Effects of photoperiod and mechanical stress on Olympia oyster physiology

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Research Proposal for FISH494: Capstone Research

Abstract
Since its decline from commercial success, the population size of Olympia oyster (*Ostrea lurida*), the native oyster species to the Pacific Northwest, has significantly diminished. As a slow growing oyster with strict settling conditions, recovery of Olympia oyster has faced significant challenges. Increasing the growth rate of these species would benefit the restoration and commercial fishery possibilities of *O. lurida*. This study aims to deduce the effects of photoperiod and mechanical stress on growth in Olympia oyster. The effects of treatment will be measured by quantifying gene expression of growth related genes in the animals. Results of this work may be useful in future policies concerning Olympia oysters.

Introduction
Olympia oyster (*Ostrea lurida*) is the native oyster species of the Pacific Northwest with natural habitat ranging from the Californian to Canadian coast (White et al. 2009). In the past, *O. lurida* have been prized as a luxurious delicacy, but at the turn of the 20th century, introductions of the Eastern oyster (*Crassostrea virginica*) and the Pacific oyster (*Crassostrea gigas*) replaced *O. lurida* as the dominant fisheries species (White et al. 2009; Beck et al. 2011; Dumbauld et al. 2011). This was largely due to the small size and slow growth of *O. lurida*, which made it less suitable for commercial aquaculture. Since this transition, *O. lurida* numbers have decreased sharply over the years. In more recent years, restoration of native species, such as *O. lurida,* has become a priority in many areas including the Pacific Northwest. Problems with this process have emerged, which include the lack of available habitat, proper nutrients and the relatively strenuous requirements for settlement (White et al. 2009; Wasson 2010). *O. lurida* require a much thicker and calmer culch bed than the dominant oyster species in the region, *C. gigas*. Additionally, the relatively slow growth and maturation have also slowed recovery efforts.

Unfortunately, due to the back seat *O. lurida* has taken to other oyster species over the last few decades, extensive research has not been invested in the species. While the restoration efforts have led to a slew of papers being published citing the ecological benefits of the Olympia oyster, physiological changes, particularly those measured by gene expression has been absent. Given the importance of growth rate in restoration

efforts and aquaculture possibilities, information on environmental factors affecting growth should be beneficial to the scientific community concerned about *O. lurida* success. Two factors that are controllable in hatchery and fishery settings, photoperiod and mechanical stress are used in this study. While both environmental effects have been shown to regulate physiological changes in other species, including vertebrates and invertebrates, no research has been presented specific to *O. lurida*. Effects of photoperiod on growth have been well documented in other species of oyster, fish, and bivalves (Fabioux et al. 2005; Domínguez et al. 2010; Türker et al. 2011). Mechanical stress has also been shown to impact regulation of certain genes in the Eastern oyster (Roberts et al. 2012). This study aims to add to the information known about Olympia oysters in the hope that they will be useful in recovering Olympia oyster stock in their natural habitat.

Questions:

1. What is the effect of photoperiod on Olympia oyster growth-related physiology?
2. What is the effect of mechanical stress on Olympia oyster growth–related physiology?
3. What are the combined effects of photoperiod and mechanical stress on Olympia oyster growth-related physiology?

Hypotheses:

The expression of growth genes will shift in a manner that suggests Olympia oysters have higher growth rate when grown in conditions with longer amounts of light. Conversely, oysters will show lower growth rate when mechanically stressed. The growth rate of oysters grown in longer light conditions will not be affected as much as those grown in short light conditions when mechanically stressed.

Methods

A total of 44 *O. lurida* were randomly split into two treatment groups. The first group of 22 oysters was subject to 12 hours of light and 12 hours of dark per day. The second group of 22 was subject to 24 hours of light per day. All oysters were grown in otherwise identical conditions at 15oC with sufficient air circulation and feed for 14 days. Each treatment group was then randomly split in half again, with one half receiving mechanical stress and the other half not. Mechanical stress consisted of 5 minutes centrifugation at 1000rpm and 15oC. This experimental design allows comparison of four treatment types: 1). control 2). photoperiod only 3). mechanical stress only, and 4). photoperiod and mechanical stress. Each treatment type has 11 oysters which should be a large enough sample size for sufficient power in statistical analysis.

After treatments, oysters were immediately shucked and tissues samples of gill and mantle were taken separately. Tissue samples were stored at -80oC. RNA was then extracted from the gill tissue samples at a later date. Extracted RNA was treated with a DNase treatment and then converted to cDNA using reverse transcription. Gene primers were designed for genes suspected to be related to growth and reproduction using Primer3Plus. These genes were normalized using primers designed for normalizing genes. Gene expression was quantified using qPCR and normalized. All samples were run in duplicate. Statistical analysis using two-way ANOVAs was performed to determine significant differences in gene expression.

Products and Timeline

Products:

- Rough Draft Scientific Paper – November 2012
- Final Draft Scientific Paper – December 2012
- Capstone Presentation – December 2012
- Honors Poster – December 2012

Timeline:

- Sign capstone contract with Dr. Roberts (faculty adviser) – September 2011
- Run experiment (grow oysters) – April 2012
- Data collection – May to October 2012
- Meet with Dr. Roberts to sign FISH 495 form - October 2012
- Data analysis – October to November 2012
- Completion of project – December 2012

Signatures

We have read and discussed the above proposal thoroughly and we believe this is an achievable yet challenging project for the student named. We have also discussed how the student will get any needed supplies, etc. for this project.

Student: Bradley Bhi Date: 10/15/12

Faculty Sponsor: Steven Roberts Date: ___10/14/12___

Works cited

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