The immune response of ribbed mussels (*Geukensia demissa*) to ocean acidification

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Introduction

The ribbed mussel, Geukensia demissa plays an invaluable role maintaining the health of salt marsh ecosystems by absorbing nitrogen and phosphorous from sea water and depositing waste products in the form of feces or ammonia to sediments which aid the health of Spartina alterniflora (Kuenzler 1961; Jordan and Valiela 1982; Bertness 1984). In addition, their byssal threads stabilize the sediment, and provide habitat for smaller invertebrates (Shand 1987; Bertness 1984). The significance of water temperature on ribbed mussel survivorship is well established (Brodsky et al. 2011; Hernandez 2015). The effects of ocean acidification by comparison, are complicated. Fitzer et al. (2015) found a decrease in aragonite layer thickness and a change in the average shell shape of M. edulis when exposed to hypercapnia (increased carbon dioxide concentrations). They speculated that the change in shell shape compensates for a weakened defense. Furthermore, when mytilid mussels were exposed to hypercapnia, the ability of their byssal threads to attach to the substratum was hindered, and as a result the overall performance declined by 40% (O'Donnell et al. 2013). This could affect distribution and abundance of G. demissa as they would be more easily swept away.

Ocean acidification has a significant effect on the immune systems of shellfish. For instance, the oyster pathogen Perkinsus marinus currently has a low pathogenicity for G. demissa (Anderson 2001). Since a mussel's first line of defense is already weakened with decreased shell thickness, pathogenicity of parasitic microorganisms may increase as well. The other significant line of defense is hemocytes, which first phagocytose pathogens and then release superoxides and degradative enzymes (Pipe & Coles 1995; Renwrantz 1990). Decreased pH levels were shown to inhibit phagocytosis levels, but not superoxide production or hemocyte density (Bibby et al. 2008). While all three are significant to the immunity of G. demissa, the focus of this study was hemocyte density. The objective of this experiment was to explain the relationship between ocean acidification and the immune system of G. demissa. Our null hypothesis is that there is no significant difference in hemocyte density between mussels exposed to ambient and lowered pH.

Materials and Methods

Ribbed mussels were collected from Bradley Point, West Haven, CT (40° 12' 8.42" N, -74° 00' 43.49" W). They were measured with a caliper and divided into size classes of small (1104-1731 mm²), medium (1858-2135 mm²), and large (2194-3155 mm²) and transferred to the control and experimental tanks. Every other day the mussels from both tanks were transferred to two separate containers functioning as basins where they were fed with Phytofeast. After each feeding each size class was rotated to different sections of their tank to account for variations in the pH within the tank. A CO_2 regulator was used to pump carbon dioxide into the experimental tank, maintaining a pH of 7.80 to 7.56. The control tank had an ambient pH that varied from 8.30 to 8.04. Three mussels, one for each size class, were taken from each tank every four days to be used in hemocyte counts. Hemolymph was withdrawn from the posterior adductor mussel using a 3-mL syringe with a 25-gauge needle. A hemocytometer was used for cell counts. A phagocytosis assay was attempted, but due to extremely high variablility in the experimental method, no reliable results were achieved. Data were analyzed using Excel and Past software.

Figure 5 Hemolymph extraction (Fenech M. & Bolognesi C. 2012)

Results

Figure 1 indicates that variation was too high within the sample groups there to be a reliable conclusion. Figures 2 to 4 show the variability in hemocyte density from a single mussel at ambient and l lowered pH. On day 8 there was significantly higher hemocyte density for the smaller mussel within ambient pH than in the lowered pH (p=0.0128). On day 12 the large mussel exposed to an ambient pH had a significantly higher hemocyte density than the large mussel exposed to lowered pH (p=0.0237), but the reverse occurred on day 24, with a significantly higher hemocyte density for lowered pH than in ambient pH (p=0.0235). Overall, hemocyte density seemed to increase over time with lowered pH, but variation of hemocyte densities within a single mussel is too high for a reliable conclusion to be drawn.

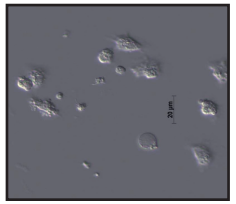

Figure 5 Hemocytes, courtesy of Dr. Eve Galimani (Milford)

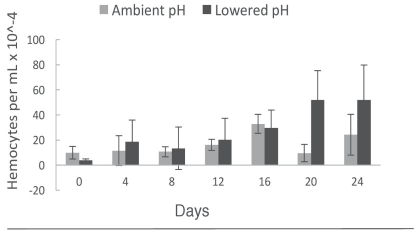

Figure 1 Average effect of lowered pH on hemocyte density of the total population (*G. demissa)* over time.

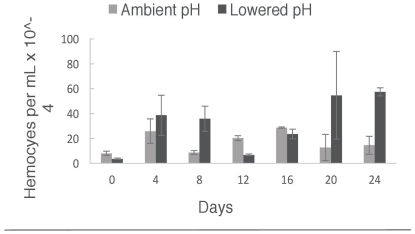

Figure 2 Effect of lowered pH on hemocyte density of large mussels (*G. demissa)* over time.

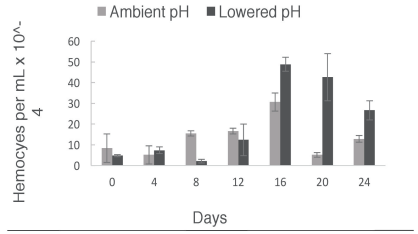

Figure 3 Effect of lowered pH on hemocyte density of small mussels (*G. demissa)* over time.

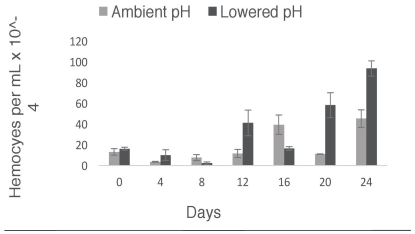

Figure 4 Effect of lowered pH on hemocyte density of medium sized mussels (*Geukensia demissa)* over time.

Discussion

The objective of this research has been to provide information that can better explain the complex relationship between the immune system of ribbed mussels (G. demissa) and ocean acidification. The results of the hemocyte counts showed no significant difference between ambient pH (8.04-8.30) and lowered pH (7.56-7.80). However, hemocyte density seemed to increase under lowered pH on days 20-24 (fig 1). Bibby et al. (2008) finds similar results in that there is no observable pattern between hemocyte density and decreased pH, although they do report a decrease in phagocytic activity corresponding to decreased pH. I attempted a phagocytosis assay, to test this observation in ribbed mussels. Unfortunately, the hemocytes did not react with the Zymosan provided, and the procedure did not give reliable results. Bibby's research involved blue mussels (M. edulis), so differences in physiology between them and ribbed mussels is a possible explanation for this.

Hemocyte density often varied greatly among counts within the same mussel (figs 2-4). This suggests that hemocytes are not evenly distributed within the hemolymph. Hemocytes were often clumped together within the hemolymph, which varied between a yellowish green and cloudy white color. Within the green hemolymph, clumping of hemocytes was observed more often, whereas white hemolymph often had several different species of microorganisms. This high variation isn't mentioned in the literature. It is possible that hemocyte density is inconsistent within the hemolymph and therefore difficult to quantify accurately with the methodology used. More research would be necessary to fully understand the effects of increased levels of CO_2 on the health of Geukensia demissa.

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