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

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Abstract!

Ocean acidification is the process in which surplus atmospheric carbon dioxide (CO_2(g)) transfers across the ocean-atmosphere boundary and becomes CO_2 (aq). This process changes the carbonate system balance leading towards increased [H^+] and decreased [CO_3^{2-}], 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 CO_2-induced low pH conditions to explore the effect of ocean acidification on growth. We used a pH-stat CO_2-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 *T-Isochrysis* algae. We changed the water in each tank every day and counted algal cells to estimate algal density. At the end of the one-week exposure, we measured survivorship, shell length and width. We evaluated shell measurements using a Matlab script to determine circularity, area and perimeter. There was no difference in mussel growth between treatments ($p>0.1$). However, there were differences in the circularity of the mussel shells between treatments ($p<0.1$). This experiment should be repeated in order to verify the results. !

Introduction!

Ocean Acidification!

- Carbon dioxide in the atmosphere increasing due to anthropogenic emissions from fossil fuels!
- Oceans absorb CO_2 (g) and induces phenomenon known as ocean acidification (OA), in which acidity of water increases (Feely 2009)!

OA and Calcification!

- Increasing acidity causes changes in the carbonate chemistry of seawater (Fig. 1)!
- Changes in ion stability pose serious threats to calcifying marine organisms that rely on [CO_3^{2-}] to build shells (Fabry 2008)!

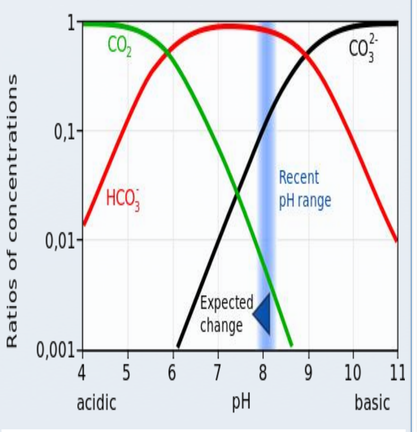

Fig. 1: (Bjerrum plot) As the pH of the ocean decreases, carbonic acid (H_2CO_3) and bicarbonate (HCO_3-) dissociate to produce H+ ions. This process increases the acidity of the water (Schubert et al. 2006).!

Mytilus edulis Juveniles!

- 6 stages of *Mytilus edulis* (Fig. 2)!
- Juveniles-early plantigrade stage!
- Shells in the juvenile stage have a transparent appearance and an ovular shape (Fig. 3)!
- Construct shells through the process of calcification!

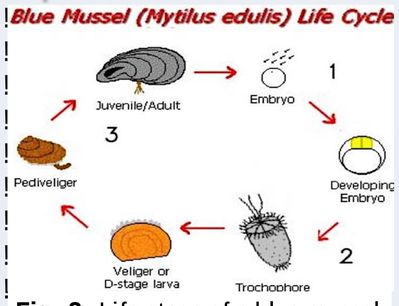

Fig. 2: Life stage of a blue mussel from embryo to adult (Cornish 2001).**!**

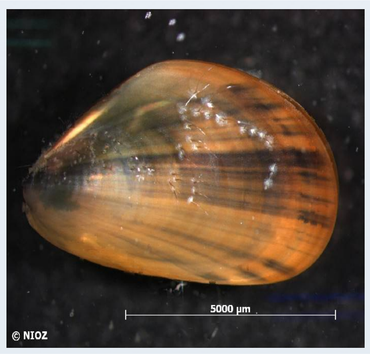

Fig. 3: *Mytilus edulis*, blue mussel juvenile. Approximately 3 months old (Phillitharp 2007)**.!**

Objectives and Hypothesis!

The goal of this experiment is to determine the effects of ocean acidification on the growth of *Mytilus edulis* juveniles. **!**

Objectives:!

-Construct ocean acidification simulation system to control pH of jars**!**
-Take measurements of the seawater to determine chemical properties**!**
-Measure size and shape of mussels to determine health and growth!

Null Hypothesis:!

There is no difference in the growth or health between *M. edulis* juveniles exposed to low-pH conditions and those exposed to ambient conditions!

Methods!

Ocean Acidification Simulation System!

- Constructed ocean acidification simulation system (Freeburg 2013) to automatically regulate and record pH of jars (Fig. 4)!

-Jars dosed with CO_2(g) to decrease pH
-4 pH treatments with 4 replicates ! totaling 16 jars!
-Treatment 1: control-outside ! OA room, 8.1 O!
-Treatment 2: control, 8.1!
-Treatment 3: 7.6!
-Treatment 4: 7.3!

- Jars randomly assigned positions! in OA room (Fig. 5)!

Fig. 4: Ocean acidification simulation system used for juvenile experiment (Freeburg 2013). pH range maintained by CO_2 dosing as programmed through the OA simulation system (Stoll 2015). !

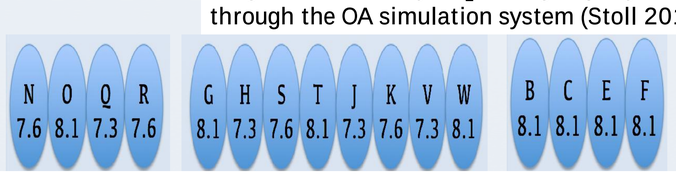

Fig.5: Top row (jars N-W) represents the relative positions of the jars and treatments inside the OA room. Jars B, C, E and F were placed outside the OA room. These jars were not connected to the system and thus did not yield continuous log of pH (Stoll 2015). **!**

-Each jar contained a pH probe ! and airstone with CO_2(g) and ! ambient air (Fig. 6)!
-Each pH probe connected to ! SL1 unit!
-Used ReefKeeper Elite ! controller (Head unit) ! to program the pH of each jar!
-Solenoid valve opened and ! closed to dose tanks with CO_2!

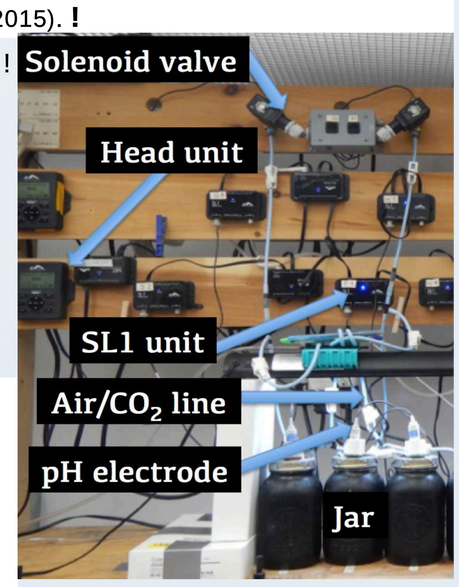

Fig. 6: Diagram of OA simulation system. pH electrodes connected to SL1 units and controlled by Head unit. Solenoid valve opens and closes to maintain pH and dose tanks with CO_2 (g) and ambient air (Stoll 2015).!

Water Chemistry Measurements!

- 4 water measurements made to determine the chemical properties of the seawater in each jar, and 1 to keep feeding consistent across experimental units!

pH!	•!All jars in OA room continuous log of pH! •!pH electrodes checked with Hach mV reader twice a day!
Salinity!	•!Measured in each jar twice a day using YSI device!
Temperature!	•!Measured in each jar twice a day using YSI device!
Total Alkalinity!	•!Water samples taken from each jar every day! •!Performed auto titrations using HI 84431 Total Alkalinity titrator !
Algal Density Counts!	•!Fed mussels *T-Isochrysis* algae twice a day! •!Water changes performed daily to regulate food availability!

Juvenile *Mytilus edulis* Measurements!

- 20 juveniles randomly selected and placed into each jar !
- Pictures of juveniles taken before and after experiment using SpotAdvanced program (Fig. 7)!
- Shell length measured in mm before and after experiment using SpotAdvanced!
- Survivorship recorded at end of experiment !
- Pictures of mussels edited in Microsoft Paint to prepare for MatLab script!
- Pictures of mussels run through MatLab script to determine area, perimeter and circularity of mussels after experiment!

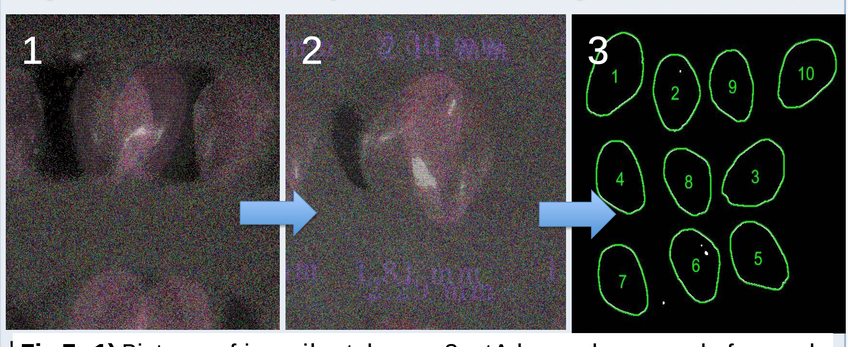

Fig 7: 1) Pictures of juveniles taken on SpotAdvanced program before and after the experiment **2)** Measurements made from umbo to posterior edge using calibrated marker and stamped onto images **3)** Pictures of juveniles then run through MatLab script to determine size properties (Stoll 2015). **!**

Results and Discussion!

Table 1: Carbonate chemistry parameters. pH_T, S, T, and A_T represent measured values and were averaged for each treatment group. pCO_2, Ω_{ar}, and DIC represent modeled values output from CO2calc (1.3.0). Standard deviation from treatment mean is listed in parentheses for each value.!

Treatment	pH_T	S ppt	T°C	A_T µmol kg^{-1}	DIC µmol kg^{-1}	pCO_2 µatm	Ω_{Ar}
Control (Outside)	8.08 (0.01)	30.61 (0.15)	21.46 (0.21)	2531.58 (31.35)	2257.29 (24.39)	421.80 (7.85)	3.33 (0.11)
Control	8.06 (0.03)	30.45 (0.31)	23.62 (0.34)	2539.99 (102.64)	2254.27 (88.24)	438.06 (30.08)	3.51 (0.26)
7.6	7.57 (0.09)	30.68 (0.42)	23.60 (0.35)	2581.85 (25.15)	2517.84 (43.83)	1644.90 (372.72)	1.34 (0.23)
7.3	7.27 (0.03)	30.40 (0.13)	23.69 (0.19)	2550.26 (31.90)	2592.17 (42.30)	3322.74 (281.87)	0.68 (0.04)

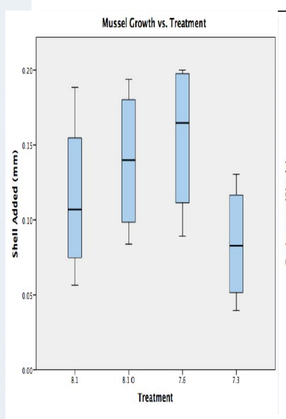

Fig 8: Length added (after-before) vs. pH treatment. ANOVA: F=1.52; p=0.26 **!**

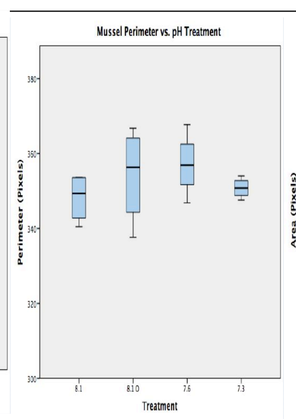

Fig 9: Mussel perimeter (mm) vs. pH treatment. ! ANOVA: F=0.84; p=0.50 **!**

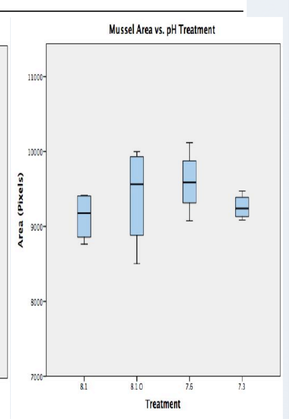

Fig 10: Mussel area (pixels) vs. pH treatment. ! ANOVA: F=0.80; p=0.52**!**

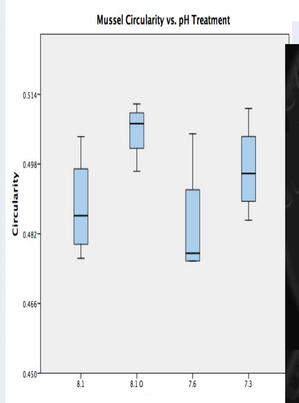

Fig 11: Mussel circularity vs. pH treatment. ! ANOVA: F=2.98; *p<0.1! Tukey: 8.1 O vs. 7.6 : p=0.07**!**

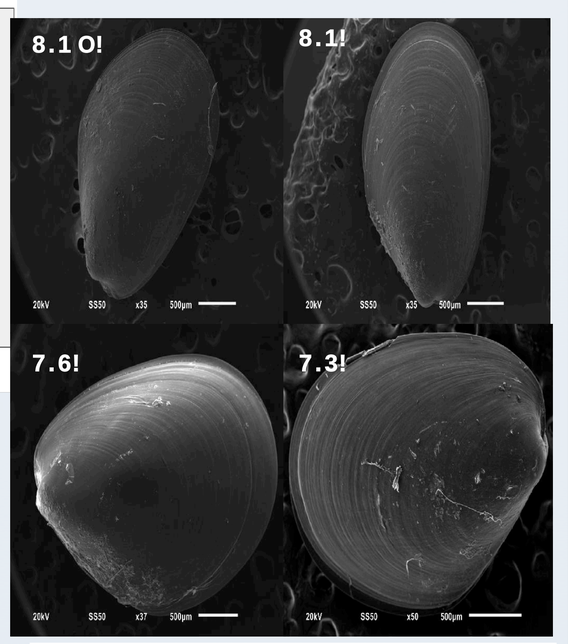

Fig. 12: Scanning electron micrographs for juveniles in treatments 8.1 O (jar B), 8.1 (jar G), 7.6 (jar S) and 7.3 (jar Q). **!**

Table 2: Pairwise T-test shell length before and after. There is a significant difference between the shell length before experiment and shell length after experiment within each treatment. **!**

Treatment!	8.1!	8.1 O!	7.6!	7.3!
Pairwise T-test results!	t(3)=-4.11; *p=0.03!	t(3)=-5.58; *p=0.01!	t(3)=-5.83; *p=0.01!	t(3)=-4.15; *p=0.03!

Mixed-Design ANOVA (Between subjects): F=0.419; p=0.743!

Conclusions!

There was no difference in mussel growth between treatments. However, we did observe unexpected differences in the circularity of the mussel shells between treatments. 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-stressor experiment to determine the effects of ocean acidification in the context of warming temperatures. !

Acknowledgements!

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