CAPACITY FOR ADAPTATION AND ACCLIMATIZATION TO OCEAN ACIDIFICATION IN GEODUCK THROUGH EPIGENETIC MECHANISMS

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INTRODUCTION

Shellfish production has been plagued by negative effects on larval shellfish associated with changes in seawater chemistry driven by ocean acidification (OA). Per unit weight, the geoduck clam (*Panopea generosa*) is the most valuable of shellfish in the WA fishery, ~4x greater than the Pacific oyster. From 1999-2013 the price per pound has increased 2.3x and geoduck contribute to a state shellfish industry valued at $91.9 mill (WA Sea Grant, 2015). Abundant evidence indicates effects of acute low pH on a variety of shellfish larvae , but little information exists for impacts on geoduck. We tested the sensitivity of early life stages and the potential for geoduck clams to display adaptation and acclimatization to OA and the role of DNA methylation in these processes.

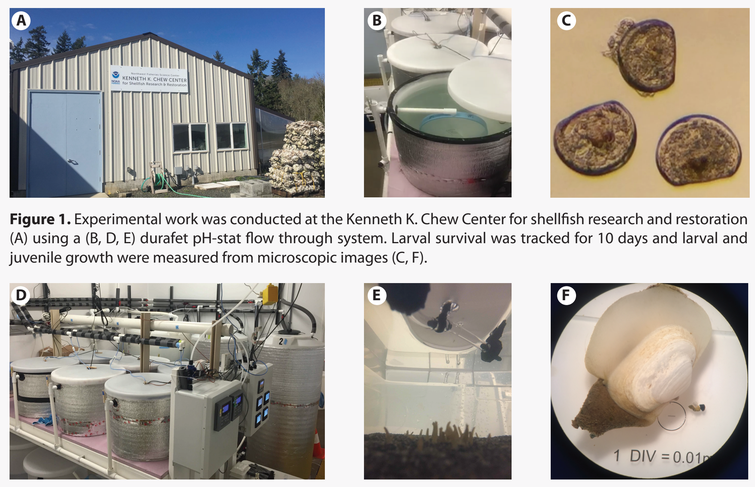

Figure 1. Experimental work was conducted at the Kenneth K. Chew Center for shellfish research and restoration (A) using a (B, D, E) durafet pH-stat flow through system. Larval survival was tracked for 10 days and larval and juvenile growth were measured from microscopic images (C, F).

RATIONALE

Waters upwelled into the Strait of Juan de Fuca enter Puget Sound and alter the nearshore seawater chemistry along with biological processes (primary production, respiration, pH, etc...). These changes to local CO_2 concentrations and aragonite saturation states alter the performance and survival of marine larvae and the population genetic composition of marine invertebrates.

The larval stage, particularly of calcifying marine organisms, is already highly vulnerable to biotic and abiotic stressors, and the response to stress is likely be exacerbated in a high CO_2 environment. However, there are indications that evolutionary adaptation (Kelly et al., 2013; Pespeni et al., 2013, Sunday et al., 2013) and rapid acclimatization (Parker et al., 2011, Putnam and Gates 2015) may be possible, at least in some species of marine invertebrates.

Here, we tested the effects of changing pH and pCO_2 on the calcifying larvae of geoduck clams. We initially exposed D-hinge geoduck clam larvae to ambient (~8.0) and low pH (~7.4) for 6 days (Fig. 2). We then split the remaining larvae at pH 7.4 into pH 7.4 and 7.0 and measured them again at 10 days. ***We found that in both portions of the experiment, larval mortality was decreased and shell size increased in the lower pH conditions (Fig. 3).***

LARVAE PERFORM BETTER AT LOWER pH

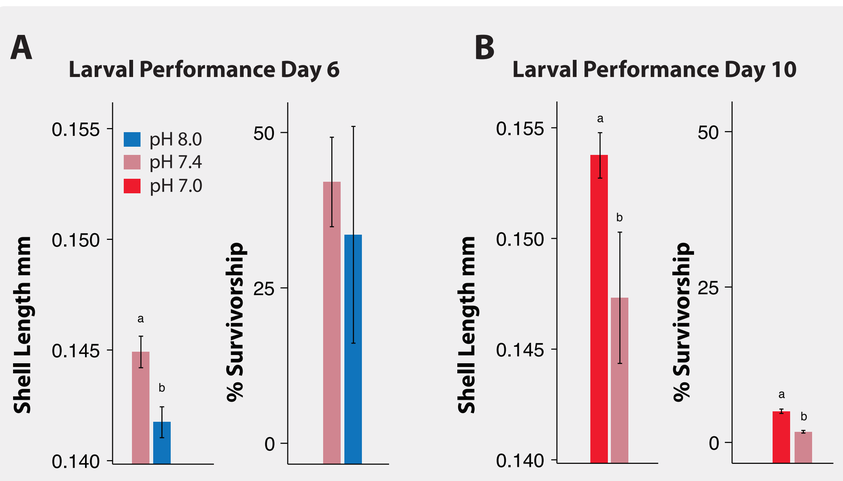

Figure 3. Larval size and survivorship at A) Day 6 and B) Day 10 of the experimental exposures.

EXPERIMENTAL DESIGN

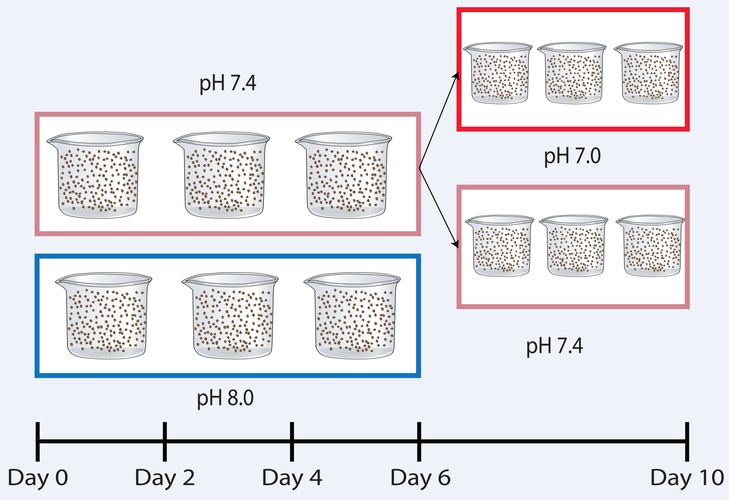

Figure 2. Experimental work was conducted in temperature regulated circular tanks. Larvae were fed a mix of flagellates and diatoms at a concentration of ~100k cells per ml. Tanks were dropped and cleaned every 2 days between day 0 and 6 for larval counting and photographs. At Day 6, the pH 7.4 survivors were split into pH 7.4 and 7.0. Final survivorship and larval size measures were made on day 10. Larval size was measured as the shell length parallel to the hinge from images taken under a comound microscope and analyzed in imageJ.

JUVENILES SHOW COMPENSATORY GROWTH AND RESISTANCE DUE TO LOW pH CONDITIONING

There is growing evidence for acclimatization to environmental change in marine organisms, where exposure to stressors can prepare organisms for future stress (Foo and Byrne, 2016). This suggests an environmental "memory" may be used to increase resistance to ocean change.

We exposed juvenile geoduck clams to low pH treatments, and found there is a benefit of preconditioning or acclimatization to low pH (Fig. 3). We exposed juvenile geoduck to ambient (~8.0), low (~7.4) and lower (~7.0) pH for 23 days and placed them in an ambient common garden for several months. In geoduck juveniles there was a size benefit of preconditioning to low pH. Juvenile growth initially declined at pH ~7.4 and 7.0 in the first exposure, but when replaced in the ambient conditions, the initial exposure to low pH resulted in compensatory growth, such that the juveniles grew larger (Fig. 3A).

After exposure to the ambient common garden conditions, juveniles from each initial pH condition were then re-exposed to ambient (~8.0) pH and low pH (~7.4) for another 23 days. The pre-exposed juveniles were more resistant to changes in growth when exposed to low pH for a second time (Fig. 3 B,C).

Our work indicates an exposure memory of the original stressor and that acclimatization to ocean acidification can result in benefits to geoduck growth. This memory may be in the form of DNA methylation.

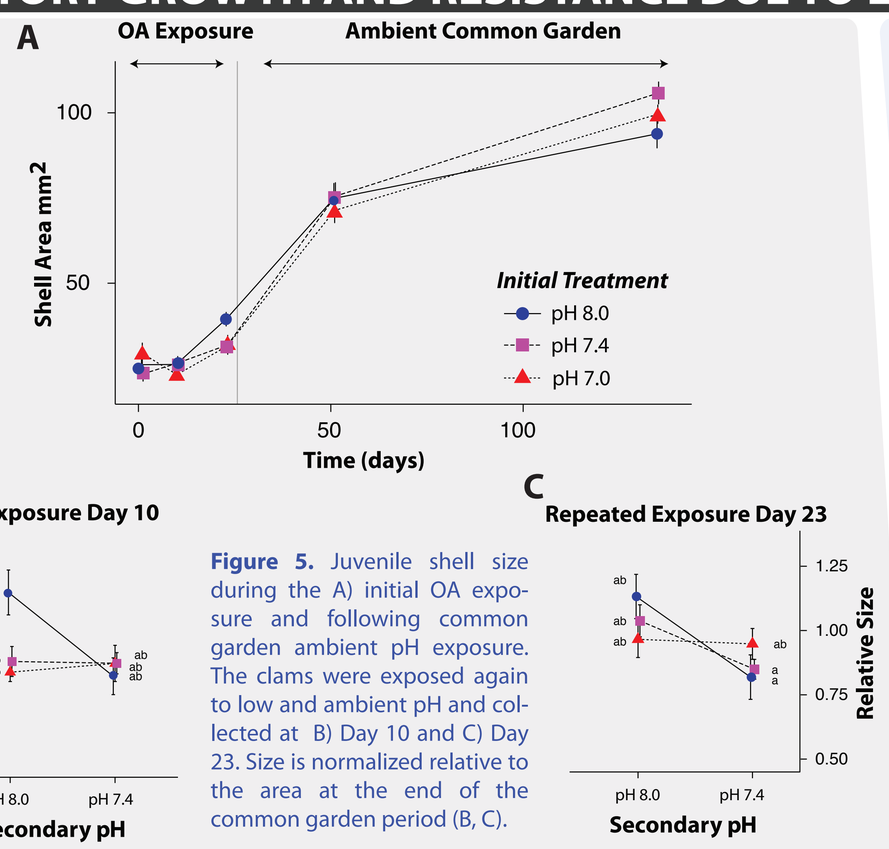

Figure 5. Juvenile shell size during the A) initial OA exposure and following common garden ambient pH exposure. The clams were exposed again to low and ambient pH and collected at B) Day 10 and C) Day 23. Size is normalized relative to the area at the end of the common garden period (B, C).

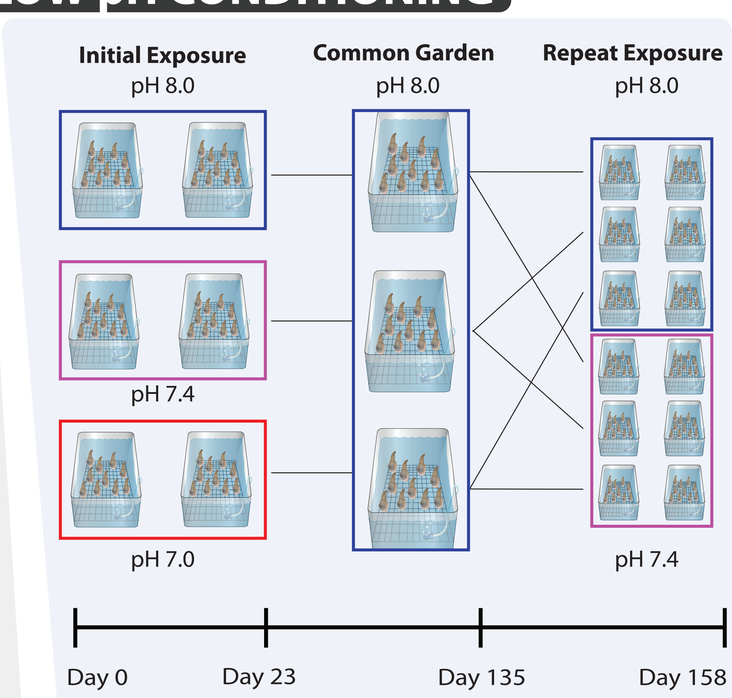

Figure 4. Juvenile geoduck seed were obtained at ~3.5 months of age and place in a layer of sand in the bottom of replicate tanks. Samples were collected and growth measures made on Day 0, Day 10, Day 23, Day 51, Day 135, Day 145, and Day 158. Size was measured as the area of one side of the shell from images taken under a dissecting microscope and analyzed in imageJ. Samples were preserved for molecular analysis to examine epigenetic response, or the relationship between DNA methylation and performance under ocean acidification treatments.

ACCLIMATIZATION AND EXPOSURE MEMORY MAY BE LINKED TO EPIGENETICS AND DNA METHYLATION

While the role of standing genetic variation has obvious implications for adaptation, we know considerably less about the role of epigenetic variation. Epigenetic mechanisms refer to phenomena that change gene activity without alteration to the underlying DNA sequence.

Common epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNA activity. Importantly, some epigenetic changes are heritable. The most well-studied epigenetic mechanism is DNA methylation, which refers to the addition of a methyl group to position 5 of cytosines (Fig. 6 A, C).

DNA methylation levels were compared among pH treatments for the juveniles. Reduced representation bisulfite libraries were generated, sequenced, and mapped to the draft geoduck genome generated for this project. The bisulfite treatment ('BS') converts any non-methylated cytosine into a uracil, while methylated cytosine are not converted. It is therefore posssible to obtain base pair resolution information on DNA methylation via next generation sequencing.

Methylation anlaysis (CpG context) identified potential regions of DNA methylation differences between the treatments. These regions will be tested and compared across the additional sampling points.

Figure 7. The DNA methylation response at Day 10 of the initial exposure is not dramatic across the whole draft genome (A), matching the physiological response above (Fig. 5A). Correlation of the methylation data across the genome between samples shows high similarity. The RRBS data provide loci of interest with regards to differential methylation (C, D, E). Further sequencing of samples from Days 135 and 145 will determine if the compensatory growth and pH resistance are linked to DNA methylation changes and "memory".

Figure 6. DNA methylation was assessed using a reduced representation bisulfite sequencing (RRBS) approach. The restriction enzyme MSPI was used to cut at the sequence C-CGG and generate small fragments for library preparation and sequencing. These fragments were bisulfite treated to convert un-methylated cytosine to uracil. Samples were barcoded and prepped to generate Illumina libraries that were sequenced 2x100bp on the Hiseq 2500 (v4 high output mode). Sequences were quality controlled, mapped, and methylation data called. These data were analyzed using clustering and genome mapping to determine location of potential differentially methylated regions.

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