Update Report
Period: 2/1/2012 - 1/31/2013

Friedman, Carolyn

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

:: STUDENTS SUPPORTED

Dorfmeier, Elene, edorfmei@uw.edu, University of Washington, School of Aquatic and Fishery Sciences, status:new, field of study:Fisheries, advisor:C. Friedman, degree type:MS, degree date:2012-06-01, degree completed this period:Yes
Student Project Title:
Ocean acidification and disease: How will a changing climate impact Vibrio tubiashii growth and pathogenicity to Pacific oyster larvae?

Involvement with Sea Grant This Period:
Funded graduate student

Post-Graduation Plans:
Environmental consulting
Metzger, David, unknown, University of Washington, School of Aquatic and Fishery Sciences, status:cont, field of study:Fisheries, advisor:C. Friedman, degree type:MS, degree date:2012-06-01, degree completed this period:Yes
Student Project Title:
¬Characterizing the effects of ocean acidification in larval and juvenile Manila clam, Ruditapes philippinarum, using a transcriptomic approach

Involvement with Sea Grant This Period:
Funded graduate student

Post-Graduation Plans:
graduate school (UBC PhD program)
Timmins-Schiffman, Emma, emmats@u.washington.edu, University of Washington, School of Aquatic and Fishery Sciences, status:cont, field of study:Fisheries, advisor:Roberts, degree type:PhD, degree date:2013-12-01, degree completed this period:No
Student Project Title:
Effects of ocean acidification on the Pacific oyster

Involvement with Sea Grant This Period:
Funded graduate student

Post-Graduation Plans:
employment

:: CONFERENCES / PRESENTATIONS

Pacific Coast Shellfish Growers Association, public/profession presentation, 150 attendees, 2012-09-30
US-Russia Workshop, public/profession presentation, 30 attendees, 2012-10-02

National Shellfisheries Association Conference, public/profession presentation, 300 attendees, 2012-03-30

:: ADDITIONAL METRICS

K-12 Students Reached: 250
GK-12 Ocean acidification curriculum

Curricula Developed: 1
Effects of ocean acidification on juvenile oysters

Volunteer Hours: 0

Cumulative Clean Marina Program certifications: 0

Acres of degraded ecosystems restored as a result of Sea Grant activities: 0

Resource Managers who use Ecosystem-Based Approaches to Management: 0

HACCP - Number of people with new certifications: 0

:: PATENTS AND ECONOMIC BENEFITS

No Benefits Reported This Period

:: TOOLS, TECH, AND INFORMATION SERVICES

Description		Developed	Used	Names of Managers	Number of Managers
Transcriptomic analyses of larval manila clams, pinto abalone, Olympia oysters exposed to increased CO2 to determine response to ocean acidification. R/OCEH-4, R/LME-5	**Actual** (2/1/2012 - 1/31/2013) :	1	1		0
	Anticipated (2/1/2013 - 1/31/2014) :	1	1		
Tool for shellfish breeders to manipulate Manila and native littleneck clam spawning through release of meiotic block at prophase in strip-spawned oocytes. R/OCEH-4	**Actual** (2/1/2012 - 1/31/2013) :	0	1		0
	Anticipated (2/1/2013 - 1/31/2014) :	0	1		

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

:: ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2012 - 1/31/2013) :

Number of fishers using new techniques
Actual (2/1/2012 - 1/31/2013) :

Anticipated (2/1/2013 - 1/31/2014) :	**Anticipated** (2/1/2013 - 1/31/2014) :
Sustainable Coastal Development	Coastal Ecosystems
Actual (2/1/2012 - 1/31/2013) :	**Actual** (2/1/2012 - 1/31/2013) :
Anticipated (2/1/2013 - 1/31/2014) :	**Anticipated** (2/1/2013 - 1/31/2014) :

:: PARTNERS

Partner Name: Baywater Inc., type: industry, scale: local
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: University of Washington, Friday Harbor Laboratories, College of the Environment

:: IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant research investigates ocean acidification's effects on important shellfish species and their larvae**

Type: accomplishment
Description:
Relevance: Ocean acidification has come sooner to the Pacific Northwest than almost anywhere else in the world, at levels surpassing end-of-century predictions. It is imperative that we understand its effects on marine shell-builders – particularly those that are ecologically and economically important, and especially in early life stages when they may be most vulnerable.
Response: Controlled laboratory studies funded by Washington Sea Grant are examining the larval responses of four important bivalves to multiple stressors: dissolved carbon dioxide (CO_2) at various levels; different water temperatures; and the pathogen Vibrio tubiashii. In 2012 they completed acidification trials on juvenile Pacific oysters, Manila clams, and geoducks, and conducted two trials on adult and juvenile Olympia oysters. They are also investigating molecular responses for future field use as predictors of environmental stress, and developing molecular assays of acidification's effects on three commercial species plus protected pinto abalone.
Results: Early results indicate that acidification impacts vary among species and life stages. Elevated CO_2 did not change lethal temperatures for larval Pacific oysters, Manila clams, or geoducks. Olympic oyster broodstock exposed to high CO_2 suffered reduced fecundity and more post-spawning mortality. Their larvae seemed resilient but showed extensively altered gene expression, suggesting a metabolic tradeoff and possible future developmental cost.
The project also created an ocean acidification curriculum presented to more than 800 students in seven high schools. Evaluations show high knowledge acquisition and retention. In addition, researchers designed a tank system for oyster-fecundity and larval-survival trials and created a dedicated experimental space that will accommodate future acidification research.

Recap:
Washington Sea Grant research is developing new molecular tools for ocean acidification research, exploring acidification's effects on five shellfish species, and teaching high school students about acidification and its effects on marine life.

Comments:
Primary Focus Area – OCEH (SSSS)
Secondary Focus Area – OCEH (HCE), COCC (HRCC)

Associated 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:

AquaTechnics, Inc.
Baywater, Inc.
Northwest Fisheries Science Center (US DOC, NOAA, NMFS, NWFSC)
Pacific Marine Environmental Laboratory (US DOC, NOAA, OAR, PMEL)
Taylor Shellfish Company
Taylor Shellfish Resources Research and Design
University of Washington, Friday Harbor Laboratories, College of the Environment (UW)
University of Washington, School of Aquatic and Fishery Sciences, College of the Environment (UW)
University of Washington, School of Marine and Environmental Affairs, College of the Environment (UW)

:: PUBLICATIONS

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: 2012
Uploaded File: Timmins_Schiffman_et_a....2.pdf, 421 kb
URL:
http://download.springer.com.offcampus.lib.washington.edu/static/pdf/478/art%253A10.1007%252Fs00227-012-2055-x.pdf?auth66=1363064359_184c5a78c84a4a28f59ea75a3659f6dc&ext=.pdf
Abstract:
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:
Timmin- Schiffman, E.B., M.J. O'Donnell, C.S. Friedman, and S.B. Roberts. 2012. Elevated pCO2 causes developmental delay in early larval Pacific
oysters, Crassostrea gigas. Marine Biology 20 October 2012. DOI 10.1007/s00227-012-2055-x

Copyright Restrictions + Other Notes:
To be reported in AR2013

Journal Title: Marine Biology

Title: **Timmins-Schiffman, EB, Friedman, CS, Metzger, DC, White, SJ, Roberts, SB 2013. Genomic resource development for shellfish conservation concern. Mol Ecol Resources**

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2012
Uploaded File: Timmins_Schiffman_et_a....3.pdf, 511 kb
URL: *none*
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, E.B., C.S. Friedman, D.C. Metzger, S.J. White, and S.B. Roberts. 2013. Genomic resource development for shellfish conservation concern. Molecular Ecology Resources 13(2): 295–305.

Reported in AR2012

Journal Title: *none*

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

No Leveraged Funds Reported This Period

WASHINGTON SEA GRANT PROGRESS REPORT

for the period 2/1/2012 – 1/31/2013

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

1. PROJECT OBJECTIVES (from original proposal)

To better understand the influence of ocean acidificiation (OA) on ecologically, economically and socially important molluscs, we will characterize the relationship between altered environmental conditions and health of larval mollusks under controlled conditions. Specific objectives include: A) Characterize responses observed in early life stages of four marine molluscan species to multiple stressors, which include increased pCO2, varying temperature and the pathogen *Vibrio tubiashii* in controlled laboratory studies. B) Characterize molecular responses to selected stressors for future use under field conditions as predictors of environmental stressors experienced. C) Describe response similarity between two species with a similar larval strategy. To meet these objectives we will test the following hypotheses: Overall Hypothesis: Environmental stressors (elevated temperature and increased atmospheric CO2 levels) and related changes in seawater chemistry will influence larval molluscan physiology, behavior and survival. Specifically, we hypothesize that molluscan early life stages will: 1) perform better at pre-industrial pCO2 levels than at current and future pCO2 levels; 2) show reduced thermal tolerance with increased pCO2 levels; 3) show reduced fertilization and hatching rates at elevated pCO2 levels; 4) show reduced larval survival at elevated pCO2 levels; 5) show reduced metabolic activity and performance at sublethal increased pCO2 levels, even those at which aragonite is supersaturated; 6) experience negative impacts on normal physiological processes at increased pCO2 levels (and reduced pH) and elevated temperatures relative to current levels; 7) show increased larval mortality and shell dissolution under highly elevated pCO2 levels observed in some nearshore areas; 8) be more susceptible to *V. tubiashii* at combined elevated pCO2 levels (and reduced pH) and elevated temperatures relative to current levels.

2. PROJECT PROGRESS

Larvae We proved the first transcriptomic resources for pinto abalone and Olympia oyster larvae (Timmins-Schiffman et al. 2013) We also published our Pacific oyster larval response to ocean acidification, which was funded by a sister project (Timmins-Schiffman et al. 2012).

We also completed our trials examining the effects of ocean acidification on juvenile Pacific oysters, geoduck clams, and Manila clams and conducted two trials on the effected of elevated pCO_2 on adult and larval Olympia oysters.

Pacific oysters *Crassostrea gigas*, juveniles were exposed to 6 different levels of pCO_2 at the Friday Harbor Ocean Acidification Laboratory. Selected conditions simulated current-day

atmospheric levels (400 µatm) through projections extending beyond the year 2100 (1400 µatm for 1 month. In separate systems, we conducted a multi-species common garden study in which we exposed Manila clams, smaller pacific oysters, geoduck clams and adult Olympia oysters to 400 and 100 µatm CO_2. At the end of the month, animals from all treatments were sampled for transcriptomics, proteomics, and histology. Groups of animals from each treatment 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 differences in LT were observed upon exposure of Pacific oyster, Manila clam or geoduck clam juveniles to elevated pCO_2.

Olympia oysters We conducted two trials on Olympia oysters, *Ostrea lurida*.

In Trial 1, oysters were held in replicate tanks at 400 and 1000 µatm for 30d at 13°C followed by acclimation to 20°C over 3d and held for an additional ~2 wk. Survival, gonadal-somatic index (GSI), gonad stage and presence/absence of brooding larvae (Fig X) were recorded and compared. No significant difference in measured parameters was observed. However, it was interesting to note that some oysters with brooding larvae had already changed into males (they are hermaphrodites).

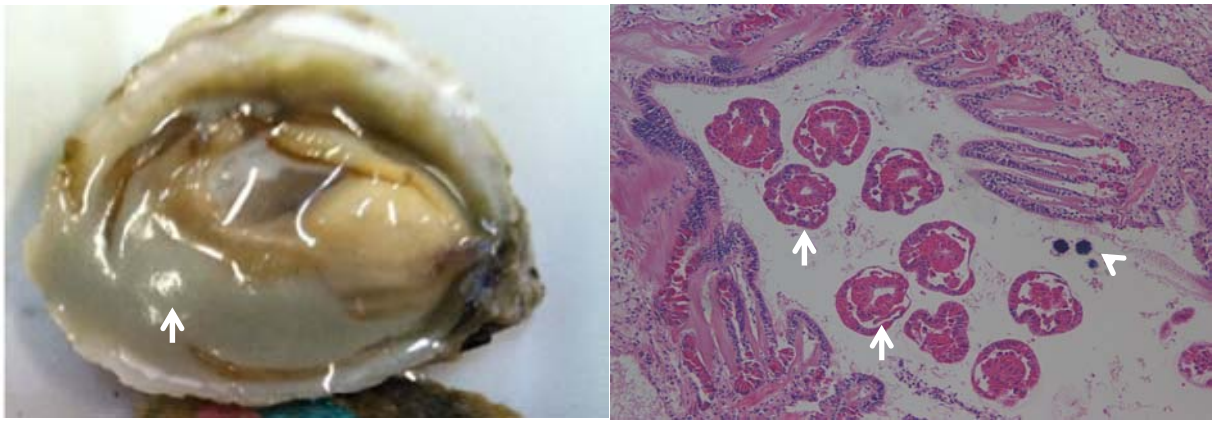

Fig. 1. Olympia oyster brooding larvae (A, arrow) and image of histological section of brooding larvae (arrows) in an oyster that had already changed into a male as evidenced by the presence of spermatophores (arrow heads) .

In Trial 2, groups of adult *O. lurida* were held at four pCO_2 levels ranging from 400 to 2200 µatm for 6 wks at ~16.5°C. Survival, gonad maturation (GSI and gamete stage), fecundity and gene expression were quantified for each oyster. In addition, larval performance was assessed using triplicate containers from a single release at each pCO_2. While oyster fecundity was inversely related to pCO_2 ($p=0.0282$, $C=-0.972$), post-spawning mortality increased at elevated pCO_2 levels ($p=0.046$, $C=0.954$) (Fig 2.). Larval size and shell calcification were not influenced by pCO_2 ($p>0.05$). In addition, the timing of larval releases was delayed with increasing pCO_2 (Fig. 3). All larvae were fully calcified upon release from brood chamber and after 11 days in culture (Fig. 4). Despite the apparent lack of observable impact of Olympia oyster larvae with increasing pCO2, genomic analysis suggests a metabolic and later developmental cost are likely. We are in the process of further analyzing these data.

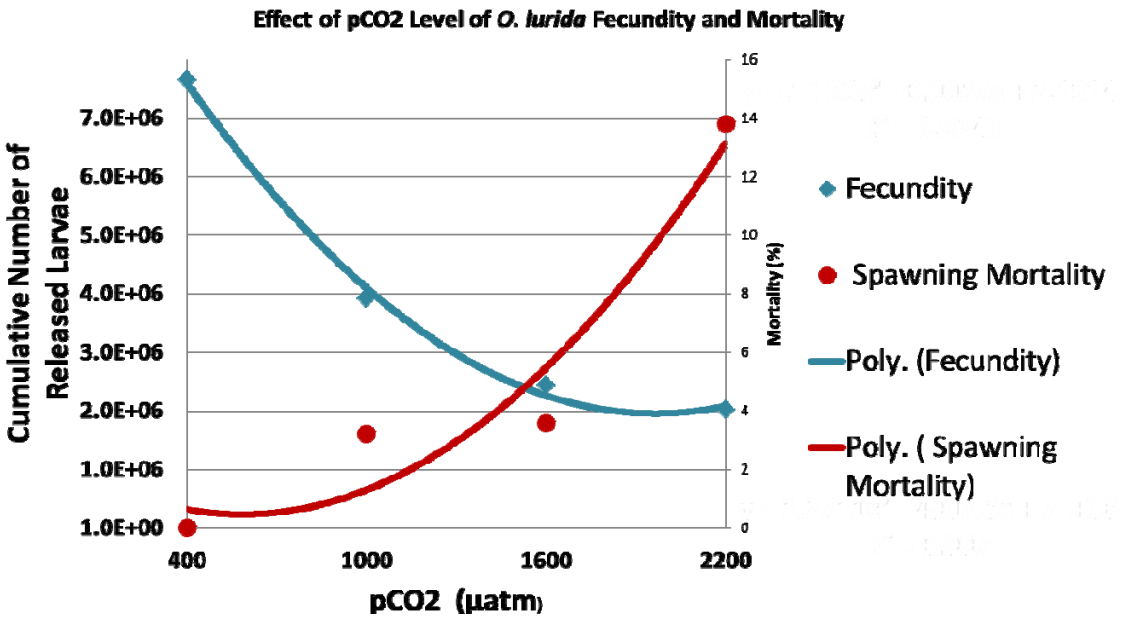

Fig. 2. Effect of pCO_2 on Olympia oyster fecundity and mortality

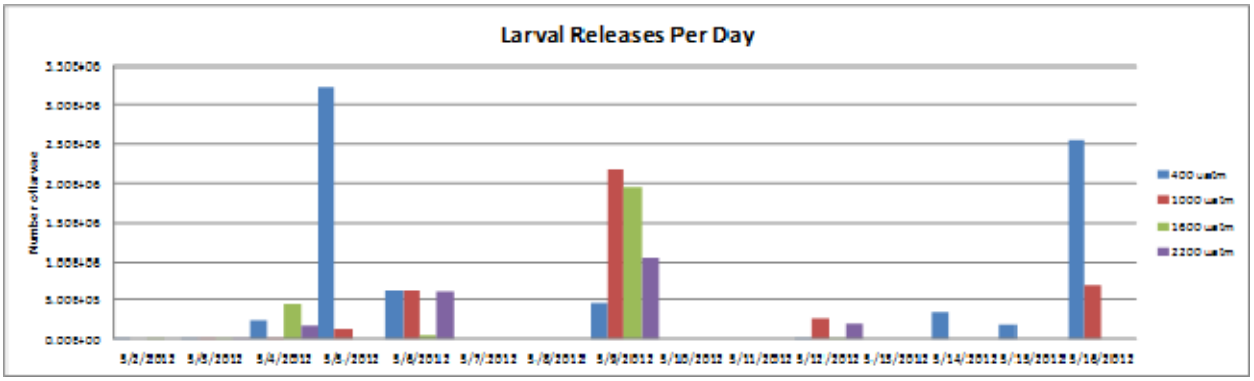

Fig. 3. Timing and number of larvae released from female Olympia oysters held at 4 pCO_2. Note that oysters reared under ambient conditions (blue) released higher numbers of larvae more frequently and earlier than those held at elevated pCO2 (1000-2200 µatm; red, green and violet bars, resp.).

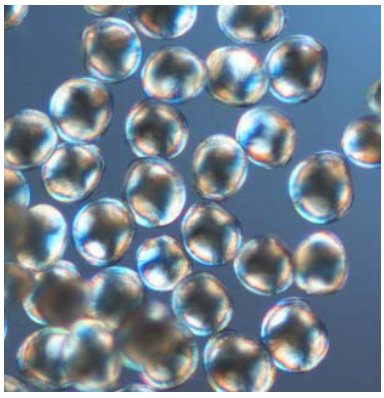

Fig. 4. Olympia oyster larvae with fully calcified shells as evidenced by the presence of a cross when illuminated under double polarized light.

Outreach

Teachers continue to incorporate the OAOP into their classes, allowing for even more 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.