LARVAL PERFORMANCE OF SELECTIVELY BRED VERSUS WILD POPULATIONS OF PACIFIC OYSTERS (CRASSOSTREA GIGAS) IN ACIDIFIED SEAWATER

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Overview

1. Oysters in the Pacific Northwest (PNW)
   - Wild and selectively bred stocks

2. Ocean Acidification (OA) and oyster larvae
   - Potential for breeding resilience

3. Methods

4. Results and Discussion

5. Future Work
Oysters in the PNW are a tale of two species

- Native oyster: The Olympia oyster (*Ostrea lurida*)
  - Natural stocks overharvested late 19th century

- 1920’s *C. gigas* imported from Miyagi region of Japan
  - British Columbia
  - Washington and Oregon States

- Natural habitats are marginal
  - Cold water (10-15°C)
  - Warm bays few and far between
  - Strong currents

- Naturalized populations limited
Selective breeding creates stronger oysters

- 1960s and 70s brought hatchery technology
  - Remote settlement= dramatically increased range
  - Controlled reproduction= ability to breed

- 1995- Molluscan Broodstock Program (MBP)
  - Family based, selective breeding program
  - Currently in 5th and 6th generation of selection

MBP Field Sites
Upwelling is exacerbated by Ocean Acidification

In the PNW, seasonal upwelling (May-October) brings deep ocean water onto the coastal shelf
- Cold (10-15°C)
- Nutrient rich
- Acidic:
  - pCO$_2$ 800-1800 ppm
  - pH (7.5-8.0)
  - $\Omega_{\text{arag}} < 1$

Introduction
Oyster Life Cycle

- Fertilized egg
- Straight-hinge veliger
- Late veliger
- Pediveliger

Egg and sperm

Spat attach to old oyster shells or other structures

1 - 3 years

Adult males and females

Introduction
OA conditions threaten oyster larvae

• Development of “D” veliger larvae (48 hr) is adversely affected by low $\Omega_{\text{arag}}$. (Waldbusser et al., 2015)

• Upwelling conditions tied to larval oyster mortalities in hatcheries (Barton et al., 2012)

• Hatcheries and wild populations have been negatively impacted for the past 10 years.

• Anecdotal evidence suggests that MBP larvae outperform wild crosses during heavy upwelling
Research questions:

• In low $\Omega_{\text{arag}}$ culture conditions:
  • What is the effect on larval performance through the entire developmental cycle?
  • Do MBP broodstock produce larvae that are more or less impacted?
  • Are there genetic factors contributing to larval performance in low $\Omega_{\text{arag}}$?
Broodstock and cross design

• Two genetic stocks:
  • MBP 5\textsuperscript{th} and 6\textsuperscript{th} generation (19♂ x 19♀)
  • “Wild” oysters from Willapa Bay, WA (5♂ x 19♀)

• Broodstock conditioned in seawater at pH>8.0 for 4 weeks

• 95 crosses from each pool

• Fertilization in seawater with ambient CO\textsubscript{2}.
Larval Culture

• 5 hours post fertilization, eggs stocked to 10 liter static tanks
  • 5x replication

• Two treatment levels:
  • Ambient CO₂: ~400ppm
  • High CO₂: ~1600ppm

• Water changed every 2 days
  • 25, 45, 64 and 80 μm screens
  • No selection on larval size
Water Quality

- Water pCO$_2$ manipulated by mass flow controllers
- Effects of respiration on total CO$_2$ of the system over 48h was minimal.

**Methods**

- ~ 1600 ppm
- ~ 400 ppm
Larval mortalities were linked to metamorphic changes.

**Results**

- Survival
  - ~ 1600 ppm
  - ~ 400 ppm

**CO₂ levels**
Initial metamorphosis negatively impacted by high pCO$_2$
Initial metamorphosis negatively impacted by high pCO$_2$
Larval mortalities were linked to metamorphic changes.

Results

CO₂ levels
- ~ 1600 ppm
- ~ 400 ppm
Pediveliger development distinctly impacted by broodstock and pCO$_2$

**Results**

- CO$_2$ levels:
  - ~1600 ppm
  - ~400 ppm

- MBP
- Wild

- Percent eyed larvae, Day 16

A

B

A

B
Pediveliger development distinctly impacted by broodstock and pCO$_2$
In Larvae, growth and development are linked

**CO₂ levels**
- ~1600 ppm
- ~400 ppm

**Larval growth**

<table>
<thead>
<tr>
<th>Size (μm)</th>
<th>Day</th>
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<tbody>
<tr>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>200</td>
<td>10</td>
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<tr>
<td>200</td>
<td>15</td>
</tr>
<tr>
<td>200</td>
<td>20</td>
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Results
High pCO$_2$ had unexpected effects on settlement.

The total production of spat and eyed larvae was measured under different CO$_2$ levels and types. The results show that:

- **MBP** (yellow) had a higher production of eyed larvae at approximately 1600 ppm CO$_2$.
- **Wild** (blue) had a higher production of eyed larvae at approximately 400 ppm CO$_2$.

The graph indicates that the settlement process was affected by the CO$_2$ levels, with MBP showing a higher production at higher CO$_2$ levels compared to Wild.
High $\text{pCO}_2$ had unexpected effects on settlement

- MBP
  - ~ 1600 ppm
  - ~ 400 ppm
- Wild

CO$_2$ levels

- 1600 ppm
- 400 ppm
High pCO$_2$ had unexpected effects on settlement

Spat size

- **MBP**
  - Type A
  - CO$_2$ levels:
    - ~ 1600 ppm
    - ~ 400 ppm

- **Wild**
  - Type B
  - CO$_2$ levels:
    - ~ 1600 ppm
    - ~ 400 ppm
Size and Metamorphosis

Larvae/Spat Size, D22

Growth curves
- ~ 1600 ppm
- ~ 400 ppm

Discussion
Larval performance

- Low $\Omega_{\text{arag}}$ culture conditions:
  - Reduced “normal” D-larvae at 48hrs
  - Hindered development to pediveliger
  - Ultimately did not effect total spat production
  - No correlation between early and late stage performance

- MBP broodstock produced larvae that:
  - Had higher growth rates
  - Were impacted less by low $\Omega_{\text{arag}}$
  - Produced more and larger spat after 22 days
Genetic factors in OA resilience

- 2bRAD analysis on DNA samples
  - SNP frequency changes from egg to spat
  - GWAS for larval resilience to high pCO$_2$

Zhang et al., 2012
Genetic factors in OA resilience

- RNAseq on transcriptome

"Nacrein-like protein"

"Carbonic anhydrase"
Breeding for Larval Traits

• Repeat spawn in a commercial hatchery 2016
• Identify key life stages for selection experiments
• Incorporate OA performance into selection program
Thank you!