RESEARCH/PD ANNUAL REPORT - FINAL REPORT

2015 annual report - final
Steven Roberts
Alleviating Regulatory Impediments To Native Shellfish Aquaculture
R/LME/N-3
Submitted On: 04/22/2016 02:11:55 PM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	
Fishermen and seafood industry personnel	4	Puget Sound Restoration Fund modified breeding practices based on our findings.
Communities - economic and environmental development	0	
Stakeholders - sustainable approaches	0	
Informal education programs	0	
Stakeholders who receive information	50	presentations
Volunteer hours	40	Estimated number of undergraduate volunteer research hours
P-12 students reached	0	
P-12 educators	0	

REQUESTED INFORMATION

Publications

Evidence of Ostrea lurida (Carpenter 1894) population structure in Puget Sound, WA
Publication Type: Non-peer-reviewed: Complete Issues of Journals, Periodicals; Magazines, Miscellaneous Reports, Papers, Special Collections
Publication Year: 2015
Publication Authors:
Publisher Info: PeerJPrePrints
Notes:
Related URLs: https://zenodo.org/record/34143
Keywords:
Publication URLs: https://peerj.com/preprints/704/
Abstract: For long-term persistence of species, population structure is important. Traits that hold adaptive advantage such as reproductive timing and stress resilience may differ among locales. Knowledge and consideration of these traits should be integrated into conservation efforts. To test for adaptive differences between Olympia oyster populations a reciprocal transplant experiment was carried out monitoring survival, growth, and reproduction using three established populations of Ostrea lurida within Puget Sound, Washington. Performance differec for each population. Ostrea lurida from Dabob Bay had higher survival at all sites but lower reproductive activity and growth. Oysters from Oyster Bay demonstrated greater proportion of brooding females at a majority of sites with moderate growth and survival. Together these data suggest the existence of O. lurida population structure within Puget Sound and provide

information on how broodstock should be selected for restoration purposes.
Citation: Heare JE, Blake B, Davis JP, Vadopalas B, Roberts SB. (2015) Evidence of Ostrea lurida (Carpenter 1864) population structure in Puget Sound, WA. PeerJ PrePrints 3:e1986 https://doi.org/10.7287/peerj.preprints.704v4
Citation for Coverpage:
SG can post PDF online?:
Uploaded File:

Differential response to stress in Ostrea lurida (Carpenter 1864) as measured by gene expression
Publication Type: Non-peer-reviewed: Complete Issues of Journals, Periodicals; Magazines, Miscellaneous Reports, Papers, Special Collections
Publication Year: 2015
Publication Authors:
Publisher Info:
Notes:
Related URLs: https://github.com/jheare/OluridaGeneExpression/tree/v1.0
Keywords:
Publication URLs: https://peerj.com/preprints/1595/
Abstract:
Citation: Heare JE, White SJ, Roberts SB. (2015) Differential response to stress in Ostrea lurida (Carpenter 1864) as measured by gene expression. PeerJ PrePrints 3:e1994 https://doi.org/10.7287/peerj.preprints.1595v1
Citation for Coverpage:
SG can post PDF online?:
Uploaded File:

Students Supported

Jake Heare (Continuing Student)
jakeheare@gmail.com
UW, SAFS

Field of Study: SAFS
Advisor: Steven Roberts
Degree Type: MS
Degree Year: 2015

Student Project Title: Response of Olympia oysters (Ostrea lurida) to changing environmental conditions.

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.):

Post-Graduation Plans (employer, grad school, etc.): Teaching at UW

Was this thesis/dissertation supported by Sea Grant?: Yes

Thesis / Dissertation: Response of Olympia oysters (Ostrea lurida) to changing environmenta conditions.

New or Continuing?: continuing

Degree awarded this reporting period?: Yes

Financially supported?: Yes

Katherine Silliman (New Student)
ksilliman@uchicago.edu
University of Chicago, Evolutionary Biology

Field of Study:
Advisor:
Degree Type: PhD
Degree Year: 2018

Student Project Title: Characterizing Olympia oyster population structure

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): U of Chicago leveraged funding - student project

Post-Graduation Plans (employer, grad school, etc.):

Was this thesis/dissertation supported by Sea Grant?: No

Thesis / Dissertation:

New or Continuing?: New

Degree awarded this reporting period?: No

Financially supported?: Yes

Narratives

Narrative
Uploaded File: Final-Report-alleviate.pdf

Partners This Period

Puget Sound Restoration Fund
Types: NGO
Scale: REGIONAL
Notes:

Northwest Fisheries Science Center, National Marine Fisheries Service
Types: Government
Scale: FEDERAL or NATIONAL
Notes:

Washington Department of Fish and Wildlife
Types: Government
Scale: STATE
Notes:

STANDARD QUESTIONS

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Economic Impacts

(1)

For each economic impact:	

(provide a description and numbers in all relevant categories)	
Description	Increased efficiency in native Olympia oyster hatchery operations based on genetic results from Washington Sea Grant-supported research project. Larval output increased from 36 million to 60 million, while technician time decreased from more than 1700 hours to 48 hours, resulting in a $40,000 savings.
Market Impacts ($)	40000
Non-market Impacts ($)	
Businesses Created	
Businesses Sustained	
Jobs Created	
Jobs Sustained	
Patents	

Impacts and Accomplishments

(1)

Type	impact
Title	Washington Sea Grant researchers confirm local adaptation in native oysters to assist restoration and commercial aquaculture production
Relevance	Restoration of Olympia oysters—the only native West Coast oyster and the first farmed in Washington—is a regional priority. Traits of this native species that hold adaptive advantage, such as reproductive abundance and stress resilience, may differ among local populations. Understanding traits could benefit restoration efforts and alleviate regulatory concerns about cultured native shellfish impacting nearby wild populations.
Response	With funding from a national strategic initiative, researchers investigated essential information on local population structure of Olympia oysters so restoration efforts could be efficiently managed in an environmentally sustainable manner. They performed reciprocal transplant experiments at three sites and grew the three populations at a fourth site. Researchers also assessed mortality, reproductive activity and growth; conducted stress response experiments; and evaluated second-generation oysters under hatchery conditions.
	Researchers confirmed that population structure exists on a relatively small geographic scale, and

Results	moving oyster populations to locations where local stocks still are present could be disadvantageous. The results make the case for caution in introducing new populations where remnant population structure exists: maladapted transplants could overwhelm locally adapted residents or not survive in a new location or interbreed with local populations, reducing overall fitness. Investigators also developed improved breeding procedures — which increased the number of broodstock while maintaining genetic diversity — and nearly doubled larval output. Procedures also reduced staffing hours substantially, saving restoration practitioners $40,000 over a 12-week period.
Recap	Washington Sea Grant researchers confirmed differences among local populations of native Olympia oysters and identified effective breeding procedures that saved thousands of dollars.
Comments	
Primary Focus Area	Healthy Coastal Ecosystems
Secondary Focus Areas	Sustainable Fisheries and Aquaculture
Goals	Ocean and coastal resources are managed using ecosystem-based approaches.|Aquaculture operations and shellfish harvests are safe, environmentally sustainable and support economically prosperous businesses.
Partners	Clam Fresh, LLC Fagergren Oyster Co. Fidalgo Marina Northwest Fisheries Science Center (US DOC, NOAA, NMFS, NWFSC) Northwest Fisheries Science Center, Manchester Research Station (DOC, NOAA, NMFS) Puget Sound Restoration Fund Washington State Department of Fish and Wildlife
	* Type impact * Title Alleviating Regulatory Impediments To Native Shellfish Aquaculture * Relevance 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. * Response This project set out to provide essential information on local population structure of Olympia oysters such that restoration efforts can be carried out in an efficient and environmentally sustainable manner. The overarching experiment associated with this project included a reciprocal transplant of oysters among three sites across Puget Sound, along with the three populations also being grown out at a

PI Draft	fourth site. Complementary studies were carried out on these oyster populations including epigenetic characterization. * Results Results from the transplant experiment indicate In summary, oysters from Dabob Bay had higher survival at all sites but lower reproductive activity and growth. Oysters from Oyster Bay demonstrated greater reproductive activity at all sites with moderate growth and survival. Together these data suggest the existence of oyster population structure within Puget Sound and provide information on how broodstock should be selected for restoration purposes. Subsequent genetic and epigenetic characterization confirms differences in populations. * Recap Oyster populations in Puget Sound posses discrete phenotypes that could be targeted for restoration activities, however risk-averse decision making would avoid mixing populations. Comments Primary Focus Area Sustainable Fisheries and Aquaculture Secondary Focus Areas Healthy Coastal Ecosystems Goals Partners Clam Fresh, LLC Fagergren Oyster Co. Fidalgo Marina NOAA Manchester lab Puget Sound Restoration Fund Washington Department of Fish and Wildlife ----------------- * Type impact * Title Develop an anesthesia procedure for non-lethal sampling * Relevance One key metric to understand adaptive divergence in Olympia oysters is reproduction, a fitness component. Reproductive timing and effort differences among populations reared in a common environment can indicate local adaptation. Targeting these metrics heretofore required sacrificing many Olympia oysters to find a subset of active brooders. * Response A simple, nonlethal field procedure to anesthetize Olympia oysters was developed, causing them to gape sufficiently to ascertain brooding status, enable collection of broods, and/or facilitate tissue sampling. * Results The anesthesia method has been used in at least 3 other Olympia oyster research projects (B. Becker, UW Tacoma, personal communication; J. Barber, Swinomish Tribe, personal communication; D. Zacherl, CSU Fullerton, personal communication). * Recap An effective oyster anesthesia method has been developed. Comments Primary Focus Area Healthy Coastal Ecosystems Secondary Focus Areas Sustainable Fisheries and Aquaculture Goals Aquaculture operations and shellfish harests are safe, environmentally sustainable and support economically prosperous businesses. Partners ------------------ * Type impact * Title Define improved breeding procedures for conservation aquaculture * Relevance Olympia oyster restoration in Puget Sound can in some cases be best realized using hatchery produced seed. It is important, however, to minimize genetic differences between hatchery seed and the wild population targeted for supplementation * Response A breeding plan was set up to try to ensure low differentiation

	between seed oysters derived from wild South Puget Sound broodstock, and a random sample of the population in South Puget Sound. The breeding plan involved creating a series of small breeding groups of wild adults, thus ensuring genetic diversity in the population of larvae released over a period of about 2 months. * Results No significant differentiation between the south sound wild Olympia oysters and the Taylor hatchery produced seed was observed. In contrast, there was low level differences between the restoration seed and the wild adults. The same pattern was evident for the restoration seed produced in the following year. Based on these results, the currently adopted method for restoration seed production has changed and now mimics the commercial production method used by Taylor Shellfish. * Recap Puget Sound Restoration Fund has modified hatchery practices to reduce effort. Comments Primary Focus Area Sustainable Fisheries and Aquaculture Secondary Focus Areas Sustainable Fisheries and Aquaculture Goals Aquaculture operations and shellfish harests are safe, environmentally sustainable and support economically prosperous businesses. Partners

Leveraged Funds

(1)

Purpose	Alleviating Regulatory Impediments To Native Shellfish Aquaculture - graduate student support
Source	University of Chicago
Amount	12000
Start Date	02-01-2015
End Date	01-31-2016

Meetings, Workshops, Presentations

(1)

Type of Event	Public or professional presentation
Description	Discussion of Olympia oyster restoration at National Shellfisheries Association Meeting
Event Date	03-24-2015
Number of Attendees	50

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	Data sharing website - http://oystergen.es/
Developed (in the reporting period)?	Yes
Used (in the reporting period)?	Yes
Used for EBM?	No
ELWD product?	No
Number of managers	0
Description/Names of managers	

R/LME/N–3

Alleviating Regulatory Impediments To Native Shellfish Aquaculture

Final Report

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. This project set out to provide essential information on local population structure of Olympia oysters such that restoration efforts can be carried out in an efficient and environmentally sustainable manner. The overarching experiment associated with this project included a reciprocal transplant of oysters among three sites across Puget Sound, along with the three populations also being grown out at a fourth site. In addition to the array of data we produced about differences in populations of oysters in Puget Sound, we also developed procedures and resources that will contribute to aquaculture practices and fundamental biological investigations. Specifically, as part of the research activities we were able to

1. Develop an anesthesia procedure for non-lethal sampling
2. Define improved breeding procedures for conservation aquaculture
3. Develop significant genomic resources for the Olympia oyster

As for the characterization of local populations of Olympia oyster in Puget Sound, we identified numerous differences that are critical for consideration of conservation efforts. For this final report we will outline the primary findings from a number of studies carried out during the tenure of this project including

1. Field performance of oysters in the transplant experiment
2. Differential response to stress of oyster populations as measured by gene expression
3. Growth and survival of F2 oysters under hatchery conditions
4. Epigenetic and genetic degree of local population differentiation

In the remainder of this report we will first describe the findings of these four core studies followed by how these combined data will be used be resources managers (Section A). Then we will describe the procedures and resources developed as part of work that have immediate impacts beyond our study area (Section B).

A.1. Field performance of oysters in transplant experiment

For this study three geographically separated, discrete groups of *O. lurida* within Puget Sound were selected. These animals were brought to a hatchery, spawned, and the offspring from each population outplanted back to the bays selected. This approach enables observations about how differing natural environments with resident oyster populations may affect both local and non local populations over time.

Three bays (ie. Fidalgo Bay, Dabob Bay, and Oyster Bay) within Puget Sound were selected for this experiment based on presence of resident *O. lurida* populations, distance from other bays, and latitudinal position. Fidalgo Bay is the most northern site and experiences cooler year round conditions. This bay is also more directly influenced by the Strait of Juan de Fuca, allowing colder sea water directly from the Pacific to mix with bay waters. Dabob Bay is located within Hood Canal, an area of Puget Sound distinctly separated from the rest of the sound. Oyster Bay is the southern most site and known for its historically large numbers of *O. lurida*.

In summary, oysters from Dabob Bay had higher survival at all sites but lower reproductive activity and growth (Figure 1 and 2). Oysters from
Oyster Bay demonstrated greater reproductive activity at all sites with moderate growth and survival (Figure 1). Together these data suggest the existence of oyster population structure within Puget Sound and provide information on how broodstock should be selected for restoration purposes.

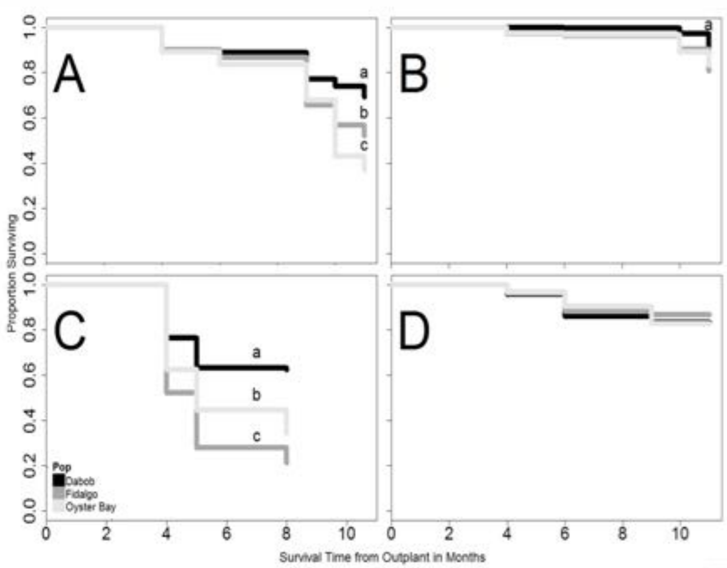

Figure 1. Proportion survival for three oyster populations at four locations; Southern site (A), Central site (B), Hood Canal site (C), and Northern site (D). Lowercase letters (a, b, c) are significant differences.

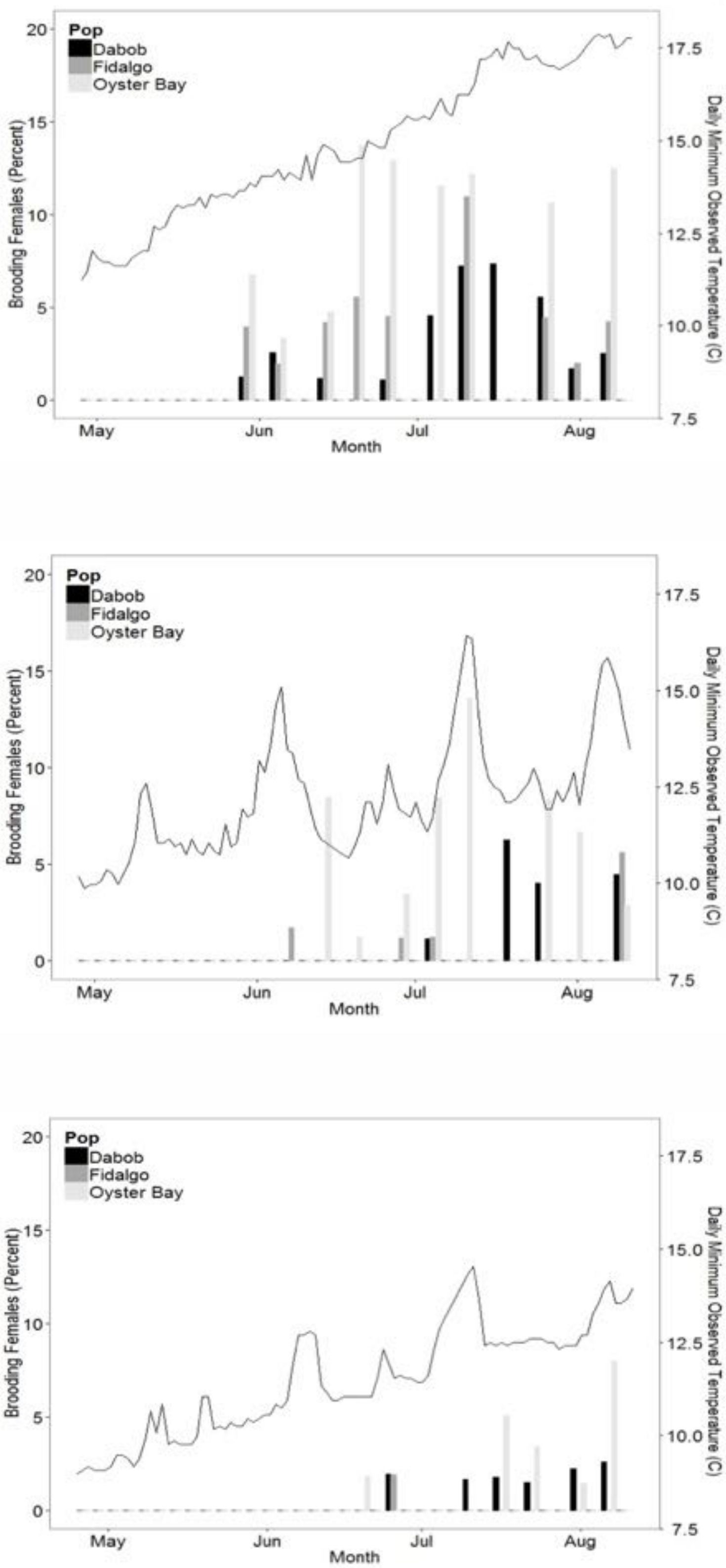

Figure 2. Percent Brooding Females from Each Population at Each Sample Date at Oyster Bay (top), Fidalgo (middle), and Manchester (bottom).
Percent determined by number of brooding females (Br) divided by number of open oysters (T) or %=(Br/T)*100.

Differences in life history traits among oyster populations grown in different locations within Puget Sound, WA suggest adaptations possibly linked with environmental cues. High survival, low growth, and low reproductive activity of the Dabob Bay population is likely due to extreme environmental variation at their home site leading to improved stress resilience. The greater proportion of brooding females in the Oyster Bay population and reduced environmental energy needed to induce peak spawning may be related to positive selection pressure for early spawners due to warmer temperature trends at their home site. Findings from this study indicate possible local adaptation in two of the three populations observed but there may be other factors dictating observed phenotypes, including the fact that we do not know broodstock used here were from local parents.

This complete work is published: Heare JE, Blake B, Davis JP, Vadopalas B, Roberts SB. (2015) Evidence of *Ostrea lurida* (Carpenter 1864) population structure in Puget Sound, WA. PeerJ PrePrints 3:e1986 https://doi.org/10.7287/peerj.preprints.704v4

A.2. Differential response to stress of oyster populations as measured by gene expression

Based on the results of the transplant experiment and apparent differences in stress responses, we examined expression of genes associated with growth, immune function, and gene regulatory activity in oysters from the three populations following temperature and mechanical stress. We found that heat stress and mechanical stress significantly changed expression in molecular regulatory activity and immune response, respectively. We also found that oysters from Oyster Bay had the most dramatic response to stress at the gene expression level.

More specifically oysters from each population were brought to the lab and subjected to acute temperature stress (38°C water for 1 hour; n=14 per population), mechanical stress (1000 rpm x 5 min; n=14) or served as controls (maintained at 8°C; n=14). Oysters were placed back in 8°C seawater and sampled at 1 hour post stress (n=8). Six oysters were also monitored daily for 14 days to assess survival. Ctenidia and mantle tissue was dissected and stored in RNAzol RT (Molecular Research Center, Inc.) at –80°C for later analysis. Quantitative PCR was carried out on the nine genes listed in table 1.

Full Sequence	Uniprot ID	Uniprot ID	Annotation	Function	Gene Abbr
comp7220_c0_seq2	Q6DC04	CARM1_DANRE	Histone-arginine methyltransferase	Transfers methyl groups to Histone 3 and 4 to change how DNA is bound up in chromatin	CARM
comp23747_c0_seq1	Q9DD78	TLR21_CHICK	Toll-like receptor 2 type 1	Assists with recognition of foreign pathogens and endogenous materials for consumptions by phagocytes in early stages of inflammation	TLR
comp25000_c0_seq1	P08991	H2AV_STRPU	Histone H2A.V	One of 5 main Histone Proteins involved in the structure of chromatin and the open reading frame of DNA	H2AV
comp24065_c0_seq1	O75594	PGRP1_HUMAN	Peptidoglycan recognition protein 1	Assists with recognition of bacteria in an immune response	PGRP
comp44273_c0_seq2	Q8MWP4	Q8MWP4_OSTED	Heat Shock Protein 70kDa	Molecular chaperone and protein preservation in heat response	HSP70
comp7183_c0_seq1	P12643	BMP2_HUMAN	Bone morphogenetic protein 2	Directs calcification in shell creation	BMP2
comp10127_c0_seq1	P62994	GRB2_RAT	Growth factor receptor-bound protein 2	Assists in signal transduction/cell communication	GRB2
comp6939_c0_seq1	P32240	PE2R4_MOUSE	Prostaglandin E2 receptor EP4 subtype	Receptor for Prostaglandin E2 which suppresses inflammation due to injury	PGEEP4
comp25313_c0_seq1	Q60803	TRAF3_MOUSE	Tumor Necrosis Factor receptor-associated factor 3	Related to immune response specifically cell death initiation	CRAF
comp30443_c0_seq2	Q8TA69	Q8TA69_CRAGI	Actin	Housekeeping gene used for baseline	Actin

Table 1. Table of qPCR Primers for genes of interest

Contrary to our hypothesis, oysters from Oyster Bay demonstrated the greatest difference in response to stress compared to the other populations. Specifically oyster originally from Oyster Bay, had an increase in H2AV expression during heat stress as compared to control, a decrease in BMP2 and GRB2 upon mechanical stress, and differences in HSP70 expression between the two treatments. While only speculation at this point, the gene expression pattern differences observed here with oysters from Oyster Bay coupled with corresponding field-based observation that this population has the greatest reproductive activity, could indicate this population has a greater ability to effectively respond to energy demands. This trait could certainly be perceived as advantageous for restoration purposes. On the other hand, the results of the gene expression analysis does suggest population differences. Caution should be used in using non-local stocks when structure exists, as it is possible to have supplemented oysters outcompete the native population or to create hybrids that are ultimately less fit than the native counter parts.

This complete study is published: Heare JE, White SJ, Roberts SB. (2015) Differential response to stress in *Ostrea lurida* (Carpenter 1864) as measured by gene expression. PeerJ PrePrints 3:e1994 https://doi.org/10.7287/peerj.preprints.1595v1

A.3. Growth and survival of F2 oysters under hatchery conditions

To test if differences in fitness observed in the reciprocal transplant experiment were consistent in F2s raised in controlled, common conditions, we conducted a common garden study in Summer 2015 with ~100 F1 oysters from each population at the K. K. Chew Center for Shellfish Restoration and Research in Manchester, WA. Concerns over transgenerational effects would be mitigated if F2 animals reared in the same environment showed similar interpopulation differences as the F1s. Oysters from each population were spawned in 5 groups of 20 oysters to promote genetic diversity. Newly released larvae were combined based on population and reared in 100 L tanks. After reaching >160 µm in size (as determined by filtering over mesh screens), larvae were moved into a second tank and raised until close to metamorphosis (> 224 µm). Juveniles reaching metamorphosis at the same time were established on 10 cm x 10 cm PVC tiles before being placed in nearby Clam Bay to grow for 10 weeks.

Reproductive output and growth rate were used as fitness proxies. Reproductive output was estimated by counting the number of spawned larvae from each group (Figure 3). Similar to the reciprocal transplant experiment, oysters from Oyster Bay showed the earliest and greatest reproductive output. To measure larval growth rate, ~700 one-day old larvae were raised in 1 L beakers for 2 weeks with daily water changes, with 6 replicates per population. Samples of 100 larvae were taken 3 times throughout this period and measured using a microscope and ImageJ. In analysis of protoshell length in 20 seven-day-old larvae from each population, size varied significantly among populations and the larvae from Oyster Bay were significantly larger than those from Oyster Bay (Figure 4). After establishing on the PVC tiles, juveniles were measured at three time points over 10 weeks, from August 2015 to November 2015. A measurement of approximately 30 juvenile oysters from each population in October was consistent with the larvae measurements and reciprocal transplant results in that oysters from South Sound were significantly larger than those from Hood Canal (Figure 5). These results are concordant with the reciprocal transplant indicating that phenotypic differences in size may indeed be due to genetic factors and not environmental effects.

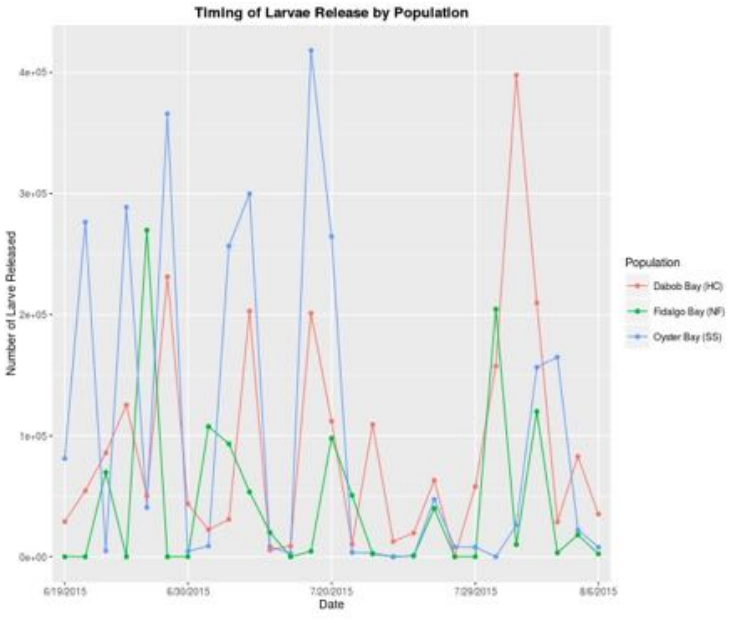

Figure 3. Reproductive output as measured by number of larvae released.

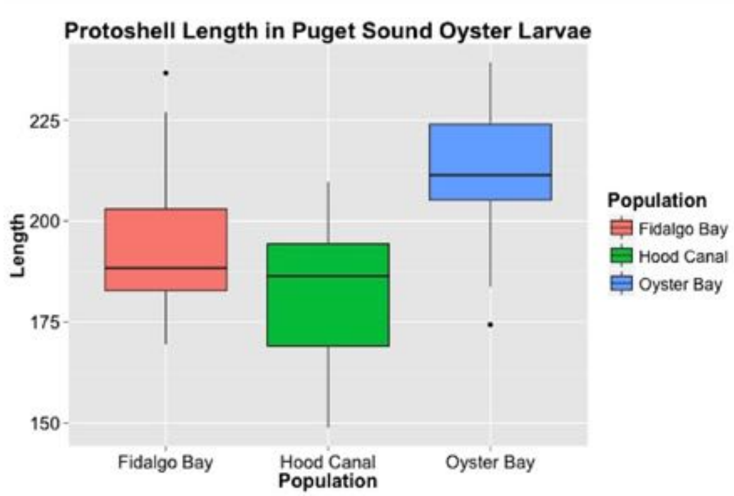

Figure 4. Protoshell length in 27 day old larvae.

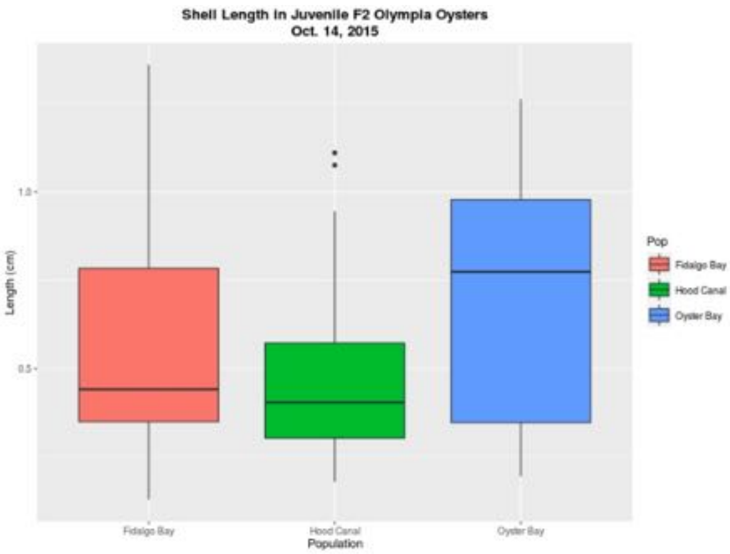

Figure 5. Size measurements of oysters on tiles.

A.4. Epigenetic and genetic degree of local population differentiation

In conjunction with examining growth and survival of F2s in order to determine if population difference were independent of environmental factors, we also characterized genetic and epigenetic differences in our target populations. To examine genetic differentiation of the three populations we characterized single nucleotide polymorphisms (SNPs). Using a 2bRAD reduced-representation sequencing approach, we sequenced 279 individuals with roughly equal representation of all three populations. To date we have identified 3,510 SNPs confidently genotyped in at least 50% of samples examined.

To get an estimate of population differentiation among our three populations, we thinned our dataset and selected 882 SNPs genotyped in at least 70% of 59 individuals. Using Weir and Cockerham's 1984 FST estimator for each SNP, we found a mean FST of 0.0528 for all 59 individuals as three subpopulations. Eight SNPs had elevated values of FST over 0.4. Average pairwise FST was highest between the Oyster Bay and Hood Canal populations (0.0668), with 24 SNPs having an FST over 0.4. Pairwise FSTs in comparing Fidalgo to Hood Canal and Oyster Bay to Fidalgo were 0.0433 and 0.0485, respectively.

Together these results indicate there is population differences, though some gene flow could exist. There are also specific loci that are segregating more among populations and could be associated with population specific traits.

For the first time in this species, we also characterized the potential epigenetic component of population differences. To confirm that the epigenetic signature of DNA methylation persists independent of environment, we characterized whole genome DNA methylation via bisulfite sequencing of Oyster Bay and Hood Canal oysters both grown out in Clam Bay. Nine individuals were sequenced and there is a clear population specific signature that likely contributes to phenotypic differences in populations (Figure 6 & 7).

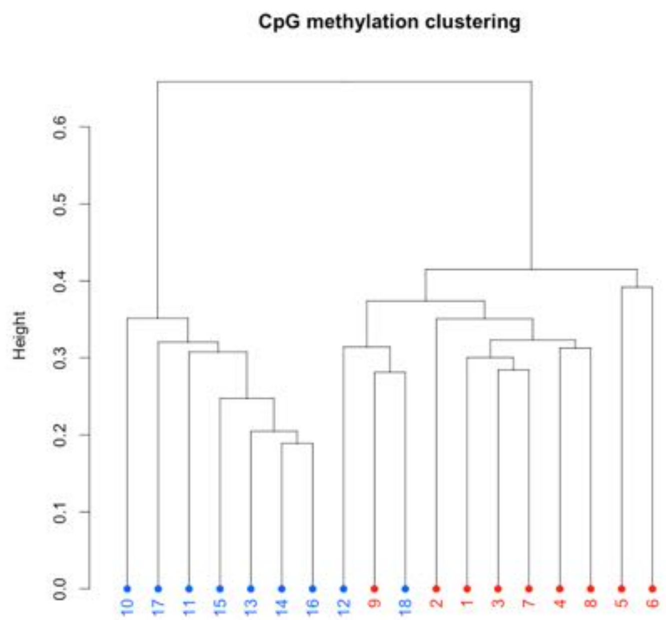

Figure 6. CpG methylation clustering cladogram of oysters from Oyster Bay (blue) and Hood Canal (red) grown in Clam Bay.

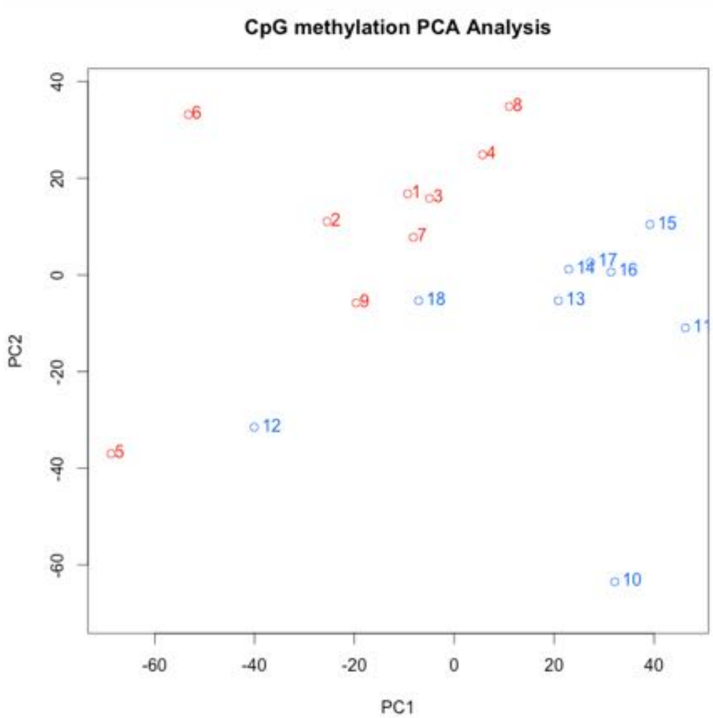

Figure 7. CpG methylation PCA analysis of oysters from Oyster Bay (blue) and Hood Canal (red) grown in Clam Bay.

A.5 Implications for resource managers

Our results certainly have important implications for restoration of Olympia oysters within Puget Sound, WA. There are a number of ways that these findings could be used in generating restoration strategies specific to Puget Sound and in the face of climate change. Based on the fact that Dabob Bay oysters had the lowest mortality, use of the most robust population for broodstock may increase chances for outplant survival. Generally, this approach would dictate organisms should be used from home environments that experience persistent stressful conditions. An alternative approach managers might take given the current findings is to take the population with the greatest reproductive output (Oyster Bay) and use it as a source of broodstock. This would increase the likelihood of juvenile recruitment and ultimate restoration of the species, while also producing more offspring for outplant. Having a strong understanding of population related phenotypes creates another option for restoration efforts. An assisted gene flow strategy that incorporates the outplanting of populations known to contain phenotypes fit for the new environmental parameter and have them interbreed with resident populations. It is highly debated whether such a strategy would have benefits that outweigh the drawbacks, such as possible outbreeding depression, but should be considered for restoration efforts facing a variety of climate change scenarios.

Ultimately, what these studies demonstrate is that population structure can and does exist on a relatively small geographic scale and thus moving oyster populations to locations where remnant stocks exist could be disadvantageous. When population structure exists, there should be caution with respect to moving populations as: 1) transplanted populations could overwhelm locally adapted remnant resident populations, and possibly not persist themselves, 2) transplanted populations might not survive in new location and thus wasting resources required for restoration, and 3) transplanted populations could interbreed with remnant population and thus result in overall reduced fitness through outbreeding depression.

B. Additional Products

In addition to the array of data we produced concerning differences in populations of oysters in Puget Sound, we also developed procedures and resources that will contribute to aquaculture practices and fundamental biological investigations.

B.1. Develop an anesthesia procedure for non-lethal sampling

One key metric to understand adaptive divergence in Olympia oysters is reproduction, a fitness component. Reproductive timing and effort differences among populations reared in a common environment can indicate local adaptation. Targeting these metrics heretofore required sacrificing many Olympia oysters to find a subset of active brooders. We developed a simple, nonlethal field procedure to anesthetize Olympia oysters, causing them to gape sufficiently to ascertain brooding status, enable collection of broods, and/or facilitate tissue sampling. Briefly, the method involves 45 min exposure to air, followed by immersion in 83 mM $MgSO_4$ for 45 min. After testing the method in the lab, the method was used in our field study on a weekly basis to obtain reproductive timing and population proportion data. Repeated exposure of Olympia oysters to the anesthetic resulted in no detectable mortality or adverse effects. The anesthesia method has been used in at least 3 other Olympia oyster research projects (B. Becker, UW Tacoma, personal communication; J. Barber, Swinomish Tribe, personal communication; D. Zacherl, CSU Fullerton, personal communication). The method and field application are currently being prepared for submission for publication.

Figure 8. Photograph of oysters subjected to anesthesia procedure prior to sampling.

B.2. Define improved breeding procedures for conservation aquaculture

Olympia oyster restoration in Puget Sound can in some cases be best realized using hatchery produced seed. It is important, however, to minimize genetic differences between hatchery seed and the wild population targeted for supplementation. A breeding plan was set up to try to ensure low differentiation between seed oysters derived from wild South Puget Sound broodstock, and a random sample of the population in South Puget Sound. The breeding plan involved creating a series of small breeding groups of wild adults, thus ensuring genetic diversity in the population of larvae released over a period of about 2 months. To further optimize genetic diversity, the larvae/juveniles from the breeding groups were reared separately until outplanting to enable even contribution from each breeding group.

We used 7 microsatellite loci to compare diversity metrics between a random wild sample and the seed destined for restoration. We also compared commercial hatchery production (Taylor Shellfish) to the wild sample, expecting to see lower diversity. We detected no significant differentiation between the south sound wild Olympia oysters and the Taylor hatchery produced seed. In contrast, we detected low level differences between the restoration seed and the wild adults. The same pattern was evident for the restoration seed produced in the following year. *Based on these results, the currently adopted method for restoration seed production mimics the commercial production method used by Taylor Shellfish*. Approximately 1200 broodstock are held in a common tank and all released larvae are collected. The primary difference between the commercial and restoration method is the period of collection: for the former, collection is terminated once sufficient larvae are released for production goals, while for the latter, the restoration hatchery collects released larvae over an extended period (ca 2 mo) to capture and retain genetic variation in spawn timing which is likely of adaptive significance.

B.3. Develop significant genomic resources for the Olympia oyster

As part of this project and complementary efforts we have been able to generate a wealth of genomic data including RNA-Seq, RAD-Seq, Bisulfite Sequencing, and Whole Genome Shotgun data. One transcriptome has been published (Timmins-Schiffman EB, Friedman CS, Metzger DC, White SJ and Roberts SB. (2013) Genomic resource development for shellfish of conservation concern Molecular Ecology Resources. doi:10.1111/1755–0998.12052) with all data publicly available at http://owl.fish.washington.edu/nightingales/O_lurida/. We have also developed a webpage to make these data more easily accessible (http://oystergen.es/olympia/). In addition we have created a genome browser where anyone can visualize these data or combine with other data (Figure 9). While much of these data provided core information for us to be able to characterize local population of Olympia oysters, we suspect these data will provide important resources for others involved in aquaculture, biological, and environmental studies.

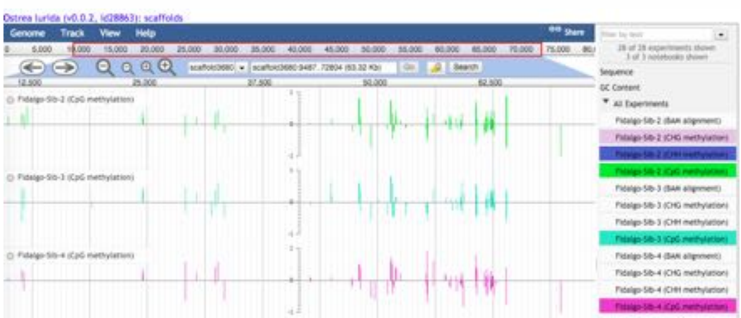

Figure 9. Screenshot of Olympia oyster genome browser available at https://genomevolution.org/coge.