A comparison of breeding methods for the Olympia oyster, *Ostrea lurida*, using microsatellite markers

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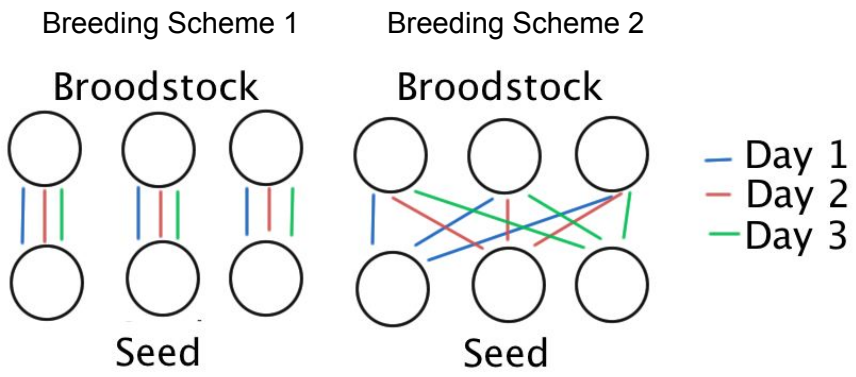
Abstract

The Olympia oyster, *Ostrea lurida*, is native to Puget Sound and the west coast of the United States. Due to overharvesting and water quality decline, the once thriving local population has decreased dramatically. In recent years, an effort to reestablish this culturally, ecologically, and economically important animal has begun. While methods have been created to breed genetically diverse and suitable oysters, there have been no quantitative studies to evaluate them. In this study I plan to use seven microsatellite loci to compare relatedness between three groups of hatchery produced oysters and wild populations. I will also compare heterozygosity, allele frequencies, and allele counts between these groups. In addition, I will be completing parentage analysis to see how many adults contribute to the subsequent generation.

Introduction

The Olympia oyster, *Ostrea lurida*, is the only oyster native to Puget Sound and the west coast of the United States. In the past, this animal has been crucial to the ecosystem because of the habitat created by oyster beds as well providing filtration services. The Olympia oyster is also culturally important to local Native American cultures and the shellfish farmers that have been growing these animals for generations. Concurrent with the human population boom in this region in the late 19th century, the *O. lurida* population faced a dramatic decrease. The oysters were unable to withstand the decrease in water quality as well as overharvest. In recent years, there has been an interest and many efforts to begin restoring native populations.

Currently there are efforts to breed “restoration grade” Olympia oysters. While we think these animals will be diverse enough to help reinstate a healthy population, no quantitative data has been collected. In this project, I plan to use microsatellite data to compare relatedness between oysters produced in a restoration hatchery using to different breeding schemes. Specifically, different methods were used to define a cohort. In Breeding Scheme 1 (2010), all of the offspring resulting from one group of broodstock were considered a family. Each family from the different groups of broodstock were kept separate. In Breeding Scheme 2 (2011), each day all of the larvae that had been released, from all groups of broodstock, were collected, combined, and considered a family. See the figure below for a visual representation. In addition to comparisons between the two restoration groups, I will also compare these to commercial hatchery produced oysters and wild oysters. Commercial hatchery oysters were produced by a series of mass spawns from a single large tank holding ca 300 adults.



In the diagram above, the top circles are the groups of broodstock and the bottom circles are what become the offspring family groups or cohorts. In Breeding Scheme 1, all of one group of broodstock's offspring comprised a single cohort. In Breeding Scheme 2, all of the larvae released in one day, from all of the broodstock, were combined into one cohort. This was repeated each day larvae were released.

Questions

- Do the cohort "families" have more related individuals when they are collected via one method over another?
- Does a group of restoration grade oysters have significantly more related individuals than a wild population?
    - Are commercial aquaculture methods producing more highly related animals than restoration methods?
- How does allele frequency, heterozygosity, and allele counts compare between these groups?
- How many of the broodstock are reproductively successful in a particular season?
    - Does a small group of parents seem to dominate a cohort?

Hypotheses

- Breeding Scheme 2 (2011) should have less related individuals, higher heterozygosity, allele count, and different allele frequencies between family groups than Breeding Scheme 1 (2010).
- The commercial animals should have a higher number of related individuals than either the restoration population or the wild. In addition, there should be lower heterozygosity, lower and a lower allele count. This is because creating a diverse group of offspring is not the goal of commercial growers.
- The wild population will likely have the least number of related individuals, highest heterozygosity, and highest allele count of any of the groups because diversity should occur naturally in a wild population.

Approach

Olympia oysters from each of two different breeding schemes from the Puget Sound Restoration Fund hatchery in Port Gamble, WA have been sampled. Wild *O. lurida* have been previously collected and processed from Totten Inlet and the data will be used to compare the relatedness between wild and restoration populations. Commercial oyster tissues that have been previously sampled may be included in the comparisons. The tissue samples are primarily gill or mantle and are stored at either -80°C or in ethanol at room temperature. The total sample size will be approximately 600 animals. The DNA extractions, PCRs, as well as genotyping runs will be completed at the NOAA station in Manchester, WA. Seven known microsatellite loci (Stick, et al. 2009) will be subjected to fragment size analysis. All tissue collection has already been completed. I will travel to Manchester for at least one day to participate in the DNA extraction, PCR, and sequencing.

Breeding Scheme	Family Name	Total Analyzed
1	2	4
1	5	10
1	14	10
1	16	10
1	17	10
1	18	10
1	19	10
1	22	4
1	23	10
1	24	10
2	11x5.002	10
2	11x5.007	10
2	11x5.011	10
2	11x5.013	10

2	11x5.014	10
2	11x5.015	10
2	11x5.018	9
2	11x5.019	10
2	11x5.022	10
2	11x5.024	10

The above table includes information about the restoration oysters that have already been processed. Family names for Breeding Scheme 2 ends with the day number that the larvae were collected. Ten animals from each family were sampled and processed except for 2, 22, and 11x5.018 which included 4, 4, and 9 samples respectively. This is approximately half of the restoration samples that we have, the rest still need to be processed.

Sample	Approximate sample size
Restoration grade - Breeding scheme 1 (2010)	100
Restoration grade - Breeding scheme 2 (2011)	100
Commercial	100
Wild	100

Above is a list of the different categories of samples that will be compared.

Data analysis:

The raw data will be binned using Tandem (Matschiner & Salzburger 2009). The groupwise Full maximum Likelihood method in Colony2 (Jones & Wang 2010) will be used to determine sibship and estimate how many parents created a particular group of offspring. Colony will output a list of full siblings, half siblings, potential parent genotypes, and a “best cluster”, which includes parentage assignment probabilities. GenAlEx (Peakall & Smouse, 2006; 2012) will be used to compute F statistics, and compare heterozygosities. Allele count rarefaction will be performed using HpRare (Kalinowski 2005).

<u>Products and timeline</u>

By the end of spring quarter:

- All of the samples will be genotyped
- All of the Colony analyses will be completed
- Organization and analysis of the data will be about 50% done

Summer quarter:

- Finish analysis of data
- Write paper
- Prepare presentation
- Present final project

We have read and discussed the above proposal thoroughly and 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.

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Steven Roberts

Literature Cited

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