Update Report

Period: 2/1/2014 - 1/31/2015

Project: R/SFA-2 - *Crossbreeding and Selection for Resistance to Ocean Acidification in Pacific Oysters*

STUDENTS SUPPORTED

Bennett, Sean, seanb80@uw.edu, University of Washington, School of Aquatic and Fishery Sciences, status: new, field of study: Aquatic and Fishery Sciences, advisor: Carolyn Friedman, degree type: BS, degree date: 2016-06-01, degree completed this period: No
Student Project Title: *none*
Involvement with Sea Grant This Period:
Work study student assistant on field sampling efforts associated with project
Post-Graduation Plans:
graduate school

Gillon, Daniel, dgillon@uw.edu, University of Washington, School of Aquatic and Fishery Sciences, status: new, field of study: Fisheries, advisor: Carolyn Friedman, degree type: MS, degree date: 2016-06-01, degree completed this period: No
Student Project Title:
Breeding Approaches to Improving Pacific Oyster Resistance to Ocean Acidification
Involvement with Sea Grant This Period:
Research Assistant
Post-Graduation Plans:
will seek employment

Matson, Catherine, matson@gmail.com, University of Washington, School of Aquatic and Fishery Sciences, status: new, field of study: none, advisor: Carolyn Friedman, degree type: MS, *no degree date*, degree completed this period: No
Student Project Title:
none
Involvement with Sea Grant This Period:
Assisted in setup of ocean acidification experiments in 2014
Post-Graduation Plans:
no longer a student

Roberts, Emily, earobert@uw.edu, University of Washington, Biology, status: new, field of study: Ocean Acidification in Mussels, advisor: Emily Carrington, degree type: PhD, degree date: 2020-06-01, degree completed this period: No
Student Project Title:
Ocean Acidification Effects in Mussels
Involvement with Sea Grant This Period:
Lead in setting up ocean acidification challenge system for 2015 experiments
Post-Graduation Plans:
Faculty Position

CONFERENCES / PRESENTATIONS

No Conferences / Presentations Reported This Period

ADDITIONAL METRICS

P-12 Students Reached:	**P-12 Educators Trained:**
Participants in Informal Education Programs:	**Volunteer Hours:**
Acres of coastal habitat protected, enhanced or restored:	**Resource Managers who use Ecosystem-Based Approaches to Management:**
Annual Clean Marina Program - certifications:	**HACCP - Number of people with new certifications:**

ECONOMIC IMPACTS

No Economic Impacts Reported This Period

SEA GRANT PRODUCTS

Description	Developed?	Used?	ELWD?	Number of Managers	Names of Managers
Novel approach for measuring dissolved carbon dioxide in seawater for use in measuring small-scale responses in pacific oyster larvae to acidic seawater	Yes	Yes	No	0	

HAZARD RESILIENCE IN COASTAL COMMUNITIES

No Communities Reported This Period

ADDITIONAL MEASURES

Number of stakeholders modifying practices:	Sustainable Coastal Development
	# of coastal communities:

PARTNERS

Partner Name: Baywater Shellfish Farms, type: Industry and Business, scale: State

Partner Name: Friday Harbor Laboratories Ocean Acidification Environmental Laboratory, type: Academic Institution, scale: State

Partner Name: Puget Sound Restoration Fund

Partner Name: Taylor Shellfish Company

Partner Name: USDA Agriculture Research Station

IMPACTS AND ACCOMPLISHMENTS

Title: **Washington Sea Grant research investigates crossbreeding and selection of Pacific oysters for resistance to ocean acidification**

Type: accomplishment

Description:

Relevance: The West Coast shellfish industry remains at risk from the threat of ocean acidification (OA) to the health of Pacific oyster broodstock. Borrowing from traditional agricultural practices, selective crossbreeding methods may hold potential for creating OA-resistant Pacific oyster broodstock lines.

Response: Washington Sea Grant-funded researchers initiated screening of up to 90 genetically distinct hybrid lines of Pacific oysters for OA resistance, making observations throughout early life stages and looking at the possibility of carryover effects from one generation to another. Experiments are focused on exposing larval oysters to acidified waters in the laboratory, leading to the creation of broodstock that may be resistant to OA.

Results: Researchers developed and installed a new system for more efficient OA screening of larvae and small oyster seed at NOAA's Kenneth Chew Center for Shellfish Research and Restoration. Oyster parents and larvae were exposed to four CO2 treatments and planted at three Puget Sound commercial shellfish aquaculture sites. Preliminary analysis from two sites, Totten Inlet and Thorndyke Bay, indicated that some treatments resulted in increased yields. Forty-five hundred oysters were measured for survivorship, growth characteristics, and total, wet, and shell weight.

Recap:

Recap: Washington Sea Grant researchers began developing OA-resistant hybrid Pacific oyster broodstock for commercial use by the Pacific shellfish industry.

Comments:

Primary Focus Area: SFA

Secondary Focus Area: HCE

Associated Goals: Aquaculture operations and shellfish harvests are safe, environmentally sustainable, and support economically prosperous businesses. (SFA)

Ocean and coastal resources are managed using ecosystem-based approaches. (HCE)

Partners:

Baywater Shellfish Farms

Friday Harbor Laboratories Ocean Acidification Environmental Laboratory

Puget Sound Restoration Fund

Taylor Shellfish Company

USDA Agriculture Research Station

Related Partners: *none*

PUBLICATIONS

No Publications Reported This Period

OTHER DOCUMENTS

No Documents Reported This Period

LEVERAGED FUNDS

No Leveraged Funds Reported This Period

UPDATE NARRATIVE

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Crossbreeding and Selection for Resistance to Ocean Acidification in Pacific Oysters

WA Sea Grant Program - reporting period (Feb 1, 2014 – Jan 31, 2015)

1. Screening for ocean acidification resistance in intraspecific hybrid lines of Pacific oysters

In Spring 2014 in collaboration with Taylor Shellfish Company (TSC) we initiated an assessment of the effects of ocean acidification (OA) on early and late development in the larvae of intraspecific hybrid lines of diploid Pacific oysters. The proposal calls for the UW team to coordinate with the Pacific Shellfish Institute (PSI) and TSC with the initial assessment of up to 90 distinct genetic lines of oysters generated by the Taylor HYBreed crossbreeding program. The project is focusing on two early life history larval stages in Pacific oysters: embryogenesis to the veliger stage and during the transition from pediveliger to early spat. The OA screening process for generating data on genetically determined resilience to OA stress calls for placing embryos and larvae from different genetic lines into replicate chambers submerged in seawater under pre-determined high and low pCO_2 conditions and comparing short-term growth, survivorship and other larval stress responses.

The OA assessment work was to be conducted at the UW Friday Harbor Laboratory Ocean Acidification Environmental Laboratory. Co-PI Friedman's graduate student, Dan Gillon spent much of the spring and summer training for the assessment work under the direction of Drs. C. Friedman, J. Davis and E. Carrington. Dr. Carrington also has OA assessment work on molluscs (*Mytilus trossulus*) that is ongoing at FHL.

Significant problems associated with rearing single oysters through the 1mm size threshold by TSC at the Quilcene, WA hatchery facility during early summer 2014 and

issues associated with genotyping parental lines proved problematic in initiating the large-scale crossbreeding work as planned. Following repeated delays, we opted to postpone the full-scale crossbreeding assessment and first conduct a pilot experiment to assess variation in growth and survivorship of larvae, under control and high pCO_2 conditions, in multiple, half-sib mated families of *C. gigas*. We added ploidy as an additional factor to test as the diploid lines generated were paired with sibling triploid lines produced by manipulating chromosome sets during early embryogenesis. Briefly, this entailed producing fifteen pair-mated half-sib lines generated from broodstock lines produced originally from wild Pipestem Inlet (B.C., Canada) Pacific oysters collected by TSC. Each broodstock oyster was *partially* strip-spawned and the gametes from male and female oysters prepared for making individual pair-mated crosses within a nested half-sib mating design. Specifically, three sets of four female oysters each were mated with a single male to produce three groups of four half-sib lines each. Half of the embryos created within each diploid line were subsequently treated with 6-dimethylaminopurine (6-DMAP) to create triploid embryos. For example, male 13 (M13) was mated with female 1 (F1). Half of the embryos generated in the diploid line were then subsequently treated to generate triploid embryos (F1-T) (Table 1). The specific treatment protocol for producing triploid embryos consisted of incubating fertilized embryos at 25 °C at 15 min. post fertilization with 300 uL 6-DMAP for 20 minutes. Following the treatment, embryos were rinsed in filtered seawater and the resulting embryos reared at the TSC facility using standard rearing protocols for Pacific oysters.

The 15 partially stripped broodstock oysters used in the crosses were wrapped in cellophane, refrigerated and stored overnight. The following morning these oysters were

transported to the FHL OA Environmental Laboratory and additional gametes obtained through the strip-spawning procedure used the day prior for the purpose of regenerating the same 12 pair mated, half-sib diploid lines (no triploid lines were produced).

M13 x F1-D	M13 x F1-T	M13 x F2-D	M13 x F2-T	M13 x F3-D	M13 x F3-T	M13 x F4-D	M13 x F4-T
M14 x F5-D	M14 x F5-T	M14 x F6-D	M14 x F6-T	M14 x F7-D	M14 x F7-T	M14 x F8-D	M14 x F8-T
M15 x F9-D	M15 x F9-T	M15 x F10-D	M15 x F10-T	M15 x F11-D	M15 x F11-T	M15 x F12-D	M15 x F12-T

Table 1. matrix of half-sib family matings made at the Taylor Shellfish Company hatchery in summer 2014. (example; M13 x F1D and M13 x F1T are sibling lines generated by mating male 13 with female 1 to produce F1D and then inducing triploidy in ½ of the embryos generated to produce F1T).

Immediately following stripping of gametes, rinsing and preparation of gametes for making pair-mated crosses and fertilizations, replicate groups of embryos (N= 50 of embryos; N=3 replicates) were placed into 12-well plates (3 replicates in 3 plates per treatment) and placed into sealed "mesocosms" under two pCO_2 treatment levels (1600 uatm vs. 800 uatm). Embryos in each well were suspended in approximately 4mL of seawater at the appropriate pCO_2 level. Premixed 1600 uatm Praxair© gas was slowly pumped into the "treatment" mescosm to equilibrate with seawater and maintain aqueous pCO_2 levels. A 1L tripour beaker filled with seawater at the appropriate pCO_2 level was placed in each mesocosm to help maintain equilibrium conditions. Each mesocosm was weighted so it would sit in a water bath at 25^{o}. Embryos were incubated for 30 hours at

25°C. After 30 hours, 10% buffered formalin was added (200uL) to all the well plates to fix developing embryos.

Embryos were incubated for 24 hours at 25°C. After 24 hours, 10% buffered formalin was added (how much) to all the well plates to fix developing embryos.

The sibling larval group being reared at the TSC were reared and sampled for size at age and survivorship at Day 14. Pediveligers from all the lines with surviving larvae were collected from the cultures and transported to FHL for a second OA screening. At FHL replicate pediveliger groups (N=3) from each surviving ploidy/family combination were placed into individual downwellers (N=500 pediveligers per silo), each silo fit with a 180-micron screen to retain the pediveligers. Silos were placed in the OA system individually as described above. Flow rates of incoming seawater were maintained at about 100ml per minute. Temperature and salinity were maintained at 20^{o}C and 30ppm, respectively. Pediveligers were maintained on a live algal diet consisting of T-ISO and *C. calcitrans* at 80-cells/uL ration. Two pCO_2 treatments were selected: 1600 uatm and 800 uatm pCO_2 and maintained continuously over the treatment window of 14 days. In order to facilitate pediveliger settlement behavior, microcultch, consisting of Pacific oyster shell chips, was added to all the downwell silos to provide substrate for pediveligers to settle and metamorphose.

After 14 days, cultch with attached oyster spat was removed from all the downwellers and preserved in 90% EtOH. Data collection consisted of the count and size (shell height) of all settled oysters attached to the microcultch and the sides of the replicate silos for the treatment and control for all the surviving diploid and triploid half-sib families.

Several issues emerged over the course of the reporting period pursuant to this project objective. First, the embryogenesis experiment was completely compromised due to the partial loss of samples during transport. Secondly, the overall settlement rate of pediveligers from all lines tested was low in both the experimental and control silos at between 10-15% on average. It was subsequently determined that the pediveligers were likely underfed during the settlement phase and may explain the low percent of larvae observed to metamorphose. Data collection for this component of the experiment remains in process.

2. Construction and optimization of new OA system for screening Pacific oyster larvae and spat

A significant amount of time during the current reporting time was spent developing and optimizing the build-out of a new OA treatment system to be installed at the Kenneth K, Chew Restoration Hatchery and Laboratory in Manchester for use in all components of the project beginning in spring 2015. This system enables rapid and efficient modulation of seawater pH via the addition of CO_2. CO_2 is delivered from a gas cylinder to a seawater tank through a venturi injector. A Honeywell® computer controls the amount of gas being added through a PID loop, responding to constant pH readings from a Durafet® pH probe sitting in the seawater tank. To reach a target pH set point, a solenoid valve opens, allowing gas to flow into the seawater; the valve closes when the target pH is reached. CO_2-free air is also pumped into the seawater tank through an air compressor and CO_2 scrubber to help maintain pH conditions. Seawater at the appropriate pH can then be delivered via pump or drippers to any larval or broodstock rearing tanks.

The system allows for independent control of two separate PID loops, so we can maintain OA conditions and control conditions in separate seawater baths. The system can also be modified for both flow-through and recirculating seawater systems.

3. Development of Pacific oyster broodstocks resistant to OA conditions

During the hatchery phase of our experiment, Pipestem Inlet Pacific oyster families were exposed to CO_2 treatments as parents (high or low) and as larvae (high or low) to generate four treatments: High high (HH), high low (HL), low low (LL) and low high (LH) respectively. The larvae were set in the hatchery and

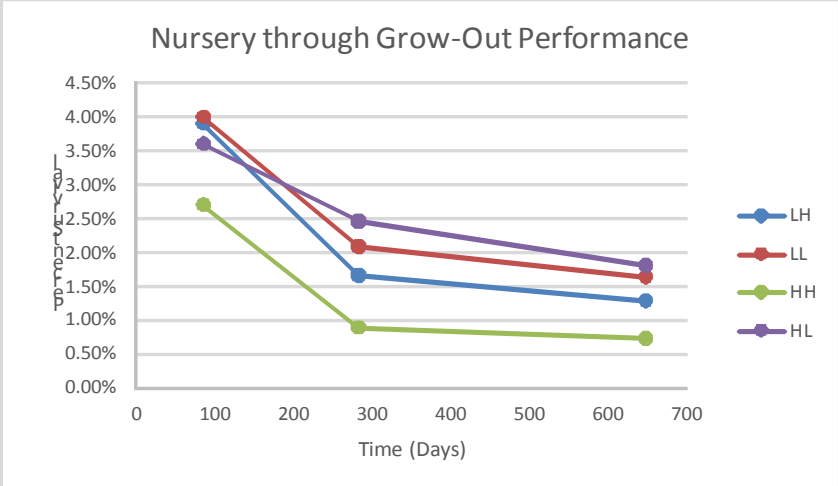

Figure 1. Survivorship of oysters to Day 647 for different treatments overall.

transitioned to the nursery prior to being planted at three sites in the greater Puget Sound area in summer 2013 and maintained the oysters through December 2014. Sites included three commercial shellfish farms located in Sequim Bay, Thorndike Bay and Totten Inlet, respectively. In December 2013 (day 282), we observed a site effect on survival. Fewer oysters in Totten Inlet survived (61%) than in the other

two sites (94-97%); p<0.0001),

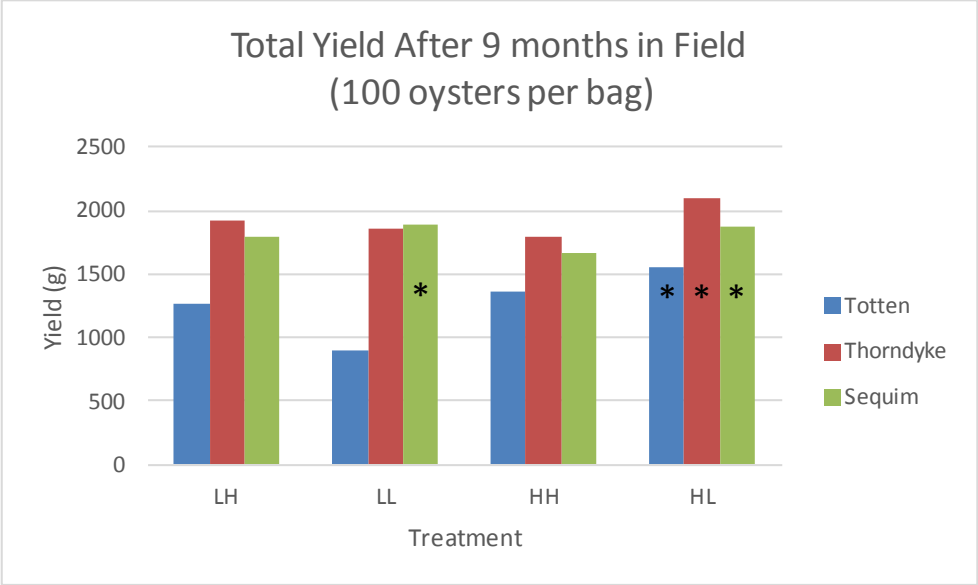

Figure 2. Yield of oysters from each treatment (all families combined) after nine months of field growout.

likely due to a thermal stress after planting. Both site and treatment impacted total weight of oysters. Those in Totten Inlet were larger than those in the other two sites ($p<0.05$). When all data was combined, treatment affected weight: oysters from the HL treatment weighed more than those from the other three treatments ($p<0.001$), which were similar to one another ($p>0.05$). In December 2014, day 647, were observed similar trends of a site effect on survival (Totten = 43% and Thorndyke = 87%; $p<0.0001$) but no effect from treatment ($p>0.05$). When all data was combined for treatments, treatment effects on total weight were marginally significant: LL > HH = HL > LH ($p=0.06$). Preliminary analysis of total survival across sites for the four treatments is shown in Figure 1. Survival was highest in the HL treatment and was similar to that in the LL treatment, while those in the LH treatment were intermediate in survival and the least survival was observed in the HH treatment.

Yield measurements, a combination of survival and live weight from hatch through 9 months in the field was estimated and preliminary analysis suggests that the HL treatment may provide increased yield at both Totten Inlet and Thorndyke Bay (Figure 2). Oysters from both the HL and LL treatments that were reared in Sequim Bay had similar yields. Highest yields for treatment groups as highlighted with an asterisk.

3. Sampling Pipestem offspring for genotypes in preparation for producing a G2 broodstock for commercial use

The highest and smallest oysters were sampled in December 2014 for measurements to examine if family lineage played a role in differences in yield observed within and between treatments. Replicate growout cages containing oysters for each treatment (corresponding to offspring produced in the original 2013 WASG breeding experiment that produced the G1 Pipestem generation) were sampled from Totten Inlet and Thorndyke Bay only. Approximately 4500 oysters were measured for total survivorship, total weight, wet weight, shell weight and morphometrics. Cages from Totten Inlet and Thorndyke Bay, corresponding to roughly 2/3 of all oysters, were then individually labeled with plastic Floy tags affixed to the shell with epoxy and returned to Thorndyke Bay to await further analysis.