

Crossbreeding and Selection for Resistance to Ocean Acidification in Pacific Oysters

WA Sea Grant Program - reporting period (Feb 1, 2016 – Jan 31, 2017)

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

In brief, the WA SG project is focusing on measuring line resistance to reduced pH during 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 called for placing embryos and larvae from different genetic lines into replicate chambers submerged in seawater under pre-determined high and low pCO₂ 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 (G₂) lines (Figure 1).

	13x5.024x.04 2	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 G₂ 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 2016 progress report to WA Sea Grant. Due to the prolonged illness of the primary graduate student on this project (Dan Gillon), little additional work was accomplished to identify oyster lines resistant to OA conditions during the current reporting period.

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).

In April 2016 in collaboration with Taylor Shellfish Company (TSC) we initiated a performance audit on 15x4 intraspecific hybrid lines of diploid Pacific oysters. The proposal called for the UW team to coordinate with the Pacific Shellfish Institute (PSI) and Taylor Shellfish Company (TSC) to collaborate on routine assessments of surviving genetic lines of oysters generated by the Taylor HYBreed crossbreeding program in 2015.

	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 combining ability.

With assistance from Dr. 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. Results of a second line assessment conducted by Taylor Shellfish and PSI in April 2016 indicate that

surviving lines continue to grow and otherwise thrive at Thorndyke Bay, Hood Canal (Figure 3). Due to low numbers of surviving lines it was determined to maintain all the oyster lines at this location for final assessment (spring-summer 2017).

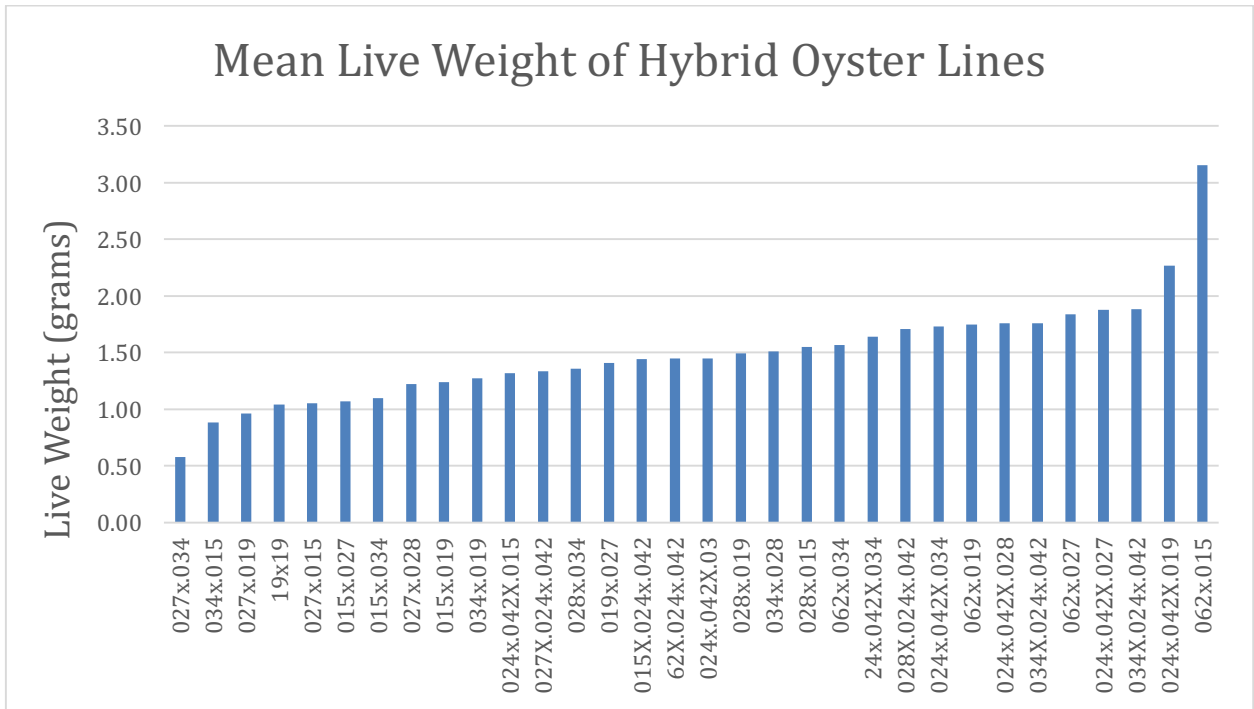


Figure 3. Results of line testing accomplished in April 2016 for surviving lines maintained at Thorndyke Bay, Hood Canal on the Baywater Shellfish Company farmsite.

Plans for the remaining year of the project include 1. correlating line performance relative to OA resistance once Dan Gillon has completed his assessment based on samples taken in 2016 and 2. replicating lines for dissemination to the shellfish industry for use as broodstock. In addition, we plan to spawn individuals from high performing lines (based on growth and survivorship) to create new broodstock lines for future industry use. In terms of an update, Dan Gillon has initiated his assessment of samples taken on early embryos and pediveligers that was initiated prior to taking leave from the University of

Washington. The sampling is currently on track to produce results and direction on line selection for continued breeding for resistance to detrimental performance due to carbonate chemistry associated with OA.

Challenges over the project period were mainly associated with tracking individuals for cohort 15x4 over the reporting period to enable full assessment in spring and summer 2017 as it became obvious in Fall 2016 that, due primarily to delinquent broodstock management by Taylor Shellfish Company, the lines they maintained in Thorndyke Bay experienced tag loss in shellfish cages holding some replicates for some family lines. Taylor Shellfish Company has subsequently withdrawn from the project. The broodstock management void has been filled by Baywater, Inc. in the meantime and the stocks are being well maintained at their farm site on Thorndyke Bay. The second major challenge has been associated with all the delays associated with Dan Gillon's health. This too has been resolved as Dan has half of all the samples assessed and he will soon have data analysis completed related to genetic line performance in play to help inform broodstock selection decisions later in 2017.