**Completion Report**

**Period 2/1/2013 - 1/31/2014**

**Project R/OCEH-4 - Effects of ocean acidification on declining Puget Sound calcifiers**

## STUDENTS SUPPORTED

No Students Reported This Period

## CONFERENCES / PRESENTATIONS

Black abalone restoration threats Ocean acidification, public/profession presentation, 10 attendees, 2013-11-19
Ocean acidification effects on marine shellfish. College of the Environment Donor Appreciation Event, public/profession presentation, 80 attendees, 2013-11-07
Ocean acidification effects on marine shellfish. 2013 Think Evolution Summer Institute at ONRC in Forks, WA, public/profession presentation, 25 attendees, 2013-08-06
Ocean acidification effects on marine shellfish. The San Juan Marine Resources Committee meeting, Friday Harbor, WA, public/profession presentation, 40 attendees, 2013-02-28
Transgenerational effects of ocean acidification effects on marine shellfish. University of Southerin Mississippi-Gulf Coast Research Lab, Biloxi, MS., public/profession presentation, 40 attendees, 2013-11-13

## ADDITIONAL METRICS

| | |
|---|---|
| K-12 Students Reached | Acres of degraded ecosystems restored as a result of Sea Grant activities |
| Curricula Developed | Resource Managers who use Ecosystem-Based Approaches to Management |
| Volunteer Hours | HACCP - Number of people with new certifications |
| Cumulative Clean Marina Program - certifications | |

## PATENTS AND ECONOMIC BENEFITS

No Benefits Reported This Period

## TOOLS, TECH, AND INFORMATION SERVICES

| Description | Developed | Used | Names of Managers | Number of Managers |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Ocean acidification biology curriculum. R/OCEH-4 | Actual (2/1/2013 - 1/31/2014) | 0 | 1 | 0 |
| | Anticipated (2/1/2014 - 1/31/2015) | 0 | 0 | |
| Transcriptome for Olympia oyster, pinto abalone, and manila clam larvae. R/OCEH-4 | Actual (2/1/2013 - 1/31/2014) | 1 | 1 | 0 |
| | Anticipated (2/1/2014 - 1/31/2015) | 0 | 0 | |

**HAZARD RESILIENCE IN COASTAL COMMUNITIES**

No Communities Reported This Period

**ADDITIONAL MEASURES**

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

Number of fishers using new techniques
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

Sustainable Coastal Development
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

Coastal Ecosystems
Actual (2/1/2013 - 1/31/2014)
Anticipated (2/1/2014 - 1/31/2015)

**PARTNERS**

Partner Name AquaTechnics, Inc.

Partner Name Baywater Inc.

Partner Name Northwest Fisheries Science Center (US DOC

Partner Name Pacific Marine Environmental Laboratory (US DOC, NOAA, OAR, PMEL)

Partner Name Taylor Shellfish Company

Partner Name Taylor Shellfish Resources, Research and Design

Partner Name University of Washington, Friday Harbor Laboratories, College of the Environment

Partner Name University of Washington, School of Marine and Environmental Affairs, College of the Environment

**IMPACTS AND ACCOMPLISHMENTS**

Title Washington Sea Grant research finds bivalve winners and losers as the ocean acidifies

Type impact

Relevance, Response, Results  Relevance Ocean acidification has arrived in the Pacific Northwest, at levels surpassing end-of-century predictions. Understanding its effects on ecologically and economically important marine shell-builders is imperative, especially in vulnerable early life stages, in adult reproduction, and via carry-over effects from parent to offspring.  Response Washington Sea Grant-funded laboratory experiments examined the responses of five bivalve species (Olympia oyster, Pacific oyster, pinto abalone, geoduck clam, and Manila clam) to combinations of three stresses dissolved carbon dioxide, elevated water temperature, and exposure to the bacterium Vibrio tubiashii. Researchers also used their work as the basis for a high school biology curriculum on acidification. Results As dissolved CO2 levels were increased, V. tubiashii reached pathogenic bloom levels faster but did not show greater pathogenicity. Larval and juvenile Manila clams and juvenile geoducks did not show any adverse effects from increased CO2 in the range tested. Exposed adult Olympia oysters saw delayed larval release and reduced fecundity. When broodstock were conditioned and held at the same CO2 levels as their parents, they suffered no effects on survival, growth or shell morphology. However, a measurable change in gene expression suggests that growing up in more acidic environments may be energetically costly. Larval Pacific oysters and pinto abalone fared worst when matured under low CO2 but exposed to high-CO2 upwelling events; such events may be more stressful than gradual increases in CO2. The curriculum the researchers developed helped more than 800 students understand acidification.

Recap  Washington Sea Grant-funded research found that bivalve species exhibit different susceptibility to increasing CO2 levels. Under the conditions tested, clams were relative winners and oysters losers. Educational outreach heightened student understanding of acidification.

Comments  Primary Focus Area OCEH (SSSS) Secondary Focus Area OCEH (HCE), COCC (HRCC) State Goals Improve understanding and management of emerging and cumulative threats to ocean and coastal health (SSSS, Supply).  Improve understanding and management of emerging and cumulative threats to ocean and coastal health (HCE, Science).  Improve understanding of coastal hazards and environmental change and develop tools and approaches for observation, prediction, planning and adaptation (HRCC, Risks).

Related Partners , , , , , , ,

**PUBLICATIONS**

Title The Effects of Ocean Acidification on Multiple Life History Stages of the Pacific Oyster, Crassostrea gigas Implications for Physiological Trade-offs.

Type Full theses / Dissertations Publication Year 2014 Uploaded File TimminsSchiffman_washi....6.pdf URL none

Abstract  As global climate change accelerates, due in large part to increasing emissions

of carbon dioxide and other greenhouse gases from fossil fuel use, agriculture, and largescale changes in land use, natural ecosystems bear the consequences. For marine systems these include increased mean seawater temperature, changes in carbonate chemistry equilibria, and increased pollutant loading due to non-point run-off, among other effects. Human-induced environmental changes will not have the same magnitude of effect in all regions, but on average the changes occurring are rapid and significant. Natural populations will either need to acclimatize and/or adapt, or shift their ranges to enable continued existence. This dissertation explores the effects of ocean acidification on the Pacific oyster, Crassostrea gigas. Oysters are sedentary and inhabit a naturally variable environment (the intertidal zone) and thus may be pre-adapted to withstand rapid environmental change. Oysters and similarly sedentary organisms are ideal for investigating the effects of environmental change on biology because they are not able to escape these changes, but must respond physiologically (acclimatize) if they are to survive. Due to this ecological history, oysters provide a model that allows us to explore potential physiological mechanisms that are needed in a response to specific environmental changes as well as the limits of these mechanisms. In the first chapter, the effects of elevated partial pressure of CO2 (pCO2, a major driver of ocean acidification) on oyster larvae are explored. Larvae were exposed to low pH during early development, a period that included the transition from energetic 4 dependence on maternally derived lipids to dependence on exogenous resources. Larvae were found to experience a developmental delay at elevated pCO2, manifested as smaller size and slower rate of shell deposition. These significant effects of ocean acidification on early larval development may indicate a bottleneck in the oyster life cycle as the pH of marine waters decreases. Subsequent research has shown that these effects at early larval stages can carry over into later stages after settlement in another oyster species (Hettinger et al. 2012). In order to better understand the effects of environmental change on oyster physiology, we developed proteomic tools to explore changes in protein pathways in oyster gill (ctenidia) tissue. The second chapter explores the gill proteome (suite of expressed proteins) of adult oysters. Characterization of the proteome provides insight into the physiological mechanisms that may be available to the oyster during response to an environmental stress. The results revealed that the ctenidia proteome includes a diverse array of proteins that accomplish many functions and that it is a metabolically active tissue. The proteome sequencing lays the groundwork for exploring how ocean acidification affects various proteomic pathways in the tissue that acts as the interface between the oyster and its environment. Lastly, the adult oyster response to ocean acidification and a second stress are explored via proteomics, fatty acid profiles, glycogen content, shell microstructure, and mortality in response to heat shock. There was a significant impact of ocean acidification on oyster shell integrity, but no effects after one month of exposure on relative amounts of fatty acid, glycogen or response to acute heat shock. Through the proteomic analysis, we revealed an active and significant proteomic response to ocean acidification exposure, uncovering some of the mechanisms behind the observed macro-phenotypic changes. Additionally, the proteomic response to mechanical stimulation was largely altered between low and high pCO2, suggesting that ocean acidification can fundamentally change how oysters respond to a second stress.

Citation Timmins-Schiffman, E. 2014. The Effects of Ocean Acidification on Multiple Life History Stages of the Pacific Oyster, Crassostrea gigas Implications for Physiological Trade-offs.University of Washington.

Copyright Restrictions + Other Notes

Journal Title PhD University of Washington

Title Characterizing the effects of ocean acidification in larval and juvenile Manila clam, Ruditapes philippinarum, using a transcriptomic approach.

Type Thesis / Dissertation abstracts Publication Year 2012 Uploaded File Metzger_D_MS_Sp12.pdf URL none

Abstract Ocean acidification as a result of anthropogenic carbon dioxide ($CO_2$) emissions and global climate change poses a risk to the ecological landscape of intertidal and shallow subtidal communities. The organisms that inhabit these waters will have to cope with changing environmental conditions through the appropriate modulation of physiological processes. Calcifying organisms are particularly at risk, as increased atmospheric levels of $CO_2$ in the atmosphere increase the partial pressure of $CO_2$ ($pCO_2$) in the oceans. Increased $pCO_2$ reduces the saturation of carbonate minerals required to form calcified structures. Being able to cope with the increased energetic demand of maintaining these structures, in addition to other vital physiological processes, will be the key driver that determines which organisms will persist. Assessment of larval and juvenile Manila clam mortality and physiology in this study suggests that this species is capable of coping with elevated $pCO_2$ conditions. The use of high throughput sequencing and RNA sequence analysis in larval clams revealed several physiological processes that play important roles in the Manila clam's ability to tolerate elevated $pCO_2$ conditions during this life stage. Exposure of juvenile Manila clams, acclimated to elevated $pCO_2$ conditions, to a thermal stress revealed that this species might also be capable of coping with multiple stressors associated with global climate change. Manila clams could therefore represent a model for studying physiological mechanisms associated with successful acclimation of populations to ocean acidification.

Citation Metzger, D.C.H. 2012. Characterizing the effects of ocean acidification in larval and juvenile Manila clam, Ruditapes philippinarum, using a transcriptomic approach. University of Washington.

Copyright Restrictions + Other Notes

Journal Title MS Thesis

Title Ocean acidification and disease How will a changing climate impact Vibrio tubiashii growth and pathogenicity to Pacific oyster larvae?

Type Full theses / Dissertations Publication Year 2012 Uploaded File Dorfmeier_washington_0....6.pdf URL none

Abstract Vibrio tubiashii (Vt) is a causative agent of vibriosis in molluscan bivalves. Recent re-emergence of vibriosis in economically valuable shellfish, such as the Pacific oyster (Crassostrea gigas) in Washington State, has increased the urgency to understand the ecology of this pathogen. It is currently unknown how predicted environmental changes associated with ocean acidification, such as elevated surface seawater temperature, increased partial pressure of $CO_2$

(pCO2), and Vt abundance, will impact marine organismal health and disease susceptibility. This study investigates how environmental cues predicted with ocean acidification influence physiological changes and pathogenicity in Vt. Using laboratory experiments to manipulate temperature and pCO2, we examined how these environmental factors influenced pathogen growth. Larval susceptibility to vibriosis was determined by exposing C. gigas larvae to a combination of elevated pCO2 and Vt concentrations. These experiments provide insight into the environmental parameters that may drive pathogenicity or influence proliferation of the bacterium. Investigation of single and multivariate parameters such as temperature, pCO2, and pathogen levels will help assess how predicted shifts in ocean conditions can impact shellfish survival and disease resistance.

Citation Dorfmeier, E.M. 2012. Ocean acidification and disease How will a changing climate impact Vibrio tubiashii growth and pathogenicity to Pacific oyster larvae? University of Washington.

Copyright Restrictions + Other Notes

Journal Title UW Thesis

Title Genomic resource development for shellfish conservation concern.

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year 2012 Uploaded File none URL DOI 10.1111/1755-0998.12052

Abstract Effective conservation of threatened species depends on the ability to assess organism physiology and population demography. To develop genomic resources to better understand the dynamics of two ecologically vulnerable species in the Pacific Northwest of the United States, larval transcriptomes were sequenced for the pinto abalone, Haliotis kamtschatkana kamtschatkana, and the Olympia oyster, Ostrea lurida. Based on comparative species analysis the Ostrea lurida transcriptome (41 136 contigs) is relatively complete. These transcriptomes represent the first significant contribution to genomic resources for both species. Genes are described based on biological function with particular attention to those associated with temperature change, oxidative stress and immune function. In addition, transcriptome-derived genetic markers are provided. Together, these resources provide valuable tools for future studies aimed at conservation of Haliotis kamtschatkana kamtschatkana, Ostrea lurida and related species.

Citation Timmins-Schiffman, EB, Friedman, CS, Metzger, DC, White, SJ, Roberts, SB. 2013. Genomic resource development for shellfish conservation concern. Molecular Ecology Resources doi 10.1111/1755-0998.12052

Copyright Restrictions + Other Notes

Journal Title . Molecular Ecology Resources

Title Elevated pCO2 causes developmental delay in early larval Pacific oysters, Crassostrea gigas.

Type Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year 2013 Uploaded File Timmins_Schiffman_et_a....3.pdf URL http //link.springer.com.offcampus.lib.washington.edu/journal/227/160/8

Abstract Increasing atmospheric CO2 equilibrates with surface seawater, elevating the concentration of aqueous hydrogen ions. This process, ocean acidification, is a future and contemporary concern for aquatic organisms, causing failures in Pacific oyster (Crassostrea gigas) aquaculture. This experiment determines the effect of elevated pCO2 on the early development of C. gigas larvae from a wild Pacific Northwest population. Adults were collected from Friday Harbor, Washington, USA (48_31.70N, 12_1.10W) and spawned in July 2011. Larvae were exposed to Ambient (400 latm CO2), MidCO2 (700 latm), or HighCO2 (1,000 latm). After 24 h, a greater proportion of larvae in the HighCO2 treatment were calcified as compared to Ambient. This unexpected observation is attributed to increased metabolic rate coupled with sufficient energy resources. Oyster larvae raised at HighCO2 showed evidence of a developmental delay by 3 days post-fertilization, which resulted in smaller larvae that were less calcified.

Citation Timmins-Schiffman, E, O'Donnell, MJ, Friedman, CS, Roberts, SB. 2012. Elevated pCO2 causes developmental delay in early larval Pacific oysters, Crassostrea gigas. Mar. Biol. 160(8) 1973-1982.



Journal Title Marine Biology

**OTHER DOCUMENTS**

No Documents Reported This Period

**LEVERAGED FUNDS**

No Leveraged Funds Reported This Period

**COMPLETION NARRATIVE**

Uploaded File Friedman_9615_completi....2.pdf

**WASHINGTON SEA GRANT PROGRESS REPORT**
for the period 2/1/2009 – 1/31/2014

WSG Project Number: R/OCEH-4
Project Title: Effects of ocean acidification on declining Puget Sound calcifiers

Principal Investigator and Affiliation:
**Carolyn Friedman** University of Washington, School of Aquatic & Fishery Sciences

**Problem addressed**

Since the beginning of the industrial revolution the release of carbon dioxide ($CO_2$) from our industrial and agricultural activities has resulted in an increase in atmospheric $CO_2$ concentrations from approximately 280 to 387 parts per million (ppm). The atmospheric concentration of $CO_2$ is now higher than experienced on Earth for at least the last 800,000 years, and is expected to continue to rise at an increasing rate, leading to significant temperature increases in the atmosphere and the ocean in the coming decades. In fact, $CO_2$ levels in the atmosphere may double over its pre-industrial levels by the year 2050; this rapid change is more dramatic than at any time in the past 20 million years. During this time, the ocean has absorbed more than 550 billion tons of $CO_2$ from the atmosphere, ~33% of anthropogenic carbon emissions. This absorption has benefited humankind by significantly reducing greenhouse gas levels in the atmosphere, thereby reducing the realized global warming. However, when the anthropogenic $CO_2$ is absorbed by seawater, chemical changes occur that increase the $CO_2$ partial pressure ($pCO_2$) and reduce seawater pH, concentration of carbonate ion ($CO_3^{2-}$) and saturation states ($\Omega$) of both calcite ($\Omega_c$) and aragonite ($\Omega_a$) minerals of calcium carbonate in a process commonly referred to as ocean acidification or OA. As a result, the pH of ocean surface waters has already decreased by about 0.1 pH units, equivalent to an overall increase in the hydrogen ion concentration or "acidity" in surface waters of approximately 30%, and is expected to decline by another 0.3–0.4 units by the end of this century. In coastal regions OA can interact with other natural and human-induced changes to hasten localized declines in pH and carbonate mineral saturation states.

Global warming and OA are expected to have important broad ranging effects on the marine environment. However, accurate predictions of these effects are not possible due to a dearth of biologically meaningful empirical data to inform models. Approaches that include the broad disciplines of oceanography, biology, and fisheries can bridge this gap, and nowhere is the need for information greater than in the inland and coastal marine waters of Washington state. Not only is Puget Sound one of the largest shellfish producing regions in the west coast industry and a key interface between coastal communities and the marine environment, it is also one of the first regions likely to experience the effects of OA on important marine calcifiers due to local upwelling events and the low buffering capacity of seawater in this region. While corals may be the sentinel organisms for tropical and subtropical seas, OA threatens to undermine foundational primary consumer taxa, such as mollusks, crustaceans, and echinoderms, in temperate ecosystems. In fact, benthic biodiversity near deep sea vent communities, where high $pCO_2$ and low pHs are found, was markedly reduced. They noted a transition from a hard corals, gastropods, urchins and coralline algae community to an area dominated by sea grasses with a

higher abundance of exotic species. Losses of calcifying marine organisms will lead to changes in local biodiversity, trophic relationships and other key processes. Here we endeavored to examine the relationship between OA conditions and declining native marine mollusc species with ecological, economic and social importance in the Pacific Northwest of the USA (see reviews by Cooley and Doney 2009, Feely et al. 2012 – Blue Ribbon Panel Report).

***We hypothesized that environmental stressors (elevated temperature and increased atmospheric $CO_2$ levels) and related changes in seawater chemistry will influence larval molluscan physiology, behavior and survival.***

**OBJECTIVES:** Our overall research objective was to: ***Characterize the relationship between altered environmental conditions and health of larval molluscs under controlled conditions.***

Specific objectives were to:

I. Characterize responses observed in early life stages of four marine molluscan species to multiple stressors, which include increased dissolved $pCO_2$, varying temperature and the pathogen *Vibrio tubiashii* in controlled laboratory studies.
II. Characterize molecular responses to selected stressors for future use under field conditions as predictors of environmental stressors experienced.
III. Describe response similarity between two species with a similar larval strategy.

Specifically, we hypothesized that molluscan early life stages will:

1) perform best at pre-industrial $pCO_2$ levels than at current and future $pCO_2$ levels;
2) show reduced thermal tolerance with increased $pCO_2$ levels;
3) show reduced fertilization and hatching rates at elevated $pCO_2$ levels;
4) show reduced larval survival at elevated $pCO_2$ levels;
5) show reduced metabolic activity and performance at sublethal increased $pCO_2$ levels, even those at which aragonite is supersaturated;
6) experience negative impacts on normal physiological processes at increased $pCO_2$ levels (and reduced pH) and elevated temperatures relative to current levels;
7) show increased larval mortality and shell dissolution under highly elevated $pCO_2$ levels observed in some nearshore areas;
8) be more susceptible to *V. tubiashii* at combined elevated $pCO_2$ levels (and reduced pH) and elevated temperatures relative to current levels.

## Methods and Results

### Larval Trials

**Geoduck clams:** Collaborating with NOAA scientists we completed two studies on larval geoduck. For both, geoduck larvae were fertilized in ambient seawater and then transported to NOAA NWFSC where they were placed in duplicate systems as fertilized embryos (n=6 larval jars per system) and maintained at 14 C. The first study lasted 3 d and the second was run for 21 d. Duplicate systems were maintained at 280 (pre-industrial), 400 (current) and 1000 µatm (future) $CO_2$. We examined survival, growth and calcification in addition to seawater chemistry.

Survival was similar among treatments ($p>0.05$; Figure 1). pH levels in the treatments and individual larval jars within treatments were consistent over the course of the study. Larvae held at 280 µatm experienced a mean pH of 8.17, while those at 400 µatm experienced a pH of 8.03 and those at 1000 µatm experienced a pH of 7.67 (Daniel Bascom, UW-SAFS, Capstone project 2011). These data suggest that larvae of the infaunal geoduck appear resilient to the effects of OA using the parameters we measured in our study.

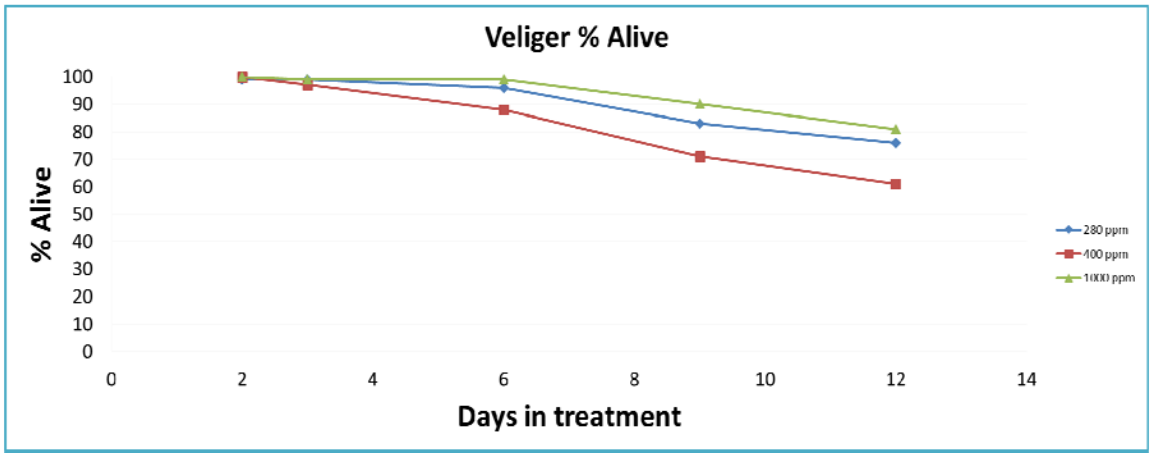



Figure 1. Survival of Geoduck clams *Panopea generosa* larvae at three $pCO_2$ levels over a 12 day period. Differences in survival were statistically similar after 12 days ($p>0.05$).

**Manila clams.** As larval native littleneck clams are not yet available, we examined the susceptibility of Manila clams, which share life history traits with littleneck clams. Manila clam gametes were fertilized in ambient seawater and then transported to NOAA NWFSC where they were placed in duplicate systems as fertilized embryos (n=6 larval jars per system) and maintained at 18 °C. Duplicate systems were maintained at 355 ± 17 µatm and 898 ± 48 µatm $CO_2$. We examined survival, growth and calcification in addition to seawater chemistry. Larvae held at 355 µatm experienced a pH of 8.07 and those at 898 µatm experienced a pH of 7.71. No difference in survival or growth was observed between treatments ($p>0.05$; Figure 2). RNA Seq analysis of clam

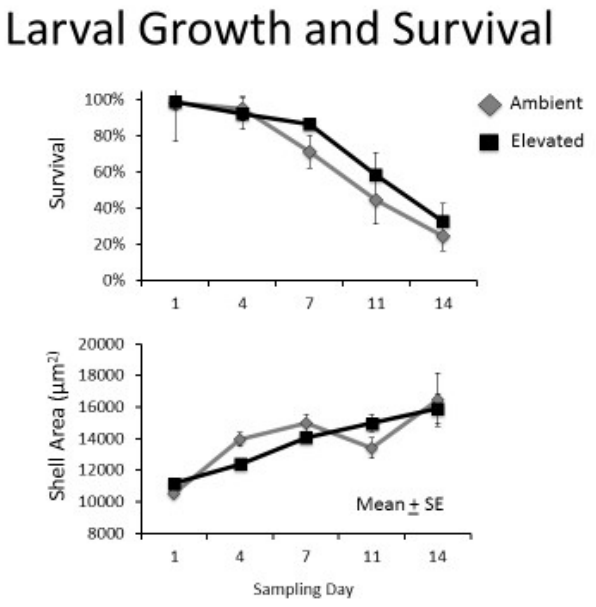



Figure 2. Manila clam survival and growth upon exposure to ambient (400 µatm) and elevated (1000 µatm) $pCO_2$. No differences in measured parameters were observed between larvae in the two treatments ($p>0.05$).

transcriptomes collected mid-way through the trials revealed that increased $pCO_2$ resulted in an overall upregulation of mRNA transcripts (96%), versus only 4% of transcripts were down-regulated relative to the control treatment (355 µatm). Candidate genes were selected for use in future studies on the physiological effects of ocean acidification on clams (Metzger 2012, MSc Thesis, UW). These data suggest that although no visible effects of OA were observed in Manila clam larvae, an energetic cost is evident based on the overall up-regulation of RNA transcripts. Thus the effects of OA may not become visible until later clam life stages.

**Pacific Oyster trials** We conducted a pilot experiment using the Pacific oyster *Crassostrea gigas* under three $pCO_2$ conditions (280 µatm, 400 µatm, and 1000 µatm). As with geoducks, oysters were fertilized in ambient seawater and then transported to NOAA NWFC where they were placed in duplicate systems as fertilized embryos (n=6 larval jars per system) and maintained at 14 C for 7 d. Duplicate systems were maintained at 280, 400 and 1000 ppm $CO_2$. We examined percent hatch, survival, growth and calcification in addition to seawater chemistry. Percent hatch was similar among treatments and ranged from 92.3 – 98.8% (280 = 97.12%, 400 = 96.09% and 1000 = 96.21%). Survival was low in all treatments due to ciliate infestations. However, survival was higher at 280 ppm than at the two higher $pCO_2$ levels. Calcification also varied among treatments. The highest percentage of larvae with calcified shells after one week exposure was observed in larvae maintained at 280 ppm $CO_2$ (83.7 $\pm$ 12.8 %) and at 400 ppm $CO_2$ (69.4 $\pm$ 14.3%). In contrast, only 33.7 $\pm$ 12.8 % of those held at 1000 ppm $CO_2$ had calcified shells. $pCO_2$ levels were very consistent throughout the study. Similarly pH of seawater in the treatments and individual larval jars within treatments were consistent over the course of the study. Mean pH was 8.17 at 280 µatm, 8.03 at 400 µatm and 7.67 at 1000 µatm. This trial demonstrated that $pCO_2$ negatively influences Pacific oyster larval survival.

**Ciliates**. We observed that ciliates, which often plague larval cultures, were found in some trials under ambient $CO_2$ conditions but were largely absent from those at elevated $CO_2$ levels. We therefore, isolated ciliates and tested their growth rates at differing $CO_2$ levels. Ciliate growth was negatively impacted by low pH conditions (Figure 3).

In a subsequent pinto abalone trial, three putative ciliate spp. (Small, Large, and Spot) that co-occurred with pinto abalone larvae were seeded into six replicate wells in tissue culture plates, prefilled with seawater at three $pCO_2$ levels: ambient (~400 µatm), 750 µatm, and 2000 µatm. Each well received 2-3 $mm^3$ of agar-based culture media as a daily ration. At 24, 48, and 72 hrs after inoculation, three separate squares on a hemocytometer were counted for each replicate for each species.

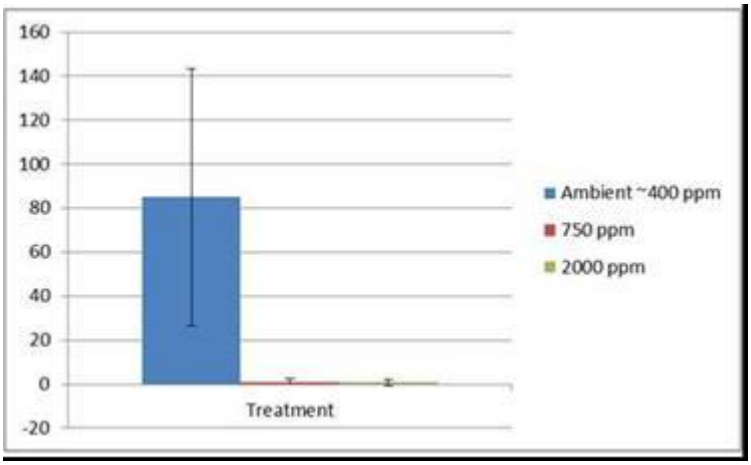


Figure 3. Survival of ciliates at different $CO_2$ levels.

The data were analyzed via ANCOVA, with hours as the covariate. There were no significant blocking effects of counts or wells. No significant difference between Large and Spot ciliate

population growth was observed (ANCOVA, t=0.193, P=0.847). Overall, the Small group population growth was significantly greater than the Large and Spot groups at both 750 µatm (ANCOVA, t=-3.415, P<0.001) and ambient (ANCOVA, t= -6.286, P<0.001). Very low population growth was observed in all groups at 2000 µatm (Figure 4).

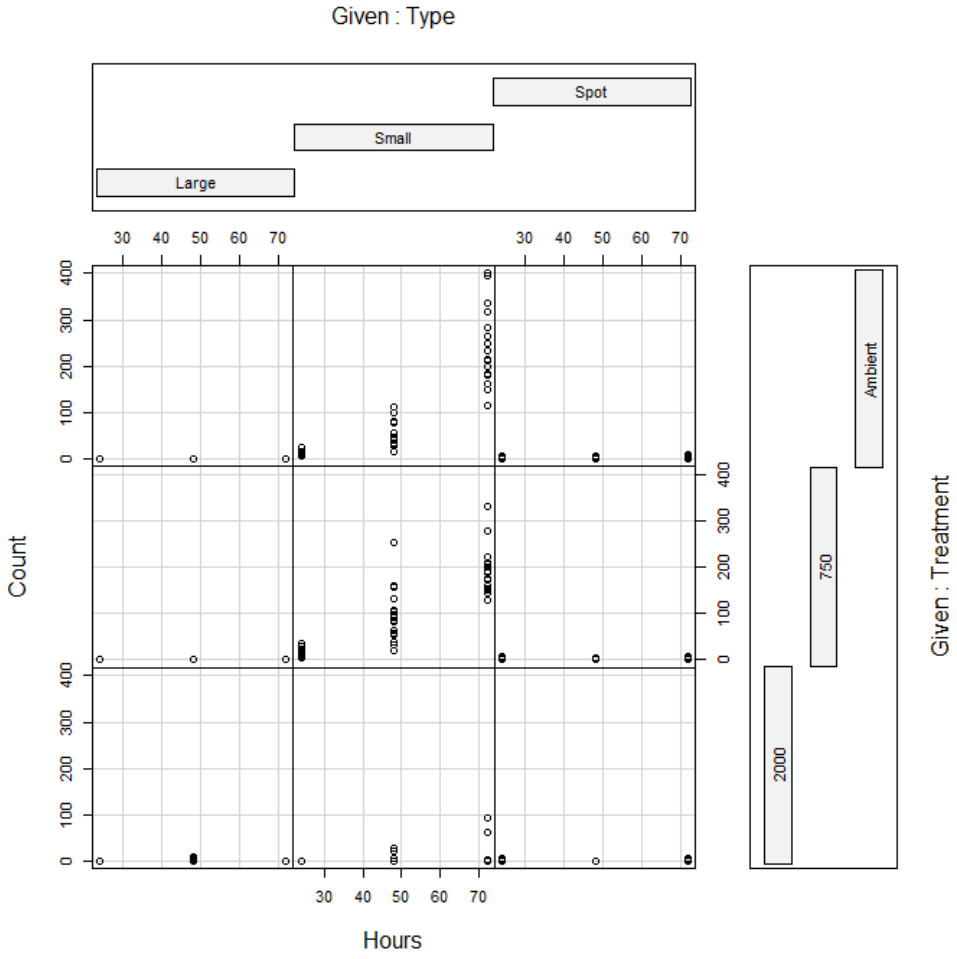



Figure 4. Population growth plot of three ciliate types (Large, Small, Spot) at three $pCO_2$ levels (Ambient, 750 µatm, 2000 µatm) over 72 hours.

### *Vibrio tubiashii* trials

**Disease susceptibility of Pacific oyster and Pinto abalone larvae at elevated $CO_2$ levels-** Disease challenges were performed exposing larvae to a combination of elevated $CO_2$ and *Vibrio tubishaii*. These experiments examined Pacific oyster and pinto abalone larval susceptibility to vibriosis caused by the bacterium at three $CO_2$ levels (ambient (~380), 750 and 2000 $\mu$atm $CO_2$)

when held at 16°C. Specialty gas mixtures of air and $CO_2$ (2000 and 750 ppm $CO_2$) (Praxair, Inc.) were used to produce elevated $pCO_2$ conditions. No significant differences in larval susceptibility were detected at elevated either elevated $CO_2$ when compared to ambient levels as shown by analysis of lethal dose to cause 50% mortality of both early and late stage veliger larvae among groups (ANOVA, $p>0.05$; Table 1).

Table 1. Calculated *V. tubiashii* $LD_{50}$ for two developmental stages of *C. gigas* larvae at 24, 48, and 72 h.

**D-veliger larvae (3 days old)**

| | Day 1 | | Day 2 | | Day 3 | |
|---|---|---|---|---|---|---|
| $pCO_2$ | $LD_{50}$ | SE | $LD_{50}$ | SE | $LD_{50}$ | SE |
| ~390 | 4.33E+07 | 5.03E+01 | 8.74E+07 | 2.84E+02 | 1.69E+05 | 4.80E+00 |
| 750 | 8.61E+05 | 1.52E+00 | 9.60E+04 | 1.37E+00 | 2.09E+04 | 1.59E+00 |
| 2000 | 6.04E+05 | N/A | 1.73E+06 | 6.26E+00 | 5.73E+03 | 4.67E+00 |

**Prodissoconch I larvae (10 days old)**

| | Day 1 | | Day 2 | | Day 3 | |
|---|---|---|---|---|---|---|
| $pCO_2$ | $LD_{50}$ | SE | $LD_{50}$ | SE | $LD_{50}$ | SE |
| ~390 | 5.85E+05 | 1.53E+00 | 4.21E+04 | 1.22E+00 | 2.70E+04 | 1.17E+00 |
| 750 | 8.00E+05 | 1.16E+00 | 6.18E+04 | 1.63E+00 | 1.96E+04 | 1.03E+00 |
| 2000 | 2.64E+05 | 2.42E+00 | 5.86E+04 | 2.19E+00 | 9.76E+03 | 1.41E+00 |

### *Vibrio tubiashii* growth under varying environmental conditions: Effect of $pCO_2$ (pH) and temperature

Trials examining differential growth of *V. tubiashii* under varying $pCO_2$ conditions (380, 840 and 2000 $\mu$atm) demonstrated differences in *V. tubaishii* growth among treatments. We examined *V. tubiashii* growth at both 16°C and 25°C to obtain growth curves for the bacterium at elevated $CO_2$ levels used in challenge trials with *C. gigas* larvae. Significant differences in bacterial growth at 16°C were seen during stationary growth at both elevated $CO_2$ levels (750 and 2000 $\mu$atm) when compared to ambient $CO_2$ conditions (Figure 5). During the exponential phase of these cultures, *V. tubiashii* grew significantly more (faster and higher level) at 2000 $\mu$atm than at ambient conditions. Differences in exponential growth at 16°C were not detected at 750 ppm when compared to ambient $pCO_2$. At 25°C however, no significant differences in bacterial growth among $CO_2$

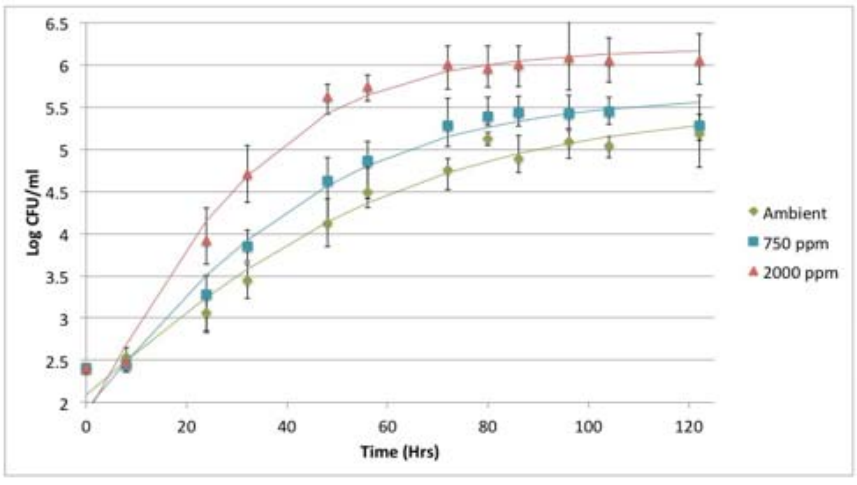



Figure 5. Growth of *Vibrio tubiashii* at 16C. Cultures were grown at three $\mu$atm $CO_2$ concentrations: ambient, 750 and 2000.

levels were detected during exponential or stationary growth after 72 hrs.

**Pinto abalone**

Pinto abalone larvae were subjected with two pCO2 levels: ambient (~400) and elevated (750 µatm) for 72 hours under static conditions. Significantly fewer abalone larvae survived elevated $pCO_2$ conditions after 48 and 72 hours (Figure 6). These data suggest that ocean acidification may reduce recruitment of pinto abalone in Washington state.

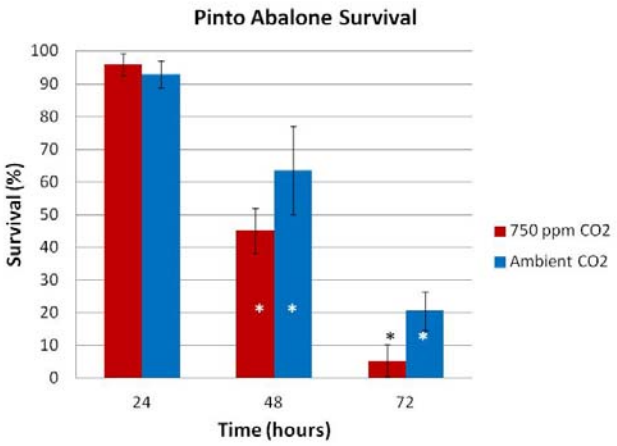



Figure 6. Differential survival of pinto abalone larvae under varying $CO_2$ levels over 72 hr period. * represents $p<0.05$.

To address concerns of the effects of ocean acidification on pinto abalone hatchery rearing, we repeated the study using the same $pCO_2$ levels but added a third concentration of 2000 µatm, a level that is slightly above those observed in Hood Canal, WA. This study revealed similar results plus an additive effect of applying a settlement inducer, gamma aminobutyric acid (GABA) to larval cultures (Figure 7). These data demonstrate that acidified conditions and GABA induction may reduce hatchery output in areas impacted by high $pCO_2$, such as Washington state.

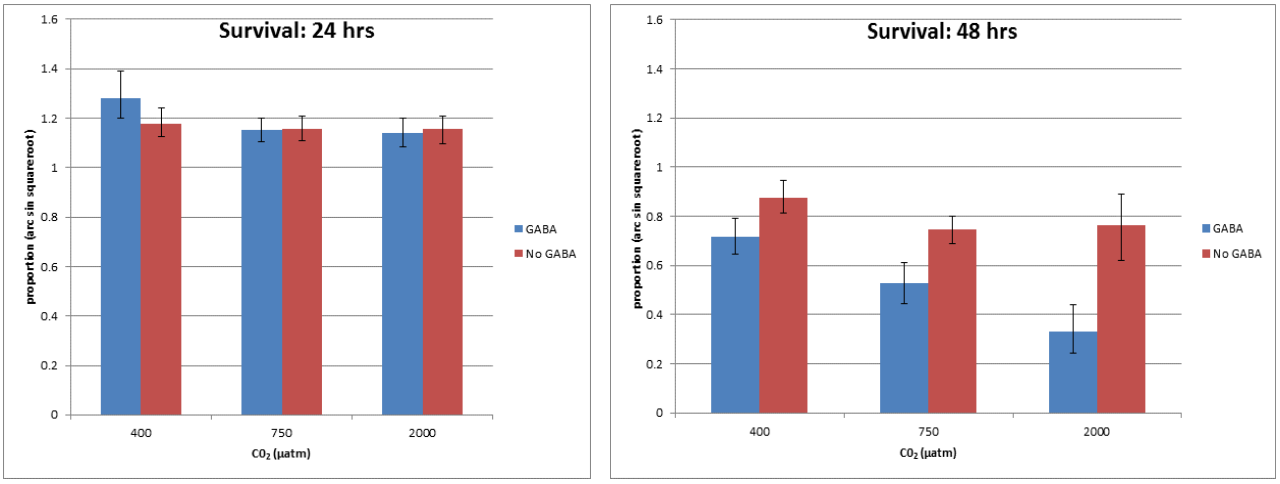



Figure 7. Pinto abalone larval survival trends. More pinto abalone survived upon exposure to near present day $pCO_2$ levels, with and without the settlement inducer gamma aminobutyric acid (GABA), at 400 µatm than high $CO_2$ treatments after 24 and 48 h of incubation ($p<0.05$ and $p<0.001$, resp.). GABA did not influence survival at 24 hours ($p>0.05$) but did influence survival after 48 hours ($p<0.001$). GABA and high $pCO_2$ combined reduced larval survival relative to elevated pCO2 alone ($p=0.017$). Error bars are 95% BCa confidence intervals.

**Transcriptomic Analyses**.

We described the transcriptome of Manila clams, pinto abalone, Olympia oysters and *Vibrio tubiashii*. As noted above, RNA Seq analysis of Manila clam transcriptomes collected mid-way through the trials revealed that increased $pCO_2$ resulted in an overall upregulation of mRNA transcripts (96%), versus only 4% of transcripts were down-regulated relative to the control treatment (355 µatm). Candidate genes were selected for use in future studies on the physiological effects of ocean acidification on clams (Metzger 2012, MSc Thesis, UW).

We used larvae from pinto abalone and Olympia oyster larvae to provide genomic resources for use in physiological studies. Figure 8 shows an example of the gene ontology groups identified from pinto abalone (Timmins-Schiffman et al. 2012).

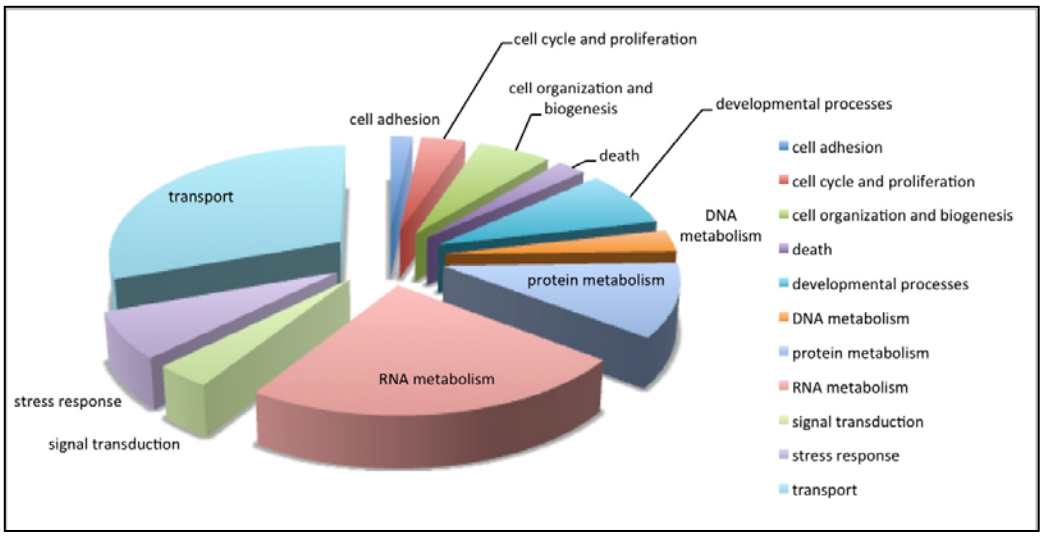


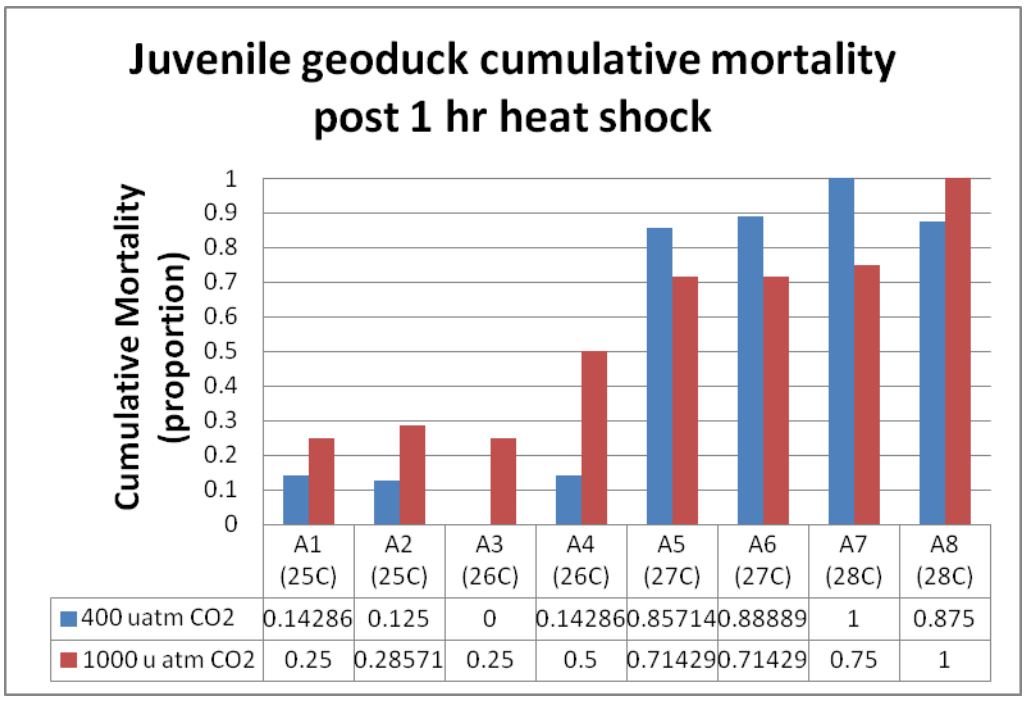

Figure 8. Pie chart of GO Slim terms for de novo assembly of pinto abalone larval transcriptome.

We also examined the genotypes of two *Vibrio tubiashii* strains: the highly pathogenic strain, RE22, and a nonpathogenic type strain, ATCC 19106. SOLiD sequencing was used to (1) describe the core genetic features of *Vibrio tubiashii* and (2) identify potential virulence proteins within the bacterial genome. Analysis of the newly sequenced genomes enabled the identification of 1,690 core *V. tubiashii* genes, including 931 associated with putative bacterial virulence. Here, we describe the genomic features of each strain and describe homologous proteins discovered with putative bacterial virulence, including pilin, secretion systems, and extracellular toxins. Three putative virulence proteins of Vt were examined in detail for variation differences: metalloprotease M6, extracellular zinc metalloprotease, and ToxR transcriptional activator. Selective protein analysis indicates high variation frequency in the RE22 genome compared to the type strain ATCC19106. Genomic data generated by this research offers important baseline genomic knowledge and the development of genomic tools that can be utilized in future functional analysis experiments with this economically important pathogen.

**Juvenile and Adult Life stages**. In addition to larval stages we also initiated trials on juvenile Pacific oysters, geoduck clams, and Manila clams.

*Crassostrea gigas*, were exposed to 6 different ocean acidification scenarios for 1 week. These 6 levels of *p*CO2 simulated current-day atmospheric levels (400 ppm) through projections extending beyond the year 2100 (1400 ppm). At the end of the 1 month exposure, oysters were sampled for transcriptomics, proteomics, and histology. Additionally, a subset of the oysters were exposed to different levels of thermal stress to assess the impacts of OA on oyster thermal response. No alternation in response to a thermal stress was observed in oysters among $pCO_2$ treatments; lethal temperature and the proportion of animals that died in each treatment were statistically similar ($p>0.05$; Timmins-Schiffman 2014, PhD dissertation).

In separate systems, we conducted a multi-species common garden study in which we exposed juvenile Manila and geoduck clams and Pacific oysters to 400 and 100 ppm $CO_2$. At the end of the month, a subset of the animals were subjected to one of three temperatures representing thermal stress: their defined lethal temperature (LT), LT-1°C, or LT-2°C. Mortality was monitored over a week and any surviving oysters sampled at the end of the week. Selected groups were also examined for feeding and metabolic rate upon termination of the study. No difference in lethal temperature was observed for any species ($p>0.05$). Figure 9 below illustrates the cumulative proportion of geoduck that died after a one hour shock of 25, 26, 27 or 28°C. In addition, feeding and respiration rates were similar between treatments within each species. These data suggest that, using the metrics we measured, that juvenile bivalves appear resilient to the effects of ocean acidification over a short term exposure.




| | A1 (25C) | A2 (25C) | A3 (26C) | A4 (26C) | A5 (27C) | A6 (27C) | A7 (28C) | A8 (28C) |
|---|---|---|---|---|---|---|---|---|
| 400 uatm CO2 | 0.14286 | 0.125 | 0 | 0.14286 | 0.85714 | 0.88889 | 1 | 0.875 |
| 1000 u atm CO2 | 0.25 | 0.28571 | 0.25 | 0.5 | 0.71429 | 0.71429 | 0.75 | 1 |

Figure 9. Cumulative mortality of geoduck juveniles one week after being given a 1 hr heat shock. After heat treatment, clams were returned to their respective $pCO_2$ conditions.

**Adult Trials**

Based on our results, we expanded our focus to include trans-generational studies to better understand the impacts of OA on the individual and population levels. We conducted experiments focusing on Olympia oysters and pinto abalone. We preconditioned Olympia oysters from several sites in Puget Sound and allowed them to mature reproductively at near current oceanic (400 µatm) and a high but currently observed $pCO_2$ level (1600 µatm; Figures 10, 11). Most of the oysters originated from near Totten Inlet, WA and showed similar trends in reproduction under near ambient conditions. However, when exposed to 1600 µatm, statistical differences in the timing and number of larvae released over time under developed (Figure 11). Collectively, these data suggest population levels effects could occur with increasing acidification. Interestingly larvae were unaffected by very high $pCO_2$ conditions based on survival and shell morphology (Figure 10 B,C). However, transcriptomic analyses suggest that post-metamorphic trade-offs may occur in order for the larvae to survive in the face of ocean acidification (Data not shown). We need to further assess the long-term effects of OA to fully understand its potential impact on this species.

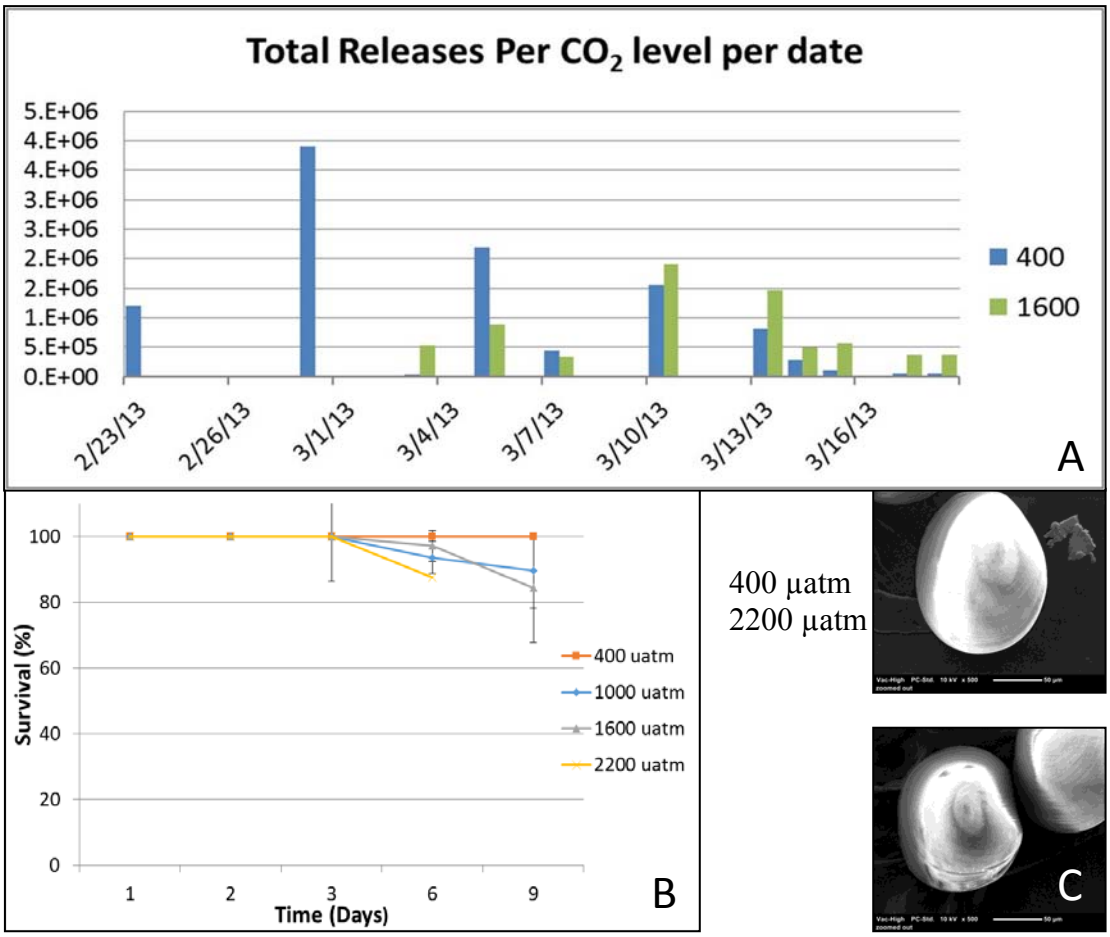



Figure 10. Increased $pCO_2$ resulted in a delay of the timing of larval release and a reduction in the fecundity of Olympia oysters (A). However larvae brooded within adults preconditioned at all four $CO_2$ levels showed similar survival when reared under the same conditions as during maturation and brooding (B). In addition, larval shells were unaffected by $pCO_2$ (C).

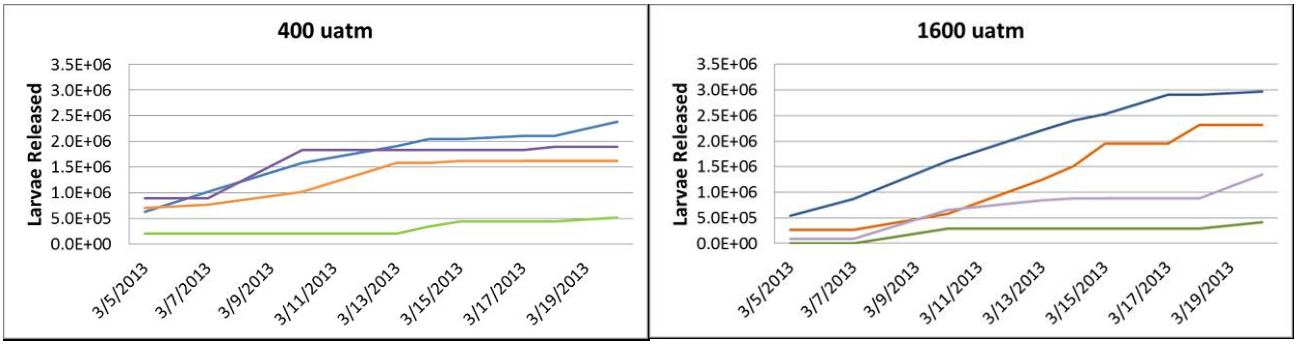



Figure 11**.** Groups of oysters from near Totten Inlet, WA (blue, purple and orange upper lines) showed differences in reproduction (timing and fecundity) at increased $pCO_2$ but not at near ambient levels. Oysters from Burley Lagoon (green lines) performed poorly in both treatments.

Pinto abalone, *Haliotis kamtschatkana*, were conditioned at two $pCO_2$ levels: ~440 (low, L) and 1200 (high, H) µatm for several weeks prior to spawning. Larvae were both held under the same conditions as during maturation and also under reciprocal conditions for a total of four treatments: HH, HL, LH and LL. In this case HH indicates larvae from parent conditioned under high $pCO_2$ and larvae from these parents were also raised under the same high $pCO_2$ conditions and so on. The latter treatment of LL is considered the control treatment.

No difference in adult maturation or spawning was observed but larval survival was reduced. Transgenerational effects of ocean acidification were seen in pinto abalone: the most rapid mortality rate and lowest survival were observed in the LH group (parents in low $pCO_2$ and larvae in high $pCO_2$) survived the worst ($p<0.01$), while all other treatments had similar survival trends ($p>0.05$; Figure 12). These data suggest that both the parental and larval conditions affect larval survival.

**Conclusions**

Collectively, these data illustrate that some species are predicted to perform well under acidified conditions, especially infaunal bivalves such as geoduck and Manila clams. These data also suggest that Pacific and Olympia oysters and Pinto abalone are negatively impacted by ocean acidification but that population effects may not be apparent when one examines a single life history stage.

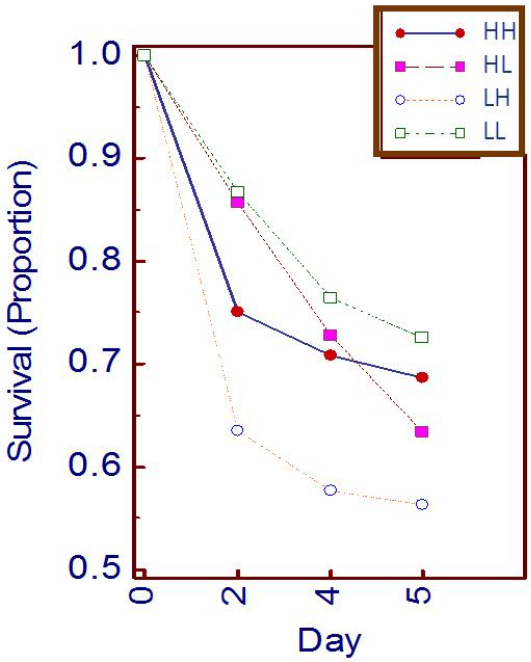



Figure 12. Pinto abalone larval survival. More larvae exposed to the LH treatment died and the mortality rate was faster than those held in the other treatments.

**Outreach**

More than eight teachers have incorporated the OAOP into their 27 classes, allowing for more than 800 high school students the access to learn current scientific issues and research approaches that we in the marine science community are presently working on. The response from both students and teachers post-OAOP has been very pleasing with assessments of student learning demonstrating a large shift in conceptual understanding of ocean acidification learning goals. Participating teachers had positive reviews of student learning and participation, and all teachers who were involved in the project want to continue using the kits and curriculum. For many of the students, this was their first exposure to "real" scientific research. Students were invested in the research outcomes of their projects and expressed feelings of accomplishment. Please see below for more information.

**PUBLICATIONS**

We have created an ocean acidification website (http://safsoa.wordpress.com/) and also provide updates on our progress on Twitter (@acid_safs). Our Twitter site (started by Steven Roberts)

has placed 4,288 tweets, and has 47 followers including local shellfish growers, the East Coast Shellfish growers Association, and Australian shellfish laboratory and an number of scientists).

**Peer-reviewed journal articles**

Timmins-Schiffman, E, O'Donnell, MJ, Friedman, CS, Roberts, SB. 2012. Elevated $pCO_2$ causes developmental delay in early larval Pacific oysters, *Crassostrea gigas*. Mar. Biol. 160(8):1973-1982.

Timmins-Schiffman, EB, Friedman, CS, Metzger, DC, White, SJ, Roberts, SB. 2013. Genomic resource development for shellfish conservation concern. Molecular Ecology Resources doi: 10.1111/1755-0998.12052

**Theses and dissertations**

Dorfmeier, E.M. 2012. Ocean acidification and disease: How will a changing climate impact *Vibrio tubiashii* growth and pathogenicity to Pacific oyster larvae?

Metzger, D.C.H. 2012. Characterizing the effects of ocean acidification in larval and juvenile Manila clam, *Ruditapes philippinarum*, using a transcriptomic approach

Timmins-Schiffman, E. 2014. The Effects of Ocean Acidification on Multiple Life History Stages of the Pacific Oyster, *Crassostrea gigas*: Implications for Physiological Trade-offs.

**Conference/Workshop activity (talk or poster), Presentation/Seminar (e.g., invited)**

**Posters**:
Influence of ocean acidification on *Panopea generosa* larvae. Dan Bascom, University of Washington and NOAA-NWFSC. Approximately 150 attendees. Pacific Coast Shellfish Growers Association

*Assessment of Manila clam larval survival and physiological changes at 400, 520, and 1000ppm pCO2 treatments* David C. Metzger, Shallin Busch, Paul McElhany, Carolyn S. Friedman, Steven B. Roberts. Approximately 150 attendees.

***Oral:***

| Year | Type | Title | Organization |
|---|---|---|---|
| 2013 | Invited symposium | Pinto abalone restoration in Washington State | NOAA |
| 2013 | Internal UW presentation | Ocean acidification effects on marine shellfish | CoEnv |
| 2013 | Invited non-professional (public) audience address | Ocean acidification effects on marine shellfish | 2013 Think Evolution Summer Institute at ONRC in Forks, WA |
| 2013 | Invited symposium | Ocean acidification effects on marine shellfish | The San Juan Marine Resources |

| | | | |
|---|---|---|---|
| | | | Committee |
| 2013 | Invited peer institution (academic) | Transgenerational effects of ocean acidification effects on marine shellfish | University of Southerin Mississippi-Gulf Coast Research Lab |
| 2012 | Submitted/awarded oral presentation | Effects of ocean acidification on the reproductive health of the Olympia oyster, *Ostrea lurida* | Pacific Coast Shellfish Growers Association-Pacific Coast Section - National Shellfisheries Association |
| 2012 | Submitted/awarded oral presentation | Effect of ocean acidification on abalone larvae and microbes | Nation Shellfisheries Association |
| 2012 | Invited symposium | Effects of ocean acidification on two declining native Washington State shellfish species: the Olympia oyster, *Ostrea lurida*, and the pinto abalone, *Haliotis kamtschatkana* | US-Russia Workshop hosted by USGS and USDA |
| 2011 | Submitted/awarded oral presentation | Influence of CO2 on pathogenicity of *Vibrio tubiashii* | American Society of Limnology and Oceanography |
| 2011 | Submitted/awarded oral presentation | Effect of ocean acidification on the health of Washington State pinto abalone | Nation Shellfisheries Association |
| 2011 | Submitted/awarded oral presentation | Restoration Aquaculture in Puget Sound | American Fisheries Society |
| 2010 | Submitted/awarded oral presentation | Effects of Ocean Acidification on Pacific Oyster (*Crassostrea gigas*) Shells: Development of a High School Curriculum | Pacific Coast Shellfish Growers Association |
| 2010 | Submitted/awarded oral presentation | Ocean Acidification and Disease: How will a Changing Climate Impact *Vibrio tubiashii* Growth and Pathogenicity | Pacific Coast Shellfish Growers Association |
| 2010 | Submitted/awarded oral presentation | Effects of Ocean Acidification on Pacific Oyster Larval Development and Physiology | Pacific Coast Shellfish Growers Association |
| 2010 | Submitted/awarded oral presentation | The development of molecular tools to monitor the physiological response of shellfish to ocean acidification | Friday Harbor Ocean Acidification Conference |

## STUDENTS SUPPORTED BY OR AFFILIATED WITH THIS PROJECT

Student Name: Samantha Brombacker
Degree track: B.S. (work study)
Whether degree was **completed** during the reporting window (NO):
New or continuing student on WSG support (NEW):
Department: School of Aquatic and Fishery Sciences
Major/Degree field: Fisheries
Capstone Professor: Friedman

Capstone title (actual or anticipated): Ocean Acidification Project: Effects of $CO_2$ on Pacific Oyster Shells and development of en educational curriculum
Date of graduation: anticipated June, 2011
If student has graduated, please provide name of current employer, if known: Sammi was supported for 3 years in large part by WSG funds.

Student Name: Annemarie Kaza Ansley
Degree track: B.S. (capstone)
Whether degree was **completed** during the reporting window (NO): Yes
New or continuing student on WSG support (NEW):
Department: School of Aquatic and Fishery Sciences
Major/Degree field: Fisheries
Capstone Professor: Friedman
Capstone title (actual or anticipated): Optimization of spawning methods for native littleneck clams
Date of graduation: June, 2011 Employer: UW

Student Name: Jessica Hale
Degree track: B.S. (work study)
Whether degree was **completed** during the reporting window (NO): Yes
New or continuing student on WSG support (NEW):
Department: School of Aquatic and Fishery Sciences
Major/Degree field: Fisheries
Capstone Professor: Friedman
Capstone title (actual or anticipated): N/A
Date of graduation: anticipated June, 2013
If student has graduated, please provide name of current employer, if known:

Student Name: Liza Ray
Degree track: Ph.D.
Whether degree was **completed** during the reporting window (NO): No
New or continuing student on WSG support (NEW):
Department: School of Aquatic and Fishery Sciences
Major/Degree field: Fisheries
Major Professor: Friedman
Dissertation title (actual or anticipated): Ocean acidification effects on native Washington state mollusks: Impacts of $pCO_2$, pathogens and microbial communities
Date of graduation: N/A

Student Name: David Metzger
Degree track: M.S.
Whether degree was **completed** during the reporting window (NO): Yes
New or continuing student on WSG support (NEW):
Department: School of Aquatic and Fishery Sciences
Major/Degree field: Fisheries
Major Professor: Roberts/Friedman

Thesis title (actual or anticipated): Physiological responses of marine shellfish to ocean acidification: Genomic approaches
Date of graduation: December 2012 and Currently PhD student at UBC

Student Name: Siri Nelson – Not directly supported by this project, partner on curriculum development
Degree track: M.S. (GK-12 Fellow)
Whether degree was **completed** during the reporting window (no): Yes
New or continuing student on WSG support (no):
Department: Department of Biology
Major/Degree field: Biology
Major Professor: Samuel Wasser
Thesis title (actual or anticipated): (?)
Date of graduation: (August, 2011)
If student has graduated, please provide name of current employer, if known: unknown

Student Name: Emma Timmins-Schiffman
Not salaried by Wa SG but a portion of her research was funded by Sea Grant.
Degree track: Ph.D
Whether degree was **completed** during the reporting window (NO): Yes
New or continuing student on WSG support (CONTINUING):
Department: School of Aquatic and Fishery Sciences
Major/Degree field: Fisheries
Major professor/Capstone advisor, if relevant: Roberts
Dissertation/Thesis/Capstone project title, if relevant (actual or anticipated): The Physiological Effects of Ocean Acidification on Multiple Life History Stages of the Pacific Oyster, Crassostrea gigas
Date of graduation (actual or anticipated): December 2013
If student has graduated, please provide any known information about their future plans (name and location of anticipated/current employer, graduate program they are entering, etc): Postdoc in Genome Sciences with Dr. Brooke Sullivan.

**PARTNERSHIPS**

| **Partner** | **Specify Type** (Academic, Government, Industry/Business, NGO, SG Program, Other) | **Specify level (**International, Federal, Regional, State, Local) | **Nature of Partnership** |
|---|---|---|---|
| *Taylor Shellfish* | *Industry* | *Local* | *Full collaboration: they provide animals for experiments and outreach, participate in studies* |

| | | | |
|---|---|---|---|
| *Baywater Inc* | *Industry* | *Local* | *Provide animals for experiments and participate in study design* |
| *Friday Harbor Marine Lab* | *Academic* | *State* | *Provide lab space for longer term trials and use of ocean chemistry laboratory* |
| *NOAA-NWFSC* | *Government* | *Federal* | *Provided lab space for Manila clam trial* |

**OUTREACH AND INFORMATION/TECHNOLOGY TRANSFER**

The Washington Sea Grant funded Ocean Acidification Outreach Project (OAOP) gave students in higher education the chance to develop experiments for incorporation into high school biology curricula. The project also gave both high school and college students the opportunity to learn in new ways. In the higher education setting, 1 undergraduate and 4 graduate students from different departments and colleges were granted the opportunity to learn through development and teaching.

The Ocean Acidification Outreach Project (OAOP) gave students in higher education the chance to develop and advertise their experiments for incorporation into high school curriculum. Since last annual reporting, the first year of in-classroom field testing came to a close with high praises from all participating students and high school teachers and all wanted to participate again for the 2011-2012 academic school year. Combining both the 2010-2011 and 2011-2012 academic school years, 1 undergraduate and 9 graduate students from varying departments and colleges assisted with development and teaching, while in the high school setting, the over 800 students and 8 teachers were given the resources to learn from an experiment about current science in real time from information and resources that may not have been readily available from normal teaching resources.

In September 2011, we presented the OAOP experimental design, curriculum, and secondary education teacher and student responses at the annual PCSGA NSA-PCS Shellfish Conference and Tradeshow in Salem, OR. The overall response from both academic and commercial attendees was very positive with many wanting to incorporate the project into their local school districts curriculum around the Pacific Northwest. Below is the list of teachers who have participated and incorporated the OAOP into their classroom(s).

| *High School* | *Teacher* | *Course* | *Dates Experiment Ran* | *Number of Classes* | *Number of Students* |
|---|---|---|---|---|---|
| Garfield HS | Paul Spangenberg | Marine Science | 11/10/2010-11/24/2010 | 5 | 150 |
| West Seattle HS | Kevin Barth | Marine Science | 1/5/2011-1/20/2011 | 3 | 90 |
| Orcas HS | Marta Branch | Marine Technology | 1/11/2011-1/22/2011 | 1 | 40 |
| Seattle Academy of Arts and Sciences | Gabe Cronin | Chemistry | 3/1/2011-3/16/2011 | 5 | 125 |
| Ballard HS | Megan Vogel | Marine Technology | 3/25/2011-4/8/2011 | 1 | 30 |
| Roosevelt HS | Margaux Isaman | Biology | 5/2/2011-5/13/2011 | 4 | 120 |
| Garfield HS | Heather Snoookal | Environmental Sciences | 5/30/11-6/10/11 | 3 | 90 |
| Garfield HS | Susan Brierly | Chemistry | 5/30/11-6/10/11 | 3 | 90 |
| Orcas HS | Marta Branch | Marine Technology | 1/3/2012-1/14/2012 | 1 | 40 |
| Ballard HS | Megan Vogel | Marine Technology | 1/3/2012-1/14/2012 | 1 | 30 |

The response from both students and teachers post-OAOP has been very positive. Below is a quote from one of the teachers after their students conducted the OA experiment:

"Thanks again for giving our students on Orcas the opportunity to do current science! The students had a very productive experience." – *Orcas HS*

**LEVERAGED FUNDS**

Ocean Acidification Outreach Project: Leveraged funds from NOAA NWFSC for curriculum development and implementation costs. Fedex costs - $150, supplies for kits - $1500 = $1650.

Threats to bivalve aquaculture and fisheries: The influence of emerging diseases and environmental change: Collaboration with a student in the Roberts lab who is funded off a Saltonstall-Kennedy proposal ($243,115) to examine the impacts of *Vibrio tubiashii* and ocean acidification on Pacific oysters. Roberts PI, Friedman co-PI.