Effects of Ocean Acidification on the Growth of Juvenile *Mytilus edulis*!

Mary Margaret Stoll1,3,4, Robert Holmberg1, Aaron Honig1,2, and Robyn Hannigan1*

1School for the Environment, University of Massachusetts Boston, Boston, MA, 2
2Biology Department, University of Massachusetts Boston, Boston, MA, 3
3Chemistry Department, Amherst College, Amherst, MA, 4
4Environmental Studies Department, Amherst College, Amherst, MA

**Abstract!**

Ocean acidification is the process in which surplus atmospheric carbon dioxide (CO₂(g)) transfers across the ocean-atmosphere boundary and becomes CO₂(aq). This process changes the carbonate system balance leading towards increased [H⁺] and decreased [CO₃²⁻], ultimately causing increased acidity of the water. Furthermore, this changes the saturation state of carbonate minerals, shifting away from stability towards dissolution. As a result, carbonate biominerals such as those in bivalve shells become thermodynamically less stable and may dissolve or shift towards a more stable form. We grew the juvenile blue mussel, *Mytilus edulis*, under different CO₂-induced low pH conditions to explore the effect of ocean acidification on growth. We used a pH-stat CO₂ dosing system designed for ocean acidification research with four replicates per treatment (n=4, control-outside room: pH=8.1, control: pH=8.1, treatment 3: pH=7.6, and treatment 4: pH=7.3). We monitored carbonate chemistry parameters including pH, salinity, temperature, and total alkalinity. Juveniles were fed Isochrysis at 8% of ocean acidification on growth. We used a pH-stat CO₂ dosing system under different CO₂ conditions, with treatment range maintained by CO₂ dosing as programmed through the CO₂ simulation system (Stoll 2015).

**Methods!**

**Ocean Acidification Simulation System**

- Constructed ocean acidification simulation system (Freeburg 2013) to be spatially regulate and record pH of jars (Fig 4).
- Jars dosed with CO₂(g) to decrease pH.
- 4 pH treatments with 4 replicates! totaling 16 jars!
- Treatment 1: control-outside.
- Treatment 2: control, pH=8.1.
- Treatment 3: 7.6!
- Treatment 4: 7.3.
- Jars randomly assigned positions in OA room (Fig. 5).
- Top row (jars N-W) represents the relative positions of the jars and treatments inside the OA room. Jars B, C, E, and F were placed outside the OA room. These jars were not connected to the system and thus did not yield continuous log of pH (Stoll 2015).
- Each jar contained a pH probe and airstone with CO₂(g) and ambient air.
- Each pH probe connected to SLS unit.
- Used ReefKeeper Elite! controller (Head Unit) to program the pH of each jar.
- Solenoid valve opened and closed to dose tanks with CO₂.

**Water Chemistry Measurements**

- 4 water measurements made to determine the properties of the seawater in each jar, and to keep feeding consistent across experimental units.
- pH:
  - Measured in each jar twice a day using YSI device.
- Salinity:
  - Measured in each jar twice a day using YSI device.
- Temperature:
  - Water samples taken from each jar every day.
- Total Alkalinity:
  - Automatic titrations using HI 84431 Total Alkalinity Reader.
- Algal Density Counts:
  - Fed mussels T.-alkalisygena algae twice a day.
  - Water changes performed daily to regulate food availability.

**Juvenile Mytilus edulis Measurements**

- Approximately 3 months old (Phillitarp 2007).
- Measurements made from umbo to posterior edge (Fig. 7).
- Pairwise T-test shell length before and after.
- There was a significant difference between M. edulis and M. edulis juvenile measurements made from umbo to posterior edge (after experiment) and between Hay and no Hay treatment. We plan to take more scanning electron micrographs and interpret the images. We would also like to build upon this study and conduct a multi-environment experiment to determine the effects of ocean acidification in the context of warming temperatures.

**Results and Discussion**

**Table 1: Carbonate chemistry parameters. pH, S, T, and A represent measured values and were averaged for each treatment group. JCO₃ and DIC represent model output values from CO2Calc (1.3.0).**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>S</th>
<th>T</th>
<th>A</th>
<th>JCO₃</th>
<th>DIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.1</td>
<td>30.15</td>
<td>19.47</td>
<td>33.1</td>
<td>8.1</td>
<td>30.15</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>8.1</td>
<td>30.15</td>
<td>19.47</td>
<td>33.1</td>
<td>8.1</td>
<td>30.15</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>7.6</td>
<td>30.15</td>
<td>19.47</td>
<td>33.1</td>
<td>7.6</td>
<td>30.15</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>7.3</td>
<td>30.15</td>
<td>19.47</td>
<td>33.1</td>
<td>7.3</td>
<td>30.15</td>
</tr>
</tbody>
</table>

**Conclusions**

- There was no difference in mussel growth between treatments.

**Acknowledgements**

Thank you to the CREST REU UMass Boston program funded by the National Science Foundation Award #1359324 to Dr. Christian and Dr. Mannigan (OCF- GEODE/Education and Human Resources (Ocean Sciences)). Thank you to Bill Robison, Chris Bokuski, James Bukowski, Catie McCabe, Silveria Costa, Amanda Atwood, Sarah Noel, Sean McCarty, Andy Rhine, Brad Ryan, Jason Sprouse, Alita Wallace-Kim, Jon Hall, and colleagues. Alan Stablein, Steve Nye, Amy Johnston, Ron Elsasser, Eric Wollin, Frederik Heinis, Peter Hursel, Florence Wurzel, Anna Lightfoot and Lauren D’Angelo for their support throughout the program.

**Fig. 1:** Bjerrum plot: As the pH of the ocean decreases, carbonic acid (H₂CO₃) dissociates to produce H₂O and CO₂. This process increases the acidity of the water (Schubert et al. 2006).

**Fig. 2:** Life stage of a blue mussel from egg to adult (Cornish 2001).

**Fig. 3:** Mytilus edulis, blue mussel juvenile, Approximately 3 months old (Phillitarp 2007).

**Fig. 4:** Ocean acidification simulation system used for experiment (Freeburg 2013). pH range maintained by CO₂ dosing as programmed through the OA simulation system (Stoll 2015).

**Fig. 5:** Top row (jars N-W) represents the relative positions of the jars and treatments inside the OA room. Jars B, C, E, and F were placed outside the OA room. These jars were not connected to the system and thus did not yield continuous log of pH (Stoll 2015).

**Fig. 6:** Diagram of OA simulation system: pH electrodes connected to SLS units and controlled by Head Unit. Solenoid valve opens and closes to maintain pH with CO₂(g) and ambient air (Stoll 2015).

**Fig. 7:** Pairwise T-test shell length before and after. (after experiment) and between Hay and no Hay treatment. We plan to take more scanning electron micrographs and interpret the images. We would also like to build upon this study and conduct a multi-environment experiment to determine the effects of ocean acidification in the context of warming temperatures.

**Fig. 8:** Length added (after-before) vs. pH treatment. **ANOVA: F=1.2, p>0.05.**

**Fig. 9:** Mussel perimeter (mm) vs. pH treatment. **ANOVA: F=2.84, p=0.05.**

**Fig. 10:** Mussel area (pixels) vs. pH treatment. **ANOVA: F=0, p=0.50.**

**Fig. 11:** Mussel circularity vs. pH treatment. **ANOVA: F=9.8, p=0.01.**

**Fig. 12:** Scanning electron micrographs for juveniles in treatments 1, 2, 3, 4 (after 8 weeks in treatment 1). 7.5 µm and 7.3 (µm).

**Table 2:** Pairwise T-test shell length before and after. There is a significant difference between M. edulis and M. edulis juvenile measurements made from umbo to posterior edge (after experiment) and between Hay and no Hay treatment. We plan to take more scanning electron micrographs and interpret the images. We would also like to build upon this study and conduct a multi-environment experiment to determine the effects of ocean acidification in the context of warming temperatures.