Crossbreeding and Selection for Resistance to Ocean Acidification in Pacific Oysters

WA Sea Grant Program - reporting period (Feb 1, 2017 – Jan 31, 2018)

Overview of Project:
Screening genetic lines of Pacific oysters for resistance to ocean acidification

In brief, the WA SG project is focusing on a). measuring resistance in genetically distinct lines of Pacific oysters to reduced pH/high dissolved carbon content/reduced aragonite saturation state (e.g. ocean acidification) during two early life history larval stages: embryogenesis to the veliger stage and during the transition from pediveliger to the early spat stage. The OA screening process for generating data on genetically determined resilience to OA stress called 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. b). Based on these results, breeding for increased resilience to OA stress utilizing crossbreeding genetic approaches to generate broodstock oysters potentially resilient to adverse carbonate chemistry conditions can proceed.

The OA assessment work was conducted at the Kenneth K. Chew Center for Shellfish Research and Restoration located on the Northwest Fisheries Science Center laboratory in Manchester, WA. Co-PI C. There, Friedman's graduate student, Mr. Dan Gillon constructed a standalone system for assessing OA response in oyster embryos and later stage larvae under low and ambient pH conditions beginning in the spring of 2015. This OA system represented 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 originally 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). Numbers of surviving replicate cages holding oysters are indicated in Figure 2. Results of the line screening have been previously described and detailed in the 2017 progress report to WA Sea Grant. *Note - the original WA SG proposal relied on a collaboration with Taylor Shellfish Farms for use of hatchery space and personnel for this project component. Since Taylor Shellfish pulled out of the project as an active collaborator in 2016, there was a necessity to provide the hatchery resources to conduct the originally proposed work.*

Progress during current reporting period: February 1, 2017 - January 31, 2018.

1. Maintenance and Production of 15x4 Broodstock Lines

Picking up from work accomplished with the 15x4 broodstock lines generated as part of this project, beginning in February oysters maintained at Thorndyke Bay, Hood Canal were reorganized into a revised, using a revised tracking system devised by PI, Joth Davis. Briefly, replicate oyster cages (*Intermas* Vexar growbags) containing up to 100 oysters were assigned a position on longlines positioned on an intertidal sand flat maintained by Baywater, Inc. Each cage has an attached label, or plastic "cowtag" imprinted with a unique assigned to individual, replicate oyster cages. All record keeping is accomplished in the field using a handheld computer with paper backup. The inventory of oyster lines was then similarly backed up to an internet based data storage system using standard protocols. Maintenance of all oyster cages assigned to this project consisted of turning cages over at two week intervals to 1. reduce fouling from encrusting invertebrates (e.g. barnacles, mussels and tunicates) and 2. redistribute oysters in the cage in order to optimize growth and survivorship. In July 2018, the project team completed a census on all surviving 15x4 intraspecific hybrid lines of diploid Pacific oysters. Table 1 describes the current inventory of lines.

	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 adult stage to be assessed for general and specific combing ability.

Results of the second assessment indicated that surviving lines continued to thrive with excellent survivorship at the Thorndyke Bay, Hood Canal Baywater, Inc. farm site.

Based on results obtained in 2017 utilizing a Bayesian diallel statistical approach, 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 (D. Hedgecock (personal communication). As a result, plans were made to initiate a double hybrid test cross in late 2017 with the intent to a) produce broodstock for the utilization by the shellfish industry and b) a limited supply of seed oysters for further testing at a commercial scale.

During summer 2017 a fully functional hatchery was developed by Baywater, Inc. under the direction of PI, Joth Davis using independent funding with the intent, however to initiate WA SG based oyster breeding utilizing the new facility as soon as practical. Over the Fall and early winter, the Sealab was built, supplied with culture gear and otherwise prepared for use in 2018. The Baywater SeaLab (now Pacific Hybreed SeaLab as of March 1, 2018) is located on the NOAA Northwest Fisheries Science Center located in Manchester, WA directly adjacent to the Kenneth K Chew Center for Shellfish Research and Restoration operated by the Puget Sound Restoration Fund (PSRF). The Sealab shares infrastructure and resources with the PSRF as both Baywater/Pacific Hybreed and the PSRF share common goals in shellfish research.

In early January, 2017 oysters from family lines maintained by Baywater, Inc. were brought into the new facility for conditioning with the intent to spawn a new

cohort of broodstock in February, 2018. Specifically, approximately 30 individuals of two of the higher performing hybrid lines within the 15x4 cohort were selected in the field and brought into the Sealab for conditioning. Specifically, individuals from

Male line: 15x4.028x.024
Female line: 15x4.034x.019

were conditioned to spawn later in the year, mating male oysters from line 15x4.**028x.024** with female oysters from line 15x4.**034x.019** to generate a *double hybrid* cohort suitable for dissemination to commercial shellfish farmers.

Unfortunately, there were inadequate numbers of broodstock oysters available from family lines 62 and 24 (originally identified as the best possible cross for yield) to utilize these families for the work. However, remaining individuals from these two lines will be used to make brother/sister matings in order to partially reconstitute genetic material present in the original inbred parent lines.

Individuals from hybrid lines 34x19 and 28x24 were nearing reproductive state by the conclusion of the reporting period (1/31/18). The intent is to spawn oysters from these high performing lines (based on growth and survivorship) in early March, 2018 to create both new broodstock lines for future industry use and double hybrid seed for commercial evaluation.

2. *Assess 15x4 line performance relative to OA resistance in embryos and pediveligers*

Over the current reporting period, UW Graduate student, Dan Gillon worked on completing an assessment of embryos and pediveligers based on samples taken in 2016. The work was not completed by the conclusion of the reporting period and

largely based on this situation, a final extension to the overall project was requested to August 31, 2018. Work to complete this critical piece of research is now on track to generate the data necessary to inform continued breeding for resistance to adverse carbonate chemistry conditions. Mr. Gillon has provided the following narrative as an update to his contribution to the research. The initial results from the embryo and pediveliger sampling appear promising at this stage of the analysis.

We have completed processing D-hinge larval samples (42 crosses x 2 CO_2 levels x 4 reps = 336) for survivorship and % normal development after 36 hours of exposure. The dataset is complete and awaiting analysis of genetic variation in performance using Bayesian statistical methods (Collaborator).

We have processed about 75% of post-metamorphic seed samples. The use of digital imaging and image analysis software is quite intensive and time consuming. Following imaging, seed samples are processed a second time to assess survivorship among genetic families at high and low CO_2. We have processed approximately 50% of samples for survivorship.

Preliminary data analysis suggests that, overall, development and survivorship are impaired at elevated CO_2 in both larvae and seed oysters, though the effects of elevated CO_2 differ among crosses, with some crosses showing little to no effect of aragonite under saturation. More robust analysis of our full dataset will likely tease out these patterns in more detail.

In summary, the broodstock management and production role vacated by Taylor Shellfish Farms was overcome and a hatchery built and poised to utilize the original genetic lines generated for this project. We look forward to utilizing the phenotypic information in embryos and seed from 15x4 in the ongoing effort to complete the pediveliger sampling. This critical work will enable a full statistical analysis of the data sets associated with breeding components associated with the phenotypic response of different genetic lines to an OA challenge and hopefully provide guidance for the direction of oyster breeding in this important area of research to the commercial shellfish industry on the US West coast.