Update Report

Roberts, Steven

Period: 2/1/2012 - 1/31/2013
Project: R/LME/N-3 - *Alleviating Regulatory Impediments To Native Shellfish Aquaculture*

:: STUDENTS SUPPORTED

Chi, Bradley, bchi@u.washington.edu, University of Washington, SAFS, status:new, *no field of study*, *no advisor*, degree type:BS, degree date:2012-12-01, degree completed this period:No
Student Project Title:
Effects of photoperiod and mechanical stress on Olympia oyster physiology

Involvement with Sea Grant This Period:
capstone

Post-Graduation Plans:
grad school

Jackson, Katie, k.e.jackson.1992@gmail.com, University of Washington, SAFS, status:new, *no field of study*, *no advisor*, *no degree type*, *no degree date*, degree completed this period:No
Student Project Title:
Genetic sample management and optimizing oyster relaxation

Involvement with Sea Grant This Period:
intern

Post-Graduation Plans: *none*

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:
OIympia oyster transcriptome characterization and genetic marker development

Involvement with Sea Grant This Period:
graduate student

Post-Graduation Plans:
employment

:: CONFERENCES / PRESENTATIONS

No Conferences / Presentations Reported This Period

:: 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
Transcriptome and genetic markers for the native Olympia oyster to inform restoration efforts. R/LME/N-3	**Actual** (2/1/2012 - 1/31/2013) :	1	0		0
	Anticipated (2/1/2013 - 1/31/2014) :	1	0		

:: HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

:: ADDITIONAL MEASURES

Safe and sustainable seafood

Number of stakeholders modifying practices
Actual (2/1/2012 - 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: NOAA Manchester lab
Partner Name: Puget Sound Restoration Fund

:: IMPACTS AND ACCOMPLISHMENTS

Title: **Transcriptome characterization of the Olympia oyster**

Type: accomplishment

Description:
Sequenced transcriptome of Olympia oyster and identified genetic markers. This will be the foundation for future research efforts.

Recap:
Characterized the Olympia oyster transcriptome.

Comments: *none*

Related Partners: *none*

:: PUBLICATIONS

Title: **Transcriptome characterization of the Olympia oyster and pinto abalone**

Type: Internet Resources, Topical Websites Publication Year: 2013
Uploaded File: *none*
URL: http://dx.doi.org/10.6084/m9.figshare.156431
Abstract:
Open Access data on transcriptome of the Olympia oyster and pinto abalone.

Data S1. Ostrea lurida transcriptome. Assembled contigs of O. lurida transcriptome sequencing.
Data S2. Haliotis kamtschatkana transcriptome. Assembled contigs of H. kamtschatkana sequencing.
Data S3. Ostrea lurida SPIDs. BLASTx results for O. lurida contig search against the UniProtKB/Swiss-Prot database. BLAST e-values and gene descriptions are also given.
Data S4. Ostrea lurida GO. Gene Ontology annotations of O. lurida contigs. GO annotations are made based on associations with a Swiss-Prot ID.
Data S5. Haliotis kamtschatkana SPIDs. BLASTx results for H. kamtschatkana contig search against the UniProtKB/Swiss-Prot database. BLAST e-values and gene descriptions are also given.
Data S6. Haliotis kamtschatkana GO. Gene Ontology annotations of H. kamtschatkana contigs. GO annotations are made based on associations with a Swiss-Prot ID.
Data S7. Ostrea lurida bitscores. Bit scores for BLASTn results of O. lurida contigs against species-specific databases of other closely related species.
Data S8. Haliotis kamtschatkana bitscores. Bit scores for BLASTn results of H. kamtschatkana contigs against species-specific databases of other closely related species.
Data S9. Ostrea lurida SNPs. SNP information for putative SNPs identified in the O. lurida transcriptome. Contig numbers are listed in the leftmost column, followed by SNP location and allele. Annotations of the contigs, as determined through a BLASTx against the UniProtKB/Swiss-Prot database, are given along with the e-value for the BLAST result.
Data S10. Haliotis kamtschatkana SNPs. SNP information for putative SNPs identified in the H. kamtschatkana transcriptome. Contig numbers are listed in the leftmost column, followed by SNP location and allele. Annotations of the contigs, as determined through a BLASTx against the UniProtKB/Swiss-Prot database, are given along with the e-value for the BLAST result.

Citation:
Transcriptome characterization of the Olympia oyster and pinto abalone. Steven Roberts, Emma Timmins-Schiffman. figshare. February 11, 2013.
http://dx.doi.org/10.6084/m9.figshare.156431

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **Effects of photoperiod and mechanical stress on Olympia oyster physiology**

Type: Workshops, Proceedings, Symposia Including Highlights/Summaries of (please note: document number reflects the year the proceedin Publication Year: 2012
Uploaded File: *none*
URL: http://goo.gl/q4io7
Abstract:
Once dominant along the North American west coast, Olympia oyster (Ostrea lurida) populations have declined significantly since the early 1900's. Restoration efforts have encountered many problems, one of which is the slow growth of O. lurida. This study aims to determine the effect of photoperiod and mechanical stress, environmental factors controllable in an aquaculture setting, on O. lurida stress and growth physiology. Natural photoperiod and absence of mechanical stress were expected to elicit a greater growth response. Oysters were separated into 12-hour light:12 dark or 24-hour light photoperiod treatments followed by mechanical stress or no mechanical stress. Tissue of oysters was analyzed using quantification of genes related to stress and growth. Results of stress genes indicated 12:12 photoperiod with mechanical stress induced greater stress. Growth genes implied 24-hour photoperiod with mechanical stress induced greater growth. Findings that 24-hour light was less stressful contradicted predictions that oysters would be better suited for conditions similar to natural lighting. The longer 24-hour light treatment could signal the growing season, which generally occurs in the summer months. Indications of growth in the 24-hour with stress treatment were also of interest. The results suggest that mechanical stress may play a role in stimulating growth in oysters. During the study, no growth was actually measured meaning quantification of genes only suggests possible physiological changes. Future work will aim to verify our results with real measured growth.

Citation:
Undergraduate Capstone Research Symposium. 2012. Effects of photoperiod and mechanical stress on Olympia oyster physiology. Seattle, WA.

Journal Title: *none*
Title: **Effects of photoperiod and mechanical stress on Olympia oyster physiology**

Type: Thesis / Dissertation abstracts Publication Year: 2012
Uploaded File: *none*
URL: http://goo.gl/pqyEE
Abstract:
Once dominant along the North American west coast, Olympia oyster (Ostrea lurida) populations have declined significantly since the early 1900's. Restoration efforts have encountered many problems, one of which is the slow growth of O. lurida. This study aims to determine the effect of photoperiod and mechanical stress, environmental factors controllable in an aquaculture setting, on O. lurida stress and growth physiology. Natural photoperiod and absence of mechanical stress were expected to elicit a greater growth response. Oysters were separated into 12-hour light:12 dark or 24-hour light photoperiod treatments followed by mechanical stress or no mechanical stress. Tissue of oysters was analyzed using quantification of genes related to stress and growth. Results of stress genes indicated 12:12 photoperiod with mechanical stress induced greater stress. Growth genes implied 24-hour photoperiod with mechanical stress induced greater growth. Findings that 24-hour light was less stressful contradicted predictions that oysters would be better suited for conditions similar to natural lighting. The longer 24-hour light treatment could signal the growing season, which generally occurs in the summer

months. Indications of growth in the 24-hour with stress treatment were also of interest. The results suggest that mechanical stress may play a role in stimulating growth in oysters. During the study, no growth was actually measured meaning quantification of genes only suggests possible physiological changes. Future work will aim to verify our results with real measured growth.

Citation:
Chi, B. 2012. Effects of photoperiod and mechanical stress on Olympia oyster physiology. FISH495 Capstone Thesis. University of Washington.

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **Katie's Notebook**

Type: Internet Resources, Topical Websites Publication Year: 2012
Uploaded File: *none*
URL: http://genefish.wikispaces.com/Katie's+Notebook
Abstract:
Lab Notebook of undergraduate student currently working on the project

Citation:
Katie's Notebook. Roberts Lab Wiki. October 23, 2012. http://genefish.wikispaces.com/Katie's+Notebook

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **Tidal Cycles**

Type: Internet Resources, Topical Websites Publication Year: 2012
Uploaded File: *none*
URL: http://oystergen.es/blog/
Abstract:
Blog

Citation:
Tidal Cycles. Oystergen Blog. 2012. http://oystergen.es/blog/

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **oystergen.es**

Type: Internet Resources, Topical Websites Publication Year: 2012
Uploaded File: *none*
URL: http://oystergen.es/olympia/
Abstract:
Website

Citation:

The Olympia oyster (Ostrea lurida) is the only native oyster on the west coast of the U.S. Steven Roberts. 2012. Oystergen.es.

Copyright Restrictions + Other Notes:

Journal Title: *none*
Title: **Genomic resource development for shellfish of conservation concern.**

Type: Reprints from Peer-Reviewed Journals, Books, Proceedings and Other Documents Publication Year: 2012
Uploaded File: men12052.pdf, 514 kb
URL: http://onlinelibrary.wiley.com/doi/10.1111/1755-0998.12052/abstract
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. 2012. Genomic resource development for shellfish of conservation concern. Molecular Ecology Resources 13(2): 295-305. doi: 10.1111/1755-0998.12052

Copyright Restrictions + Other Notes:

Journal Title: Molecular Ecology Resources

:: OTHER DOCUMENTS

No Documents Reported This Period

:: LEVERAGED FUNDS

No Leveraged Funds Reported This Period

R/LME/N-3 - Alleviating Regulatory Impediments To Native Shellfish Aquaculture

Progress Report: September 2012 – January 2013

A significant impediment to sustainable aquaculture is the lack of proper information to predict the impacts of culturing native shellfish species for restoration and commercial production. As a result, expansion and growth of domestic aquaculture is constrained and may be halted by management directives that restrict distribution of hatchery derived native shellfish until the potential interactions are better understood. The overall goals of this project are to increase our knowledge of local adaptation in Olympia oysters to address concerns that interbreeding between potentially maladapted cultured and wild stocks could negatively impact wild populations. Over the current reporting period a majority of our effort has focused on 1) developing genomic resources, 2) preparing for oyster outplanting, and 3) procedure optimization. The remainder of this report will describe the details associated with each of these activities.

Genomic Resources

One of the major accomplishments over this reporting period was the characterization of the Olympia oyster transcriptome [Timmins-Schiffman, E. B., Friedman, C. S., Metzger, D. C., White, S. J. and Roberts, S. B. (2012), Genomic resource development for shellfish of conservation concern. Molecular Ecology Resources. doi: 10.1111/1755-0998.12052]. Here we have annotated the transcriptome and identified single nucleotide markers that will be further developed as part of the molecular analysis conducted during this project.

During this reporting period we have not initiated any genetic or epigenetic population level characterization. There has been significant progress in obtaining samples and sample processing. Samples of adults from the three populations (Table 1) held in common conditions for 3 months to reduce ephemeral differences have been sampled for initial characterization of epigenetic differences using methylation-sensitive AFLP (MS-AFLP).

Table 1. Olympia oyster samples for genetic and epigenetic comparisons. Asterisk indicates 10 samples are currently being processed for epigenetic characterization.

Population	**Stage**	**Total**
Fidalgo Bay (North Sound)	adults	93*
Fidalgo Bay (North Sound)	seed	100
Dabob Bay (Hood Canal)	adults	38*
Dabob Bay (Hood Canal)	seed	83
North Bay (South Sound)	adults	79*

As part of a Capstone student thesis, "*Effects of photoperiod and mechanical stress on Olympia oyster physiology*", several gene expression assays were developed. Specifically, assays were developed for BCL2- associated athanogene 2 (*bag*), heat shock protein 90kDa alpha (*hsp*), U2 small nuclear RNA auxiliary factor 1-like 4 (*u2a*), muscle glycogen phosphorylase (*pygm*), insulin-like growth factor 1 receptor (*igfr*), and protein kinase, cGMP-dependent, type 1 (*prkg*). The latter three genes are all involved in growth whereas the former are associated with the stress response. Although limited in certain aspects, this study found two important implications in *O. lurida* growth physiology and restoration efforts. First, photoperiod may have an impact on stress and growth rate of *O. lurida* with longer photoperiods associated with increase growth. Secondly, mechanical stress may stimulate growth under certain conditions. Both of these findings are based on gene expression and confirmation by additional studies would be ideal. Regardless, these assays could be implemented in our future work.

Outplanting preparation

A major component of this project overall is to evaluate fitness components and performance of seed from different origins in a reciprocal transplant experiment. This transplantation is planned for this summer. The three source populations are Fidalgo Bay, Dabob Bay, and North Bay. Broodstock from all three populations/sites (Table 2) were collected in December and initially placed in conditioning tanks at Puget Sound Restoration Fund's Port Gamble Hatchery. The first release of larvae occurred on January 23 from one of the Fidalgo Bay breeding groups, with approximately 205,000 larvae. Larvae will be maintained until outplanting later this year.

Table 2. Olympia oyster samples used as broodstock

Site	**Total specimens collected**	**Number of breeding groups that produced larvae**	**Approximate number of larvae**
Fidalgo Bay	516	10	41k-1,333k
Dabob Bay	205	7	68k-496K
North Bay	332	0	0

Procedure optimization

To enable nonlethal assessment of fecundity, we have initiated experiments to optimize anaesthetization of Olympia oysters with minimal mortality. To our knowledge, this is the first attempt to transfer anesthesia methods used successfully with *Ostrea edulis, Saccostrea glomerata, Crassostrea gigas, and Nodipecten subnodosus*. Based on information from these other bivalve species, we have designed the following experiment to determine the optimal dosage and treatment duration using $MgCl_2$ (subject to change based on new information): Olympia oysters held at 12-14 C will be treated with 20 - 80 mg * L^{-1} $MgCl_2$ at both ambient and elevated (~18-19 C) temperatures. We recently initiated an investigation using $MgSO_4$ as an alternate anaesthetic. Preliminary results are promising: 60% of oysters anaesthetized using a one hour immersion regained responsiveness after an additional 1.5 hrs. We continue to monitor this group of oysters for post-treatment mortality.