A Comparison of Stress Responses in Sea Urchins and Sea Cucumbers Exposed to Salinity and Handling Stress

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A COMPARISON OF STRESS RESPONSES IN SEA URCHINS AND SEA CUCUMBERS EXPOSED TO SALINITY AND HANDLING STRESS

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Introduction

- Sea urchins are echinoderms that are generating interest in aquaculture
- Sea urchins are particularly valuable as:
  - Research models
  - Delicacy food item
- Many sea urchin populations have been greatly overfished
  - Depletion of European and Asian stocks has reduced sea urchin quality

Bertocci et al. 2014; Botsford et al. 2004
Introduction

• Animals can be raised in aquaculture conditions
  ○ Prevents:
    ▪ Overfishing
    ▪ Pollutant contamination
    ▪ Loss to predation
    ▪ Low quality product
    ▪ Damage to ecosystem

• However, organisms encounter stress in intensive aquaculture environments
  ▪ Some species are hardier than others
  ▪ Hardy species make better aquaculture candidates
    ○ More resistant to disease
    ○ Better production
    ○ Easier to raise

Kiew & Don 2012
Research Objectives

- To compare the stress responses of echinoderms in conditions that might be encountered in an aquaculture environment.
  - Handling
    - Animals are moved from tank to tank
    - In research facilities, they must be handled for sampling
  - Salinity change
    - Rain water can dilute salinity of culture ponds
    - Purchasing ocean mimicking salt mixes is expensive

Photo by Navid Ayon
Materials and Methods

- Adult purple sea urchins (*Stronglyocentrotus purpuratus*) and giant California sea cucumbers (*Parastichopus californicus*) were obtained from Bodega Marine Laboratory Station in Bodega Bay, California.
- Three treatment groups were established
  - Handling
    - Three times daily for 5 minutes; kept at optimal salinity (34 ppt)
  - Low salinity
    - Kept at 28 ppt
  - Controls
    - Optimal salinity (34 ppt) and never handled
- After 72 hours in treatment conditions, coelomic fluid was collected for analyses of cells (coelomocytes)
Materials and Methods

- Coelomocytes were counted via hemocytometer for differential and total coelomocyte count (Braak, 2002)
- Total protein content was read via protein refractometer (Mustafa et. al. 2000)
- Lytic activity was determined by lysozyme turbidity assay (Chia & Xing, 1996)
Material and Methods

- Phagocytic capacity of cells was determined by counting cells with engulfed bacteria (formalin-killed) bacteria (Mustafa et. al. 2000)
- Respiratory burst activity was determined by spectrophotometer

\[
\text{Phagocytic capacity} = \frac{\text{Number of cells with engulfed bacteria}}{\text{Total number of cells}} \times 100
\]

(Mustafa et. al. 2000)
Coelomocyte Studies

- Changes in coelomocytes
  - Total and differential sea urchin cell count
    - Phagocytic
    - White spherule
    - Red spherule
    - Vibratile
  - Coelomic fluid protein
  - Phagocytic capacity
  - Lytic activity

(Smith et. al. 2010)
Types of Sea Urchin Cells

- A—White spherule cell
- B—Vibratile cell
- C—Phagocytic cell
- D—Red spherule cell

Photo by Regina Shannon

Sea urchin cell types
Types of Sea Cucumber Cells

A—Lymphocyte
B—Type 1 and Type 2 spherule cell
C—Phagocytic cell
Results: Total Coelomocyte Count

### Total Coelomocyte Count

#### Sea Urchin

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Total Number of Coelomocytes (x10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
</tr>
</tbody>
</table>

Group 1 = Control
Group 2 = Low Salinity
Group 3 = Handling

#### Sea Cucumber

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<tr>
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<td>2</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

Group 1 = Control
Group 2 = Low Salinity
Group 3 = Handling
Results: Differential Cell Counts

Number of Phagocytic Cells

Sea Urchin

Experimental groups

<table>
<thead>
<tr>
<th>Group</th>
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<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Low Salinity</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Handling</td>
<td>8</td>
<td>10</td>
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Sea Cucumber

Experimental groups

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Results: Coelomic Fluid Protein

Sea Urchin

Sea Cucumber

Group 1 = Control
Group 2 = Low Salinity
Group 3 = Handling
Results: Phagocytic Capacity

Phagocytic Capacity of Phagocytic Cells

Sea Urchin

Sea Cucumber

Group 1 = Control
Group 2 = Low Salinity
Group 3 = Handling
Results: Lytic Activity

Lytic Activity of Coelomic Fluid

Sea Urchin

Sea Cucumber

Experimental groups

1 2 3

Lytic Activity of Coelomic Fluid (Difference in absorbance)

0.00 0.01 0.02 0.03 0.04 0.05

Group 1 = Control
Group 2 = Low Salinity
Group 3 = Handling

Experimental groups

1 2 3

Coelomocyte Lytic Activity (Difference in absorbance)

0.00 0.01 0.02 0.03 0.04 0.05

Group 1 = Control
Group 2 = Low Salinity
Group 3 = Handling
Results: Respiratory Burst Activity

Sea Cucumber Respiratory Burst Activity

- Control
- Salinity
- Handling

Graph showing Absorbance (OD 600) against Cell Number x 10^5.
Results: Respiratory Burst Activity

Sea Urchin Respiratory Burst Activity

- **Control**
- **Salinity**
- **Handling**
Conclusion

- It appears that both handling and low salinity produce significant stress responses in sea urchins, though not significant stress in sea cucumbers.
- This would indicate that sea cucumbers are a hardier aquaculture candidate than sea urchins.

Photo by Stephen Shannon
Impact

- Our study of the physiological and immunological parameters in invertebrate aquaculture can be used for increased production and to make better pharmaceuticals in the future.
- An increase in culturing these species will help reduce the risk of overfishing.
- Intensive aquaculture will prevent problems associated with off-show culturing.
Further Research

- Areas for further research include:
  - Effects of varying salinities on animal health
  - Longer term study
  - Studies utilizing the impact of nutraceuticals on animal immune function

Photo by Tazin Fahmi
Acknowledgments

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Questions?