Hydrogen Peroxide and its Effects on the Regenerative Abilities of Genotypically Diverse Nematostella vectensis

Introduction – Hydrogen Peroxide (H₂O₂) in Nature

- UV-dependent reaction with dissolved organic carbon in the water

\[ \text{sunlight} + \text{DOC} = \text{O}_2^- \]

Introduction – H₂O₂ Effects on Marine Biota

- Decrease in metabolic rate and intracellular pH of the shrimp, Crangon crangon (Abele-Oescher et al. 1997)

- Oxidative stress and antioxidant response in the limpet, Nacella concinna (Abele-Oescher et al. 1998)

- Common in intertidal areas and estuarine ecosystems
- Experiences tidal submersions and emersions
- Extreme conditions for marine life
  - Salinity, Temperature, pH and O₂ concentrations
Introduction – Nematostella vectensis

• Common infaunal sea anemone found in estuaries along the Atlantic and Pacific coasts

Regeneration Ability
Adaptive Mechanism
Fitness Indicator

Introduction – Research with Regeneration

Temperature

• Assessed Nematostella’s survival across a temperature range (Unpublished, Reitzel et al.)

• Growth rate and regeneration rate increased at high temperatures

Hydrogen Peroxide

• Assessed Nematostella’s survival/regenerative success rate in the presence of H₂O₂ (Unpublished, Sullivan, J)

• 0.0005% H₂O₂ had substantial effects on regeneration of certain anemones - Polymorphism

Introduction – Hypothesis & Predictions

© Based on previous studies, we believe that hydrogen peroxide will retard or inhibit the regenerative capabilities of Nematostella vectensis.

© In addition, we expect to see a higher rate of regenerative failure at higher hydrogen peroxide concentrations than lower concentrations.

Methods & Materials

• Mixed lab population
• Blind study
• Bisected longitudinally
Methods & Materials

• Pictures taken before and after decapitation:

METHODS & MATERIALS

• Peroxide Treatments
  • 0.0003% and 0.0006%
  • Control (0%)
  • Changed daily

• Corresponding heads placed in separate trays
• Heads allowed to regenerate in artificial sea water

Methods & Materials – Serial Dilutions

1 ml 3% H$_2$O$_2$ + 99 ml ASW

2 ml 0.003% H$_2$O$_2$ + 98 ml ASW

30 ml 0.0006% H$_2$O$_2$ + 30 ml ASW

60 ml 0.0006% H$_2$O$_2$

60 ml 0.0003% H$_2$O$_2$

Concentration 0.003%

Concentration 0.0006%

Methods & Materials

• Regeneration recorded at the same time everyday for 8 days
  • Compared to earlier pictures
  • Compared to corresponding head
Results – Final Regeneration Count

<table>
<thead>
<tr>
<th>Successful Regeneration</th>
<th>Failed Regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 of the 32 anemones regenerated (most fully) and were capable of eating brine shrimp when fed after completion of regeneration analysis</td>
<td>1 of the 32 anemones analyzed in this regeneration experiment did not successfully regenerate. It was treated in a low concentration of hydrogen peroxide</td>
</tr>
</tbody>
</table>

Results – Final Regeneration Count

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Low Concentration H₂O₂ (0.0003%)</th>
<th>High Concentration H₂O₂ (0.0006%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regenerated</td>
<td>9/9</td>
<td>10/11</td>
<td>12/12</td>
</tr>
<tr>
<td>Successfully</td>
<td>Individuals Regenerated Successfully</td>
<td>Individuals Regenerated Successfully</td>
<td>Individuals Regenerated Successfully</td>
</tr>
</tbody>
</table>

Results – Tentacle Count Over 8 Days

Average Tentacles Per Day for Each Treatment

Average Number of Tentacles

- Based on the standard deviation bars, within a day the means for each treatment are not significantly different from each other.

Results – First Tentacle Emergence

Percentage of Individuals Showing Tentacles

Between treatments:
p-value = 0.98

Within treatments:
- Control: p-value < 0.05
- Low: p-value < 0.05
- High: p-value < 0.05
Results – Final Tentacle Count

**CONTROL: Frequency of a Certain Number of Tentacles**

- By the last day, all anemones in the control and low H$_2$O$_2$ treatment had 8 or more tentacles.
- In the high H$_2$O$_2$ treatment, there were still three anemones with low tentacle counts.

**LOW H$_2$O$_2$: Frequency of a Certain Number of Tentacles**

**HIGH H$_2$O$_2$: Frequency of a Certain Number of Tentacles**

Discussion – Regenerative “Success”

**Old Definition of Regenerative Success:**
“Regeneration was considered successful if the animal possessed a pharynx and tentacles”

(Unpublished. Sullivan, J)

**Does possession of pharynx and tentacles does mean that it can eat?**

**New Definition of Regenerative Success:**
Regeneration was considered successful if the animal had the ability to eat.

Discussion – Old Methods vs. New Methods

Our data contradicts the study by James Sullivan, but why?

Differences between previous study and our study:
- Blind Study vs. Known Gene Pool
- Changing Water Daily vs. Using Same Water

Discussion – Ambiguous Data

Looking at this graph, there seems to be no correlation between treatments and the day that tentacles first started showing up in individuals.
### Discussion – Ambiguous Data

#### Average Tentacles Per Day for Each Treatment

![Average Tentacles Per Day for Each Treatment](image)

#### Discussion – Issues Encountered

- Pictures are not always reliable!
- Anemones will only keep regenerating or not regenerate at all, i.e. there will not be less tentacles this day than were present the day before.

### Discussion – Regeneration in Oxidative Environments

#### Data Supporting Hypothesis

- Some of the anemones in high concentrations of hydrogen peroxide grew less tentacles by day 8 than those at lower concentrations.
- One anemone subjected to low concentrations of hydrogen peroxide did not regenerate at all.

#### Data Invalidating Hypothesis

- 12/12 anemones in the high concentration of hydrogen peroxide treatment regenerated successfully.
- 10/11 anemones in the low concentration of hydrogen peroxide treatment regenerated successfully.
- 96.8% of anemones regenerated (all 3 treatments)
Future Work

- Genotyping heads of individuals
- Conducting experiment again, with populations from different locations
  
  - How do anemone’s originating from Nova Scotia fare against Hydrogen Peroxide compared to anemone’s originating from North Carolina?
  - How fast does hydrogen peroxide break down in lab experiments?

Bigger Picture

- Inferences about evolution on the micro- and macro- evolutionary scale
  
  - In reference to adapting to oxidative and other stressful environments

- Nematostella is becoming an even more useful model for tracking and understanding human genomics

Acknowledgements

- John Finnerty
- Nikki Traylor-Knowles
- James Sullivan
- Justin Scace
- Kayak Nazi

Literature Cited


