RESEARCH/PD ANNUAL REPORT - PROGRESS REPORT

2015 annual report - progress
Jonathan Davis
Crossbreeding and Selection for Resistance to Ocean Acidification in Pacific Oysters
R/SFA-2
Submitted On: 04/16/2016 09:54:32 PM

METRICS & MEASURES

Metric/Measure	Value	Note
Acres of coastal habitat	0	
Fishermen and seafood industry personnel	0	
Communities - economic and environmental development	0	
Stakeholders - sustainable approaches	0	
Informal education programs	0	
Stakeholders who receive information	0	approximate number at annual Pacific Coast Shellfish Growers Assoc. who attended talk describing early results of project (presented by Dan Gillon, September 2015. KL NOT COUNTING BECAUSE WE ALREADY COUNT SHELLFISH GROWERS CONF IN TERI REPORT
Volunteer hours	0	
P-12 students reached	0	
P-12 educators	0	

REQUESTED INFORMATION

Publications

No **Publications** information reported

Students Supported

Daniel Gillon (Continuing Student)
dgillon@uw.edu
University of Washington, School of Aquatic and Fishery Sciences

Field of Study: Fisheries Sciences
Advisor: Carolyn Friedman
Degree Type: MS
Degree Year: 2016

Student Project Title: NA

Involvement With Sea Grant This Period (capstone, fellow, intern, etc.): Research Assistantship under C. Friedman

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

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

Thesis / Dissertation: under development

New or Continuing?: continuing

Degree awarded this reporting period?: No

Financially supported?: Yes

Narratives

WA SG Progress Report
Uploaded File: WA_SG_Progress_Report_Narrative.pdf

Partners This Period

Taylor Shellfish Company
Types: Industry/Business
Scale: LOCAL
Notes:

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

University of Southern California
Types: Academic Institution
Scale: STATE
Notes:

NOAA Manchester
Types: Government
Scale: FEDERAL or NATIONAL
Notes:

STANDARD QUESTIONS

Impacts and Accomplishments

(1)

Type	accomplishment
Title	Washington Sea Grant-supported researchers and shellfish industry experts collaborate to develop Pacific oysters that are genetically resistant to ocean acidification
	In the face of climate change and ocean acidification (OA), Washington’s shellfish industry is challenged

Relevance	to cultivate enough Pacific oyster seed to satisfy market demands. Implementing controlled crossbreeding could create genetic lines of OA-resistant Pacific oysters.
Response	Washington Sea Grant-supported researchers teamed with industry partners to produce lines of Pacific oysters through a comprehensive breeding process that focused on testing OA responses in early- and late-development larvae. They successfully produced 42 lines of hybrid Pacific oysters by crossing genetically distinct inbred lines at two Washington locations. The team also began field testing previously screened, surviving hybrid lines to evaluate their potential for commercial production. In related testing, researchers assessed how embryo and juvenile-stage Pacific oysters responded to different levels of OA using the 42 oyster lines generated from crossbreeding. After several months of growth, they then evaluated survival of the experimental groups' seed.
Results	Initial analyses indicated several lines with high breeding potential for hybrid commercial production. To better measure response to different OA conditions, investigators developed an enhanced, more reliable apparatus for assessing response of oyster embryos and late-stage larvae to low pH conditions. The improved apparatus allowed flow-through seawater control that enabled cultured microalgae to be added without changing water chemistry.
Recap	Washington Sea Grant researchers produced hybrid Pacific oyster broodstock that was potentially resistant to ocean acidification (OA) and developed enhanced technology for measuring oysters' OA response.
Comments	
Primary Focus Area	Sustainable Fisheries and Aquaculture
Secondary Focus Areas	Healthy Coastal Ecosystems
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	Agriculture Research Service (USDA, ARS) Baywater Shellfish Company Puget Sound Restoration Fund Taylor Shellfish Company University of Washington, Friday Harbor Laboratories, College of the Environment (UW)
	* Type impact * Title Results of assessment of OA response in the larvae of genetically distinct lines of

PI Draft	intraspecific hybrid Pacific oysters * Relevance Climate change and ocean acidification are seriously impacting the shellfish industry's capacity to produce enough Pacific oyster seed to satisfy market demands. There is a need to address this problem through genetics and the capacity for oysters to be bred for increased survivorship under OA conditions. * Response The Pacific Shellfish Institute, Taylor Shellfish Farms and the UW are together assessing the response of oyster larvae from distinct genetic lines to an OA challenge in the laboratory at two larval life history stages. Major accomplishments include the production of a full factorial matrix of pairmated Pacific oyster lines from seven inbred lines in 2015. An OA apparatus was also built to assess the response of oyster larvae to a PC02 challenge and installed at the NOAA (PSRF) Manchester laboratory. The system affords precise control of pH at challenge (7.5) and control (8.0) levels while affording flow through control of algae. * Results Measurement of larval oyster response to different pC02 levels in embryos and (later) pediveligers for surviving crosses was undertaken in August and September 2015 by WA SG supported graduate student, Dan Gillon. After 24 hours of exposure in replicated cultures larvae for all 42 crosses was fixed for later analysis of development to the straight hinge (D-hinge) stage. Measurement of pediveliger reponse to different pC02 levels was also accomplished for larvae and seed from surviving 15x4 lines. Seed from 15x4 was assessed in January 2016 with statistical analysis indicating several superior lines based on overall yield measurements. * Recap The availability of brood oysters that produce seed with resistance to OA will hopefully assist the industry to thrive in the face of climate change. Comments Primary Focus Area Healthy Coastal Ecosystems Secondary Focus Areas Healthy Coastal Ecosystems,Sustainable Fisheries and Aquaculture Goals Partners

Tools, Technologies, Information Services / Sea Grant Products

(1)

Description	A system to assess response to exposure of elevated PC02 in seawater for multiple genetically distinct lines of Pacific oysters.
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	

Economic Impacts

No **Economic Impacts** information reported

Community Hazard Resilience

No **Community Hazard Resilience** information reported

Meetings, Workshops, Presentations

(1)

Type of Event	Public or professional presentation
Description	2015 WSG Shellfish Growers Workshop. Graduate student Dan Gillon reported on the project progress.
Event Date	3/3/2015
Number of Attendees	200

Leveraged Funds

No **Leveraged Funds** information reported

Crossbreeding and Selection for Resistance to Ocean Acidification in Pacific Oysters

WA Sea Grant Program - reporting period (Feb 1, 2015 – Jan 31, 2016)

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

In Spring 2015 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 conducted at the Puget Sound Restoration fund laboratory located on the Northwest Fisheries Science Center laboratory in Manchester, WA. 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 and J. Davis. Mr. Gillon also built a standalone system for assessing response in oyster embryos and later stage larvae to low pH conditions beginning in the spring of 2015. The new OA system represents a new state of the art apparatus for simultaneously assessing OA

response in pelagic marine invertebrates under flow through seawater conditions and is suitable for a suite of projects investigating OA response in marine organisms generally.

Full-scale genetic breeding work was simultaneously being conducted at the Taylor Shellfish Farms hatchery facility in Quilcene, WA under the direction of PI, Joth Davis to establish a full factorial set of genetically distinct oyster lines created from all possible combinations of matings from individuals from seven inbred oyster lines. In late August 2015 a successful full set of crosses was created (15x4), constituting 42 intraspecific hybrid and 7 inbred (G_2) lines (Figure 1).

	13x5.024x.042	13x5.019	12x3.062	12x3.028	08x3.027	08x2.034	08x2.015
13x5.024x.042	X	X	X	X	X	X	X
13x5.019	X	X	X	X	X	X	X
12x3.062	X	X	X	X	X	X	X
12x3.028	X	X	X	X	X	X	X
08x3.027	X	X	X	X	X	X	X
08x2.034	X	X	X	X	X	X	X
08x2.015	X	X	X	X	X	X	X

Figure 1. Full diallel crosses made between individual male and female oysters from seven inbred lines. Forty-two hybrid (including male by female and female by male) were made plus 7 G_2 inbred lines were established.

Oyster embryos were subsequently raised at the TSF hatchery and nursery facility to the seed stage and transferred to for testing and maintenance at Thorndyke Bay (Hood Canal).

For the OA stress response work, *gametes from the same set of oysters* utilized for the 15x4 test cross were utilized later the same day at the PSRF laboratory to reformulate the set of crosses made earlier in the day. This experimental cohort was immediately exposed to low $PC0_2$ and control (ambient $PC0_2$) seawater at the facility and replicate cultures placed in culture vessels into the OA apparatus in static culture for each of the 42 hybrid lines tested. After 36 hours all embryos for all crosses from treatment and control

replicates were immediately preserved in 10% formalin for later analysis of survivorship to the D-hinge stage, shell mineralization and other parameters.

Approximately 16 days later an OA stress response was similarly measured in replicated cultures at the PSRF facility using pediveliger larvae obtained from the 15x4 cohort reared in Quilcene. In this case, late term larvae were transported to Manchester and established in replicate cultures under flow through conditions. $PC0_2$ conditions were similar to those established for embryos earlier in the experiment. Pediveligers were monitored for about a week to enable the oysters to settle and metamorphose in the replicated chambers. Unfortunately, the experiment was terminated due to the failure of an immersion heater that resulted in loss of temperature control for the experimental cultures. As a result, the experiment was terminated and all surviving oysters from all replicates preserved for later analysis of survivorship, settlement rate and early spat growth.

In January 2016 the seed from 15x4 that had been maintained at Thorndyke Bay (Hood Canal) was assessed for growth and survivorship. Unfortunately, a number of replicates among some lines were lost due to a winter storm event but adequate numbers of replicates still enabled an analysis of performance to be made (Figure 2).

	13x5.024x.042	13x5.019	12x3.062	12x3.028	08x3.027	08x2.034	08x2.015
13x5.024x.042	0	5	0	4	5	5	2
13x5.019	0	5	0	1	1	0	0
12x3.062	2	5	0	0	3	2	1
12x3.028	3	5	0	0	0	4	1
08x3.027	3	4	0	3	0	3	2
08x2.034	3	2	0	1	0	0	2
08x2.015	3	1	0	0	2	2	0

Figure 2. Surviving lines (number of replicates indicated) to the seed stage assessed for general and specific combing ability.

With assistance from Dennis Hedgecock (University of Southern California) a preliminary analysis was made based on a Bayes Diallel statistical approach. Results indicate that high general combining ability was observed in lines involving parents 62 and 24 with high specific combining ability observed in a number of other crosses. The indication therefore is that genetically distinct lines having evidence for both GCA and SCA will be available to make high performing double hybrid crosses for the production of commercial oyster seed.

Challenges over the project period were mainly focused on having produced enough individuals per genetic line to enable full testing at multiple sites with adequate replication. Equipment breakdowns (e.g. immersion heater) during the OA assessment were frustrating, however the sampling during the time the experiment was running was not compromised. The major challenge that has been unavoidable has been delays on getting the samples analyzed and data on line performance acquired. These issues are being currently resolved.