manzello *et al.* 2008). Ocean pH levels are predicted to decrease by approximately 0.4 units by 2100 (Feely *et al.* 2004). As oceanic pH drops, the saturation states of minerals essential for shell building decrease. While it is unlikely that saturation states (Ω) will decrease below the threshold

that causes these minerals to spontaneously dissolve (Ω <1), the physiological cost of shell building and maintenance may decrease calcification rates even when Ω is >1 (Feely *et al.* 2004). Calcified shells, a synampomorphy of the phylum Mollusca, are functionally important structures that protect them from predation and desiccation (e.g., Amaral *et al.* 2012). Thus, OA may exert considerable selective pressures on a large and diverse group of marine animals in the near future.

Chitons (class Polyplacophora) are important algal grazers (Dethier & Duggins 1984) that have a mineralized dorsal shell composed of eight overlapping 'valves'. Chiton valves are constructed from aragonite, a mineral that, while not predicted to be undersaturated for hundreds of years, has one of the lowest saturation states of all biologically relevant calcifying minerals (Feely *et al.* 2004). Thus, as oceans absorb increasing amounts of CO_2 and the Ω of aragonite is brought lower, chiton valves are likely to be weakened. Intertidal chitons are exposed a variety of environmental perturbations, such as biofilms and wave action. Wave action likely abrades the valves with sediment, while living biofilms may release metabolic by-products that lower the effective pH around chiton valves (Nienhuis *et al* 2010). These forces, among others, likely work to weaken valves and make chitons more susceptible to predation and desiccation. Because OA may increase the detrimental effect of current shell-weakening forces, it is important to understand how chitons will respond to this future environmental change.

Previous work on the effects of OA on *Nucella lamellosa* found that OA decreases shell weight by increasing shell dissolution, as opposed to by decreasing shell deposition. The authors suggest that testing for the separate impacts of dissolution and

deposition will show specifically how OA impacts calcification (Neinhuis *et al* 2010). To test for the effects of OA on the strength of chiton valves, and to measure the effects of shell dissolution alone, I quantified the force required to break the valves of chitons that have been kept in control conditions or conditions of raised pCO_2 (hereafter, 'acidified conditions'). In doing so, I looked to see if and how OA impacts the strength of chiton valves – through shell deposition, shell dissolution, or both.

Although they seem morphologically similar, chitons show a wide range of diversity in morphology and physiology (Sigwart 2008, Carey *et al.* 2012). Even superficially similar chiton species may show differences in shell strength, and different responses to ocean acidification. I compared the force required to break valves between two species of chiton common to the Pacific Northwest: *Mopalia muscosa* and *Mopalia lignosa*. While the two species are closely related (Kelly & Eernisse 2008), they may have different valve strengths. *M. muscosa* is more common in intertidal zones around the study site (P. Green, *pers. obs.*). Differences in valve strength may be related to differences in valve and girdle morphology, and may give a functional grounding to observed species abundances.

I predicted that chiton valves would fracture at lower forces when kept in acidified conditions. I predicted that OA would have a greater effect on shell dissolution than shell deposition, following the findings of Neinhuis *et al.* (2010). Finally, I compared the effects of OA on valve strength between *M. muscosa* and *M. lignosa.*

METHODS

Collection

I collected 20 individuals of *M. muscosa* and 18 individuals of *M. lignosa* from intertidal zones around San Juan Island (38 total individuals). I measured the total length of each individual. Half of the individuals from each species were then sacrificed and had their eight valves removed through dissection. These valves were separated; half were placed in control conditions ('valve control'), and half were placed in acidified conditions ('valve experimental'). Of the remaining live animals, half were placed in acidified conditions ('live animal experimental'). The other half were immediately sacrificed and their valves tested ('live animal control'). The experimental design is summarized in Table 1. Since water temperatures are relatively stable near the study site (NOAA), and due to time constraints, immediately testing the valve strength for these live animals acted as a direct control.

Ocean Acidification Conditions

Animals in the acidified conditions, and valves in the control or acidified conditions, were kept for ten days in the OA facilities at the University of Washington Friday Harbor Labs on San Juan Island, WA, USA. Previous studies have measured acidification effects successfully in molluscan shells after short (< 1wk) exposure to acidification (Nienhuis *et al*. 2010). The laboratory design consisted of large coolers subdivided into 10 small containers. Water was taken from the local water supply, filtered, scrubbed of CO_2, and released into the coolers. CO_2 from tanks was released into the coolers to bring the pH values down to desired set points. pH was maintained by digital pH meters, which were automatically set to signal for a release of CO_2 when the pH rose above a certain set point. To ensure the pH values accurately represented true p CO_2 conditions, total alkalinity (TA) and total dissolved inorganic carbon (DIC) were

measured weekly. Control conditions were maintained at 8.07 +/- 0.15 pH units (374.52 +/- 110.9 pCO_2) and acidified conditions were maintained at 7.495 +/- 0.05 pH units (1507.77 +/- 163.10 pCO_2) for the duration of the experiment. Power outages and lab malfunctions experienced during the experiment disrupted the pH monitoring process and may have contributed to drift in water chemistry conditions.

Materials Testing

I tested the force and energy required to break all valves using a Materials Testing System (MTS) available at the comparative biomechanics lab at Friday Harbor Labs. Valves were removed from the laboratory conditions, or dissected from the live animals, and photographed from their dorsal and posterior surfaces (Fig. 1). I also measured the thickness of each valve at its apex and the width of each valve at its widest point. I submerged whole valves in seawater, on a surface of silicone, with light support provided by small pieces of modeling clay. The clay allowed for the valves to stand upright, with their vertical axis perpendicular to the ground, while still breaking naturally. A direct downward force from the MTS was applied to the top of the valve until the valve fractured. Values of force to fracture were recorded for each valve.

Statistical Tests

Data for specimen total length, valve width, valve thickness, and force to fracture were analyzed with R statistical computing software (R 2.8.1). Force to fracture correlated significantly and positively with total specimen length (Fig. 2), so all subsequent analyses of force used size-corrected values of force divided by total length (hereafter 'force/length').

I used an ANOVA to test for a significant difference in force/length between valves 2-7. The relationship was not significant (Fig. 3), thus, I combined specimen data for valves 2-7 for all animals. I tested for a difference in force/length between the two *Mopalia* species over all treatments with a two-way T-test. To test for the effect of OA on valve strength in each species, between treatments, I used an ANOVA with post-hoc Tukey HSD comparison.

RESULTS

Mopalia muscosa valves were significantly stronger than *M. lignosa* valves; that is, it took significantly more force/length to fracture *M. muscosa* valves when compared to *M. lignosa* valves (T-test $p<0.001$, Fig. 4). Valves from *M. muscosa* took 7.175 N greater force to fracture (0.256 N/mm total length) than valves from *M. lignosa.*

Within *M. muscosa* alone, valves from the valve control group were significantly stronger than valves from the valve experimental group ($p=0.0024$, Fig. 5). Valves from the valve control group took 6.972 N greater force (0.149 N/mm total length) to fracture than valves from the valve experimental group. There was no difference in force/length in the live animal groups. Surprisingly, control valves were the most resistant to fracture out of any group (including live animal groups). This contributed to a statistically significant difference between valves from the valve control group and the live animal experimental group. ANOVA and Tukey HSD results are summarized in Table 2. It is likely that the high variation in the data contributed to these unexpected results.

The trend for *M. lignosa* was similar to that of *M. muscosa*. However, there was no statistically significant difference between groups. Valves from the valve control

group took 1.999 N greater force (0.034 N/mm total length) to fracture than those from the valve experimental group. Valves from the control valve group were also the strongest out of all groups. There was a statistically significant difference between the valve control group and the live animal control group, as well as the valve control group and live animal experimental group (Table 2). Again, these results are likely influenced by high variation in the data.

DISCUSSION

Ocean acidification significantly decreases valve strength in *Mopalia muscosa* by increasing valve dissolution. However, there is no change in valve strength when live animals are subject to ocean acidification. This result shows that *M. muscosa* may increase shell deposition to maintain shell strength under ocean acidification stress. *M. lignosa* is not significantly affected by ocean acidification. However, there is a trend toward decreased strength caused by valve dissolution, although this is nonsignificant. It is likely that increased sampling would establish a significant difference in force to fracture. Finally, *M. muscosa* valves are, throughout all treatments, significantly stronger than *M. lignosa* valves. Combined with my results from the OA treatments, it is evident that *M. muscosa* is currently more resistant to crushing force, but that this species may be more greatly impacted by future ocean chemistry conditions.

My data also shows that, in both *Mopalia* species, live animals are able to compensate for the deleterious effects of shell dissolution by depositing new shell. That is, there is no difference in valve strength between animals from control and experimental conditions. This physiological ability to compensate for increased dissolution matches results seen in

other mollusks (Nienhuis *et al*, 2010), and has implications for future research. Increasing shell deposition likely comes with a physiological cost, and it unknown for how long chitons can maintain increased deposition rates. For example, increased deposition may come with a cost to feeding, growth, or reproduction rates. Selective pressures in a high-CO2 world may select for chitons that have faster deposition rates, but at a cost of growth or reproduction. Especially in the Northeast Pacific, chitons are important algal foragers (Dethier & Duggins 1984). With decreased population or individual sizes, there could be an increase in algal biomass that has repercussions for the entire benthic community. This hypothesis is supported by other research on the community-level effects of ocean acidification (e.g., Landes & Zimmer 2012, Doney *et al.* 2012, Hall-Spencer *et al.* 2008).

Mopalia muscosa valves take more force to fracture than do *M. lignosa* valves. This result could stem from differences in the material properties of each species' valves, or from morphological differences between the species. Chiton valves are penetrated throughout with sensory nerves (Vendrasco *et al.* 2008), so it is possible that *M. lignosa* has a greater proportion of these 'asthetes' than *M. muscosa*, and is thus weaker. While there seems to be little superficial difference in valve structure between the two species, there may be morphological differences contributing to the differences in force to fracture. If, for example, *M. lignosa* valves are longer than *M. muscosa* valves (for a given width), this could create a longer valve surface, which is likely to be weaker in compression (Vogel 1988). Clearly, a study of the morphological differences between the two species is in order.

My results show that chiton valves become weaker when exposed to ocean acidification treatments, but that live animals are able to compensate for this, likely by

depositing more shell material. Further work is needed to see if this effect changes with longer exposure, or if there are other physiological or functional detriments to exposure that may occur alongside increased deposition. Importantly, it is clear that functional changes occur with exposure to ocean acidification, and that these changes may be drastically different amongst species that seem similar.

REFERENCES

- Amaral, V., & Cabral, H. (2012). Effects of estuarine acidification on predator-prey interactions. *Marine Ecology Progress Series*, *445*
- Andersson, AJ *et al.* (2008). Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. *Marine Ecology Progress Series 373*
- Carey, N., Sigwart, J.D., Richards, J.G. (2012). Economies of scaling: Allometry of metabolism is linked to activity, metabolic rate, and habitat. *Journal of Experimental Marine Biology and Ecology* (in submission).
- Dethier, M.N. & Duggins, D.O. (1984). An "Indirect Commensalism" between Marine Herbivores and the Importance of Competitive Hierarchies. *The American Naturalist* 124(2)
- Doney, S.C., Ruckelshaus, M., Duffy, J.E. (2012). Climate Change Impacts on Marine Ecosystems. *Annual Review of Marine Science* 4.
- Feely, R. A., Sabine, C. L., Lee, K., *et al.* (2004). Impact of Anthropogenic CO2 on the CaCO3 System in the Oceans. *Science*, *305*(5682)

- Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S. (2008). Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454.
- Kelly, R.P. & Eernisse, D.J. (2008). Reconstructing a radiation: the chiton genus *Mopalia* in the north Pacific. *Invertebrate Systematics* 22.
- Landes, A. & Zimmer, M. (2012). Acidification and warming affect both a calcifying predator and prey, but not their interaction. *Marine Ecology Progress Series* 450.
- Langdon, C., Takahashi, T., Sweeney, C. *et al.* (2000). Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. *Global Biogeochemical Cycles* 14(2)
- Manzello, D.P., Kleypas, J.A., Budd, D.A. *et al.* (2008). Poorly cemented coral reefs of the eastern tropical Pacific: Possible insights into reef development in a high-CO_2 world. *Proceedings of the National Academy of Sciences* 105(30).
- Nienhuis, S., Palmer, R., & Harley, C.D.G. (2010). Elevated CO2 affects shell dissolution rate but not calcification rate in a marine snail. *Proceedings of the Royal Society (B)*, *277*(1693)
- R 2.8.1. R Foundation for Statistical Computing. http://www.R-project.org
- Sigwart, J.D. (2008). Gross anatomy and positional homology of gills, gonopores, and nephridiopores in "basal" living chitons (Polyplacophora: Lepidopleurina)*. *American Malacological Bulletin* 25.
- Vendrasco, M.J., Fernandez, C.Z., Eernisse, D.J. *et al.* (2008). Aesthete canal morphology in the Mopaliidae (Polyplacophora). *American Malacological Bulletin* 25.

- Vogel, S.J. (1988). Life's Devices. Princeton University Press, Princeton, New Jersey.
- Water temperature ranges found from NOAA website: http://tidesandcurrents.noaa.gov, Station ID # 9449880.

FIGURES AND TABLES

Table 1 – Design of control and increased pCO2 (experimental) conditions. Shows temperature and pH values for each treatment, as well as number of animals from each species in treatment.

Control Ambient sea temperature and pH Live animals (4 *M. muscosa*, 3 *M. lignosa)*	Control 14C, 8.0pH Valves (5 *M. muscosa*, 6 *M. lignosa)*
Increased pCO2 14C, 7.5pH Live animals (5 *M. muscosa*, 5 *M. lignosa)*	Increased pCO2 14C, 7.5pH Valves (6 *M. muscosa*, 4 *M. lignosa*)

Table 2 – Results of ANOVA and Tukey HSD tests on within-species force/length values between OA treatments. Values in **bold** are significant to $p < 0.05$.

Species	ANOVA p-value	Tukey Comparison	Mean difference (N/mm)	Lower 95% (N/mm)	Upper 95% (N/mm)	p-value
M. muscosa	**0.0040**	LA_OAExp | LA_OAC	-0.0222	-0.1300	0.0857	0.9496
		Valve_OAC | LA_OAC	0.0908	-0.0236	0.2052	0.1688
		Valve_OAExp | LA_OAC	-0.0580	-0.1674	0.0515	0.5121
		Valve_OAC | LA_OAExp	0.1130	0.0079	0.2180	**0.0299**
		Valve_OAExp | LA_OAExp	-0.0358	-0.1354	0.0638	0.7842
		Valve_OAExp | Valve_OAC	-0.1487	-0.2554	-0.0421	**0.0024**
M. lignosa	**0.0019**	LA_OAExp | LA_OAC	-0.0109	-0.0796	0.0579	0.9760
		Valve_OAC | LA_OAC	0.0730	0.0043	0.1418	**0.0329**
		Valve_OAExp | LA_OAC	0.0387	-0.0326	0.1100	0.4911
		Valve_OAC | LA_OAExp	0.0839	0.0248	0.1430	**0.0019**
		Valve_OAExp | LA_OAExp	0.0496	-0.0125	0.1117	0.1648
		Valve_OAExp |	-0.0343	-0.0964	0.0278	0.4749

Valve_OAC

Figure 1 – Comparison of *Mopalia lignosa* (left) and *Mopalia muscosa* (right) individuals. From top, images show dorsal surface of whole animal, posterior surface of one representative valve, and dorsal surface of the same valve.

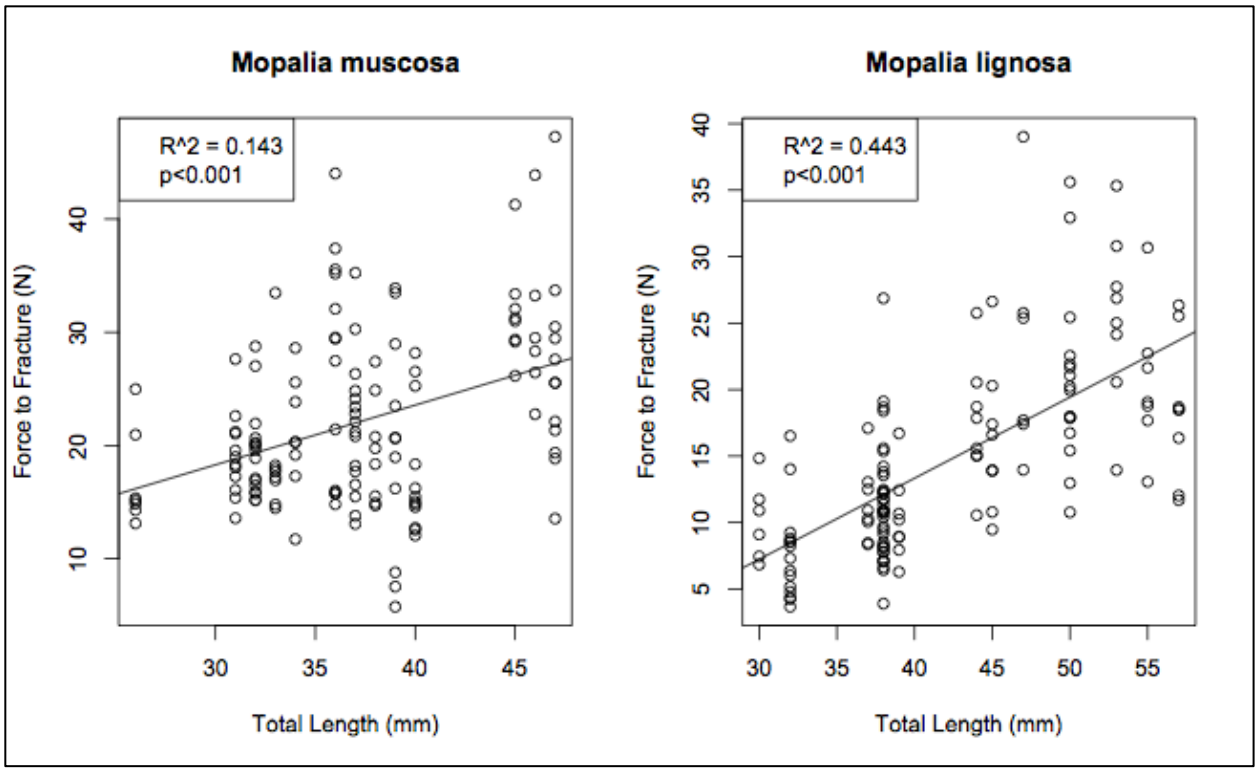

Figure 2 – Scaling relationships of force to fracture (N) as a function of total length (mm) for both species studied. Both scaling relationships have significantly positive slopes, thus subsequent force data is corrected for total specimen length (force/length, N/mm).

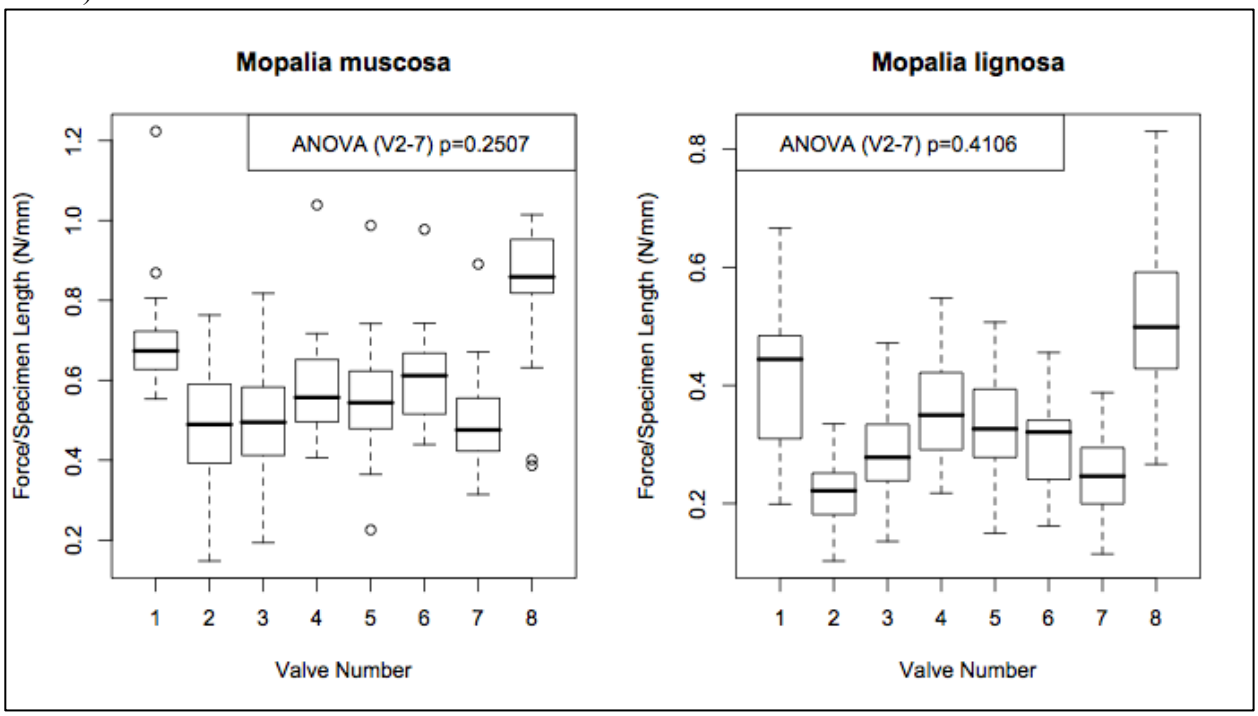

Figure 3 – Boxplots of force/length over all eight valves for each chiton species. ANOVA results show a non-significant difference in force/length between valves 2-7. Subsequent data are summed between valves 2-7.

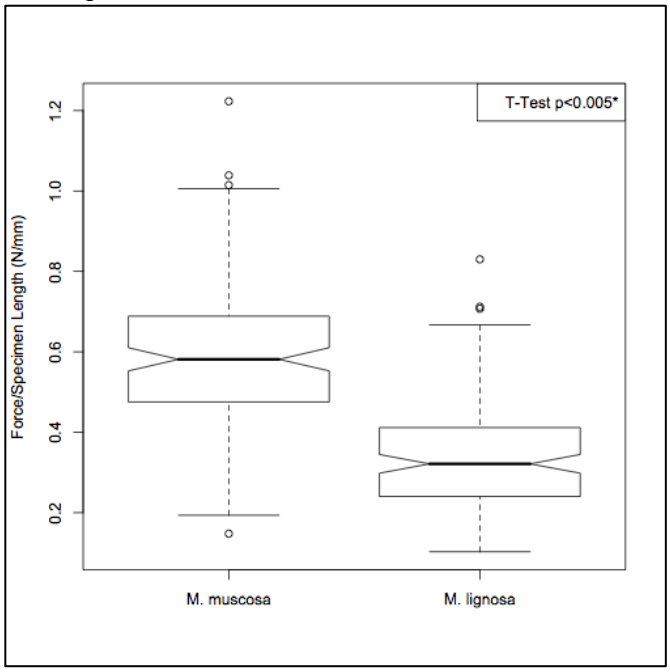

Figure 4 – Boxplot showing difference in force/length between both species studied, summed over all treatments. T-test p-value < 0.005 shows that *M. muscosa* has significantly stronger valves than *M. lignosa*.

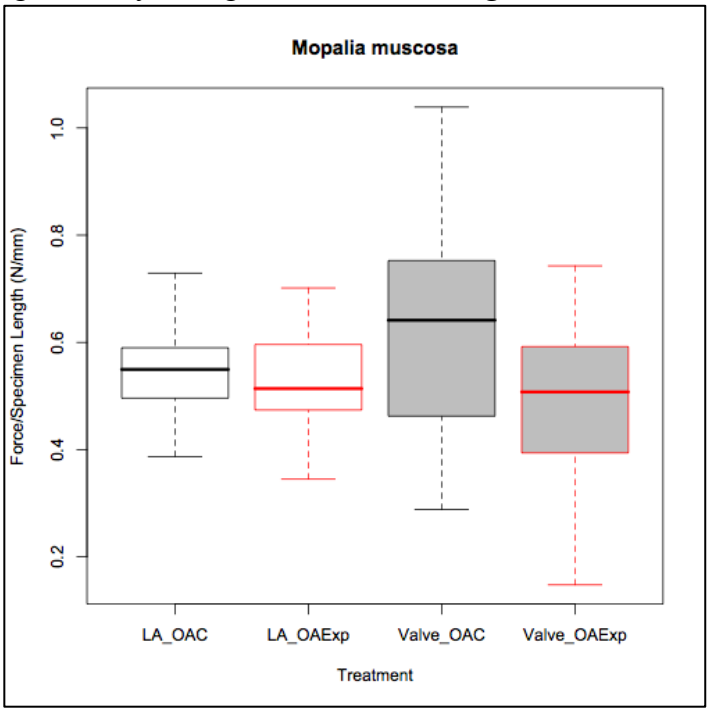

Figure 5 – Boxplot of force/length results for *M. muscosa* over all ocean acidification treatments. Valve treatments are shaded in gray. Statistically significant differences between treatments are shown in Table 1.

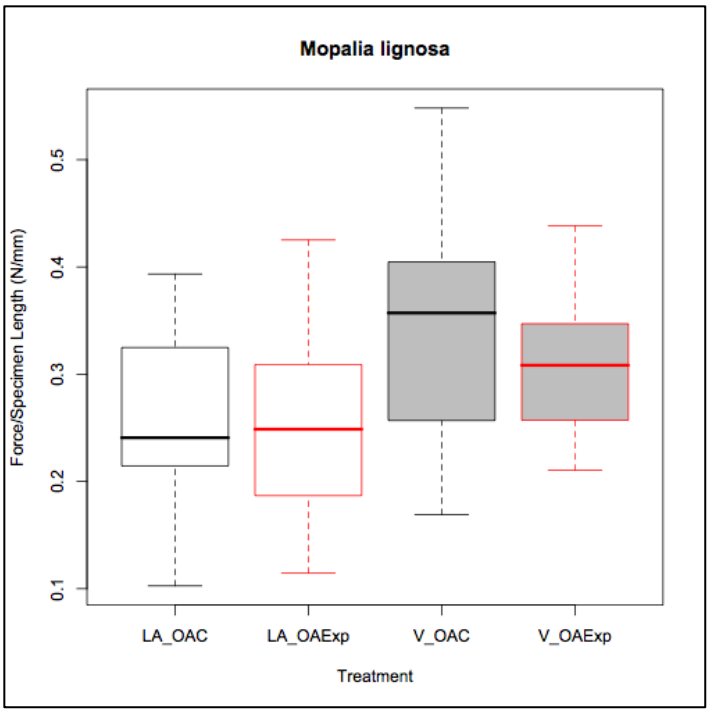

Figure 6 - Boxplot of force/length results for *M. lignosa* over all ocean acidification treatments. Valve treatments are shaded in gray. Statistically significant differences between treatments are shown in Table 1.