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

Friedman, Carolyn

Project: R/LME-5 - *Effects of early exposure of Pacific oysters to ocean acidification on subsequent performance*

:: STUDENTS SUPPORTED

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:
The Physiological Effects of Ocean Acidification on Multiple Life History Stages of the Pacific Oyster, Crassostrea gigas

Involvement with Sea Grant This Period:
Funded off leveraged funds to participate in the selection of broodstock and genetic analysis

Post-Graduation Plans:
employment

:: CONFERENCES / PRESENTATIONS

No Conferences / Presentations Reported This Period

:: ADDITIONAL METRICS

K-12 Students Reached:0

Curricula Developed:0

Volunteer Hours:6
Alan Trimble and 3 WDFW employees assisted in the cleaning and inspection of broodstock for use in this study

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
Portable CO2 delivery system to control broodstock rearing conditions in hatchery for use in assessing response of Pacific oysters to ocean acidification. R/LME-5	**Actual** (2/1/2012 - 1/31/2013) :	1	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) :
Anticipated (2/1/2013 - 1/31/2014) :

Number of fishers using new techniques
Actual (2/1/2012 - 1/31/2013) :
Anticipated (2/1/2013 - 1/31/2014) :

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

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

:: PARTNERS

Partner Name: Baywater Inc., type: industry, scale: local
Partner Name: Taylor Shellfish Company
Partner Name: USDA Agriculture Research Station, type: government, scale: federal
Partner Name: Washington Department of Fish and Wildlife

:: IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant-funded research explores the effects of larval exposure to ocean acidification on adult oysters, and seeks genetic keys to acidification tolerance**

Type: accomplishment
Description:
Relevance: Much research has explored the effects of ocean acidification on shellfish larvae, but little has examined the subsequent performance of shellfish that survive larval exposure. Larval Pacific oysters are famously vulnerable to elevated levels of dissolved carbon dioxide (CO2), and growers routinely discard batches that have suffered high mortality. But survivors might show enhanced performance in later life. Identifying resilient strains and their distinguishing genetic factors would enable selective breeding for CO2 tolerance of the Pacific Coast's most commercially important and most severely acidification-impacted shellfish.
Response: Washington Sea Grant-funded researchers are examining the effects of high dissolved CO2 on oyster gametes and larvae and on survivors' subsequent performance, using both a common garden experiment and full factorial crosses of nine broodstock families from a genetically diverse wild population. They will investigate the

genetic potential for breeding acidification tolerance, and any potential tradeoffs between resilience and other commercially important traits.
Results: In this, the project's first year, researchers worked with the Taylor Shellfish Company to develop CO_2 delivery and degassing systems for controlled oyster rearing; these will be available for other projects. They optimized genetic analysis (PCR) conditions for several dozen microsatellite primer pairs and identified and selected the most promising loci for analysis. Taylor, the largest U.S. shellfish grower, has provided additional support in hopes of developing acidification-tolerant stocks,

Recap:
Washington Sea Grant-supported research explores the effects of early exposure to acidified waters on later-life performance of commercially important Pacific oysters and seeks genetic factors for breeding acidification-tolerant lines.

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 Capacity).

Related Partners:

Agriculture Research Service (USDA, ARS)
Baywater, Inc.
Taylor Shellfish Company
University of Washington, School of Aquatic and Fishery Sciences, College of the Environment (UW)

:: PUBLICATIONS

No Publications Reported This Period

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

Type: influenced Period: 2012-04-16::2013-01-31Amount: $29700

Purpose:
Genotyping of parental and select G1 oysters

Source: US Department of Agriculture (USDA)

Effects of early exposure of Pacific oysters to ocean acidification on subsequent performance, R/LME-5 (Friedman et al.)

We hypothesize that exposure to increased pCO_2 as adults or larvae will affect subsequent performance (growth, survival, and reproduction) and that Pacific oyster populations harbor high levels of genetic variation for performance traits under high CO_2 conditions.

Climate change is influenced by the release & increase of atmospheric carbon via burning of fossil fuels & other natural & human-induced processes. CO_2 levels are predicted to rise & are likely to critically affect marine ecosystems by shifting pH (ocean acidification (OA), carbonate chemistry & bioavailability of nutrients & other compounds & will affect a myriad of physiological processes we are only now beginning to understand. The ability of populations to respond to environmental change depends on the presence of sufficient genetic variation for selection to act upon & occurs over generations. Research on OA often focuses on specific (and often early) life history stages of marine invertebrates as a means to understand species responses and predict community impacts (e.g. Reviews by Fabry 2008, Doney et al. 2009, O'Donnel et al. 2009, Moulin et al. 2011). Recent meta-analyses illustrate the need for hypothesis-driven experiments in which **all life history stages are evaluated** in order to forecast OA effects Doney et al. 2009, Hendricks et al. 2010. In fact, Dupont et al. (2010) stressed that "***a species response to ocean acidification (or any other stressor) should be assessed at different levels from individual to ecosystem***." and that "***At the individual level, the whole life-cycle of the organism should be investigated, including various fitness related biological processes (e.g. fecundity, fertility and survival) and all life-cycle stages (e.g. gonads, embryos, larvae, juveniles and adults). This is particularly true for mero-benthic or mero-planktonic species***".

Few studies have examined the long-term impacts of OA on animals that survive exposure as developing gametes (via parental exposure) or larvae. Parker et al. (2012) illustrated effects of elevated CO_2 conditions on the Sydney Rock oyster. Larvae of parent oysters conditioned under high pCO_2 grew faster, larger & more survived to the eyed larval stage compared to those of parents held under ambient conditions. This effect was heightened in hatchery selected oyster populations over wild type (Parker et al. 2012). Thus, the potential for adaptation to OA is present in marine populations and can vary within and among species. Whether a species can adapt depends on the extent of genetic variability present in populations, which directly affects the ability to evolve in relation to the rate of change in ocean conditions.

Pacific oysters are naturalized in several locations in the State, where they are important for the aquaculture industry, tribes & general public, & are the most important farmed shellfish in Washington State. Recruitment of wild Pacific oysters has failed in Willapa Bay for several years & hatchery production, the main source of seed for this crucial State industry, has been severely impacted by ocean acidification (OA) since 2005. State culturists produce ~75% of the *C. gigas* in the US & provide key jobs & income to our rural communities.

To better understand the ability of Pacific oysters to adapt to changing conditions and utility of selection to advance the success of the shellfish industry in the face of climate change, we will:

1. Investigate effects of broodstock exposure to high pCO_2 seawater on gamete and larval quality.

2. Determine if larvae exposed to high pCO_2 seawater subsequently exhibit compromised performance (growth, survival, and maturation) as post-set juveniles and adults under a range of field conditions.
3. Estimate genetic parameters required to implement an effective breeding program for enhanced tolerance of OA conditions:
    a. genetic potential (heritability) for selective breeding to improve larval tolerance of high CO_2 seawater.
    b. trade-offs (negative genetic correlations) with other economically important traits.

Progress to date

Due to the unexpected departure of one of our collaborators, initiation of our study was delayed as he was supplying, gratis to the project, all of the genotyping of the progeny, which is essential for identification of superior families. The USDA Agricultural Research Station (ARS) graciously agreed to complete as much of the project as possible.

Once this collaboration was confirmed we began selecting broodstock lines. We selected 9 families from Pipestem Inlet, Vancouver Island, BC, Canada, which is a new broodstock source of interest for the Washington State shellfish industry. Families were produced by crosses from wild-caught Pipestem oysters in 2010 and were held in a common environment in south Puget Sound.

We developed a CO_2 delivery system to control conditions in which broodstock would mature in the hatchery. Seawater degassing was developed to reduce the partial pressure of CO_2 (pCO_2) via degassing columns. A Honeywell controller connected to a Durafet pH probe controls the delivery of CO_2 via solenoid valves into seawater using Venturi injectors. Our system contains an ambient condition (~600 µatm CO_2) and an elevated condition with a target of 1500 µatm CO_2. In January 2013, we selected the following experimental families: 24x42; 57x57; 17x17; 5x31; 58x58; 49x49; 55x85; 3x3; and 19x19. Nine families were chosen with a goal of having adequate ripe oysters from 7 families. Twelve oysters from each family were placed into each CO_2 treatment system. Water chemistry is assessed daily: Durafet pH, temperature and salinity are recorded. In addition, 3x per week, pCO_2 is measured with a Licor gas analyzer and samples are taken from both broodstock tanks for alkalinity and total CO_2 analysis. Spectrophotometric pH is also measured weekly.

Spawning is scheduled for mid-March 2013 with a plan to settle larvae 17 days later followed by field planting in June or July 2013.

We have optimized PCR conditions for several dozen microsatellite primer pairs, identified the ~30 microsatellite loci that amplify and score best, and have selected over 20 loci that are expected to work best and have the potential to be co-loaded or multiplexed for high throughput analysis of microsatellites. We have genotyped Pipestem individuals at the selected microsatellite loci, mentioned above. Pipestem oysters appear to be genetically diverse, with a good amount of allelic richness and heterozygosity per loci.

References

Doney, S. C. et al., *Annual Review of Marine Science* 1, 169 (2009)

Dupont, S., Olga-Martínez, O., Thorndyke, M., *Ecotoxicology* 19, 449-462 (2010)

Dupont S, Dorey N, Thorndyke M *Estuarine Coastal and Shelf Science* 89 (2) 182-185 (2010)

Fabry, V. J. et al., *ICES Journal of Marine Science* 65 (3), 414 (2008)

Hendriks IE, Duarte CM, Alvarez M. *Estuarine Coastal and Shelf Science* 86 (2) 157-164 (2010)

Moulin L, Catarino, A, Claessens T, et al. *Marine Pollution Bulletin* 62 (1) 48-54 (2011)

O'Donnell, M., Hammond, L., and Hofmann, G., *Marine Biology* 156 (3), 439 (2009)

Parker L.M., Ross P.M., O’Connor W.A., *Marine Biology* (158) 689–697 (2011)