R/LME/N-3 - Alleviating Regulatory Impediments To Native Shellfish Aquaculture

Progress Report: September 2012 – January 2013

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. The overall goals of this project are to increase our knowledge of local adaptation in Olympia oysters to address concerns that interbreeding between potentially maladapted cultured and wild stocks could negatively impact wild populations. Over the current reporting period a majority of our effort has focused on 1) developing genomic resources, 2) preparing for oyster outplanting, and 3) procedure optimization. The remainder of this report will describe the details associated with each of these activities.

Genomic Resources

One of the major accomplishments over this reporting period was the characterization of the Olympia oyster transcriptome [Timmins-Schiffman, E. B., Friedman, C. S., Metzger, D. C., White, S. J. and Roberts, S. B. (2012), Genomic resource development for shellfish of conservation concern. Molecular Ecology Resources. doi: 10.1111/1755-0998.12052]. Here we have annotated the transcriptome and identified single nucleotide markers that will be further developed as part of the molecular analysis conducted during this project.

During this reporting period we have not initiated any genetic or epigenetic population level characterization. There has been significant progress in obtaining samples and sample processing. Samples of adults from the three populations (Table 1) held in common conditions for 3 months to reduce ephemeral differences have been sampled for initial characterization of epigenetic differences using methylation-sensitive AFLP (MS-AFLP).

Table 1. Olympia oyster samples for genetic and epigenetic comparisons. Asterisk indicates 10 samples are currently being processed for epigenetic characterization.

Population	**Stage**	**Total**
Fidalgo Bay (North Sound)	adults	93*
Fidalgo Bay (North Sound)	seed	100
Dabob Bay (Hood Canal)	adults	38*
Dabob Bay (Hood Canal)	seed	83
North Bay (South Sound)	adults	79*

As part of a Capstone student thesis, "*Effects of photoperiod and mechanical stress on Olympia oyster physiology*", several gene expression assays were developed. Specifically, assays were developed for BCL2- associated athanogene 2 (*bag*), heat shock protein 90kDa alpha (*hsp*), U2 small nuclear RNA auxiliary factor 1-like 4 (*u2a*), muscle glycogen phosphorylase (*pygm*), insulin-like growth factor 1 receptor (*igfr*), and protein kinase, cGMP-dependent, type 1 (*prkg*). The latter three genes are all involved in growth whereas the former are associated with the stress response. Although limited in certain aspects, this study found two important implications in *O. lurida* growth physiology and restoration efforts. First, photoperiod may have an impact on stress and growth rate of *O. lurida* with longer photoperiods associated with increase growth. Secondly, mechanical stress may stimulate growth under certain conditions. Both of these findings are based on gene expression and confirmation by additional studies would be ideal. Regardless, these assays could be implemented in our future work.

Outplanting preparation

A major component of this project overall is to evaluate fitness components and performance of seed from different origins in a reciprocal transplant experiment. This transplantation is planned for this summer. The three source populations are Fidalgo Bay, Dabob Bay, and North Bay. Broodstock from all three populations/sites (Table 2) were collected in December and initially placed in conditioning tanks at Puget Sound Restoration Fund's Port Gamble Hatchery. The first release of larvae occurred on January 23 from one of the Fidalgo Bay breeding groups, with approximately 205,000 larvae. Larvae will be maintained until outplanting later this year.

Table 2. Olympia oyster samples used as broodstock

Site	**Total specimens collected**	**Number of breeding groups that produced larvae**	**Approximate number of larvae**
Fidalgo Bay	516	10	41k-1,333k
Dabob Bay	205	7	68k-496K
North Bay	332	0	0

Procedure optimization

To enable nonlethal assessment of fecundity, we have initiated experiments to optimize anaesthetization of Olympia oysters with minimal mortality. To our knowledge, this is the first attempt to transfer anesthesia methods used successfully with *Ostrea edulis, Saccostrea glomerata, Crassostrea gigas, and Nodipecten subnodosus*. Based on information from these other bivalve species, we have designed the following experiment to determine the optimal dosage and treatment duration using $MgCl_2$ (subject to change based on new information): Olympia oysters held at 12-14 C will be treated with 20 - 80 mg * L^{-1} $MgCl_2$ at both ambient and elevated (~18-19 C) temperatures. We recently initiated an investigation using $MgSO_4$ as an alternate anaesthetic. Preliminary results are promising: 60% of oysters anaesthetized using a one hour immersion regained responsiveness after an additional 1.5 hrs. We continue to monitor this group of oysters for post-treatment mortality.