The affect of pH on the Regeneration of Nematostella vectensis, and the Ability of N. vectensis to Regulate the pH of its Environment

BI 547-Research Project
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Nematostella vectensis:
- Sea anemone found in salt marshes or estuaries
- Native to the Atlantic Coast of North America

Morphological Characteristics:
- Diploblastic
- Blind gut
- Simple neuromuscular system
- Bilaterally symmetrical

Development
Nematostella vectensis:
- Regeneration
- Asexual reproduction
- Sexual reproduction

J.R.W. Finnerty, "The Starlet Sea Anemone" 7/18/07
http://people.bu.edu/jrf3/B1505/

http://cumm005-0b01-dhcp-144.bu.edu/~FinnLab/FinnertyLab/FinnertyLab_RESEARCH.html
Why use *Nematostella* as a model system?

- Morphologically simplistic
- Easily cultured
- Abundant in nature, therefore, easily obtained
- Sequenced and conserved genome throughout evolution

J.R.W. Finnerty, “The Starlet Sea Anemone” 7/18/07

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pH Range at Sippewissett Marsh:

<table>
<thead>
<tr>
<th>Pool #</th>
<th>pH Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>7.73</td>
</tr>
<tr>
<td>Tidal Creek</td>
<td>6.882</td>
</tr>
<tr>
<td>3A</td>
<td>6.52</td>
</tr>
<tr>
<td>3B</td>
<td>8.38</td>
</tr>
<tr>
<td>16</td>
<td>8.04</td>
</tr>
<tr>
<td>16A</td>
<td>8.91</td>
</tr>
</tbody>
</table>

- pH range varied from pool to pool
- pH range of 6.52 to 8.91
- Based on research performed by Ogburn et. al. the average salt marsh pH is 6.7 +/- 0.3

Collection-9/5/07

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*Nematostella*

http://www.coris.noaa.gov/exchanges/coralgenome/sup_digest.html

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Sippewissett Marsh:

http://cumm005-0b01-dhcp-144.bu.edu/~FinnLab/FinnertyLab/FinnertyLab_RESEARCH.html
The Ecological Importance of Estuarine Environments:

- Protect shorelines from storm damage
- Act as biochemical filters of runoff
- Many species of marine fish and crustaceans use salt marshes as a nursery for their young

Bertness et al., *Marine Biology: An Ecological Approach*

Estuaries-A Dynamic Environment:

- Human Impacts-
  - Eutrophication (use of Nitrogen fertilizers)
  - Shoreline development
  - Flow of human waste into estuaries

Bertness et al., *Marine Biology: An Ecological Approach*

Estuaries-A Dynamic Environment:

- Variable salinity
- Oxidative stress
- Competition
- Temperature
- pH

Bertness et al., *Marine Biology: An Ecological Approach*

Factors Influencing the pH of an estuary:

- Precipitation
- Runoff
- Ability of organisms living within an estuary to regulate the pH of their environment

Bertness et al., *Marine Biology: An Ecological Approach*

http://www.mccullagh.org/db9/10d-16/assateague-salt-marsh.jpg
Collection Pools:

Broader Context:

• Our research provides insights into an organism's ability to survive and reproduce in an environment with pH extremes.

Potential Research:

• Further research could be performed to determine if Nematostella vectensis expresses a gene that enables it to regulate the pH of its environment.

Hypothesis:

• Nematostella is unable to regenerate in a pH lower than 7.0 or greater than 10.0.
  - Possible outcomes:
    • Nematostella are able to regenerate in pH extremes
    • Nematostella are unable to regenerate in pH extremes
    • Null Hypothesis: An extreme pH value has no affect on Nematostella regeneration.

http://www.nematostella.org
**Sippewissett Marsh Video:**

**Methods: Collection**
- Collection of anemones at Sippewissett Marsh
  - Screens were used to sift through sediment and collect anemones
  - Anemones were collected from two different pools: 16 and 4

**Methods: Solutions**
- Sodium Hydroxide (NaOH) was used to make seawater more basic
- Hydrochloric Acid (HCl) was used to make seawater more acidic
- HCl and NaOH were chosen because Na and Cl ions are naturally present in seawater
  - Therefore limiting contamination

**Methods: Can Nematostella survive in extreme pH values?**
- 2 Nematostella were placed at each pH value:
  - (6.5, 7.0, 8.3(control), 10.0, and 10.5)
- Nematostella remained in their different solutions from Friday afternoon to Monday afternoon
**Methods: Can Nematostella survive in extreme pH values?**

- The anemones were photographed at the start of the experiment, and at the end
- pH of the solution containing the anemone was recorded at the end of the experiment
- Nematostella were able to survive at the extreme pH values, therefore, we proceeded with the regeneration experiment

**Methods: Regeneration**

- Anemones were bisected
- Placed in a wash solution:
  - pH 6.5
  - pH 7.0
  - pH 8.30 (control)
  - pH 10.0
  - pH 10.5

  **Since Nematostella retain water from their environment, we placed them in a wash solution in order to reduce contamination of the well-plates.**

**Methods: Regeneration**

- Removed from the wash solution, and placed in a well-plate
- Anemones from different pools were kept separate
- 4 or 5 anemones were placed in solution at each pH value (6.5, 7.0, 8.30 (control), 10.0 and 10.5)
**Methods: Regeneration**

- Anemones were photographed throughout regeneration process
- Anemones were kept in solution and observed for 6 days
- The presence of a mouth, pharynx, and functioning tentacles were indicators of a successful regeneration

**Figure #1:** ANOVA Analysis of Size of Adult Anemones as a Function of pH

F = 0.3520
DF = 4

**Figure #2:** ANOVA Analysis of Fragment Size of Nematostella as a Function of pH

F = 0.4204
DF = 4

**Results**

**Figure 3:** Average growth of regenerating Nematostella in a pH solution of 6.5.

**Figure 4:** Average growth of regenerating Nematostella in a pH solution of 7.0.

**Figure 5:** Average growth of regenerating Nematostella in a pH solution of 10.0.

**Figure 6:** Average growth of regenerating Nematostella in a pH solution of 10.5.
Figure 7: Contingency analysis of the appearance of a pharynx by pH level on 9/22/2007
ChiSquare: 4.904
DF: 4
R-square: 0.1816

Figure 8: Contingency analysis of the appearance of a pharynx by pH level on 9/24/2007

Figure 9: Contingency analysis of the appearance of tentacles by pH level on 9/22/2007
ChiSquare: 4.904
DF: 4
R-square: 0.1816

Figure 10: Contingency analysis of the appearance of tentacles by pH level on 9/24/2007
ChiSquare: 3.312
DF: 4
R-square: 0.3453

Multivariate Graph

- Growth as a function of pH
  - pH p-value: 0.0372
  - Pool p-value: 0.2553
  - Size is a function of pH but not of pool
- Growth as a function of pH by date
  - More basic pH levels have greater affect on size
  - No significance on 9/18
  - High significance on 9/22
    - P-value: 0.0053
    - No significance on 9/24
      - P-value: 0.5910

\[ y = -0.8365x + 6.6258 \]
\[ R^2 = 0.9989 \]
Conclusions

• Size is a factor of pH, but not pool
• pH does not inhibit growth, but may slow it down
• At some point, pH must buffer to a value that Nematostella is able to tolerate
• Is the hypothesis supported?
  – No
• Can we throw out the null hypothesis?
  – No

Hypothesis:

• Nematostella is able to regulate the pH of its environment.
  – Possible Outcomes
    • Nematostella is able to regulate the pH of its environment.
    • Nematostella is unable to regulate the pH of its environment.
  – Null Hypothesis
    – Nematostella has no affect on the pH of its environment.

Methods: pH Regulation

• Whole anemones were placed in a well-plate
  – 4 anemones at each pH value
  – 6.5, 7.0, 8.3(control), 10.0, and 10.5

  • Control
    – 2 wells at each pH contained water only
**Methods: pH Regulation**

- Whole Anemones
  - pH 6.5
  - pH 7.0
  - pH 8.3 (control)
  - pH 10.0
  - pH 10.5

- 4 anemones
- 2 control

- Potential Problem:
  - To obtain an accurate pH reading using the pH meter, we had to combine water from at least two wells. Therefore, there was cross-contamination between wells.

**Results**

- Figure 12: Difference in starting pH and ending pH levels for water with and without adult Nematostella.

\[ y = -0.7802x + 6.1202 \]

\( R^2 = 0.9893 \)

- Figure 14: Difference in starting pH and ending pH levels for water with adult Nematostella present.

\[ y = -0.8246x + 6.4984 \]

\( R^2 = 0.994 \)
Conclusions

• pH buffered itself
• There is a linear relationship in pH change from basic to neutral levels
• Greatest absolute change is present in the more basic pH values
• Nematostella have no affect on the pH of their surroundings
• Is the hypothesis supported?
  – No
• Can we throw out the null hypothesis?
  – No

Discussion

• What does this mean?
  – Nematostella adapts to different pH levels
  – Does not effect pH of surroundings
  – Does suffer under more basic pH conditions

Improvements?

• pH level constant
• Intervals testing pH
• Greater volume of water
• More data points
• Monitor growth at closer intervals

Future Research

• Pdr12
  – Saccharomyces cerevisiae
  – ABC transporter
  – Essential for growth in the presence of weak organic acid stress
  – Found in Nematostella genome using Stellabase.org
    • First found human homolog using Blast

Coote et al. 2000.
Future Research

- Amplify gene using PCR
- Test to see if it is more/less active in differing pH levels
  - Leave organism in pH solution for 30-60 min
  - Extract DNA
  - Run PCR with primers targeting Pdr12
  - Gel electrophoresis

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References