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

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

http://people.bu.edu/jrf3/BI505/

Morphological Characteristics:

- Diploblastic
- Blind gut
- Simple neuromuscular system
- Bilaterally symmetrical

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

Nematostella vectensis:

Development

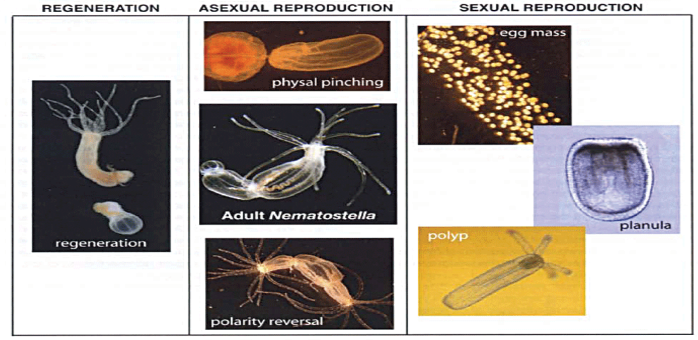


Figure 2. Life history of *Nematostella vectensis.* Regeneration subsequent to injury (left) represents a potential mode of passive asexual reproduction, but it is unknown what role this process may play in natural populations. The primary mode of active asexual reproduction is through transverse fission: contraction in the body column ultimately results in separation of the pedal end of the animal, which then regenerates head structures (top middle). Alternatively (but less frequently), asexual reproduction may occur through "polarity reversals", in which a new head forms, typically at the pedal end of the anemone, and the animal divides at its midpoint and regenerates new pedal structures (bottom middle). Sexual reproduction (right) begins with external fertilization of eggs extruded in large jelly masses (top). Eggs develop into swimming planulae (middle), which settle and form juvenile polyps (bottom). Development from fertilized egg to sexual maturity takes roughly 2–3 months.

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

pH Range at Sippewissett Marsh:

Collection-9/5/07

Pool #	pH Value
4	7.73
Tidal Creek	6.882
3A	6.52
3B	8.38
16	8.04
16A	8.91

•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

Nematostella

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

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

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

Factors Influencing the pH of an estuary:

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

Collection Pools:

Broader Context:

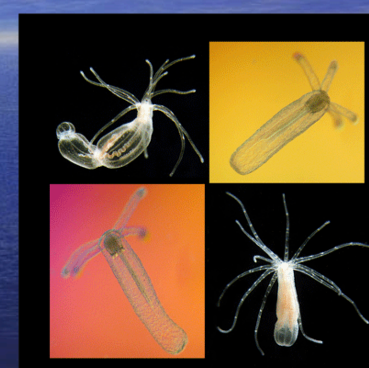
- Our research provides insights into an organisms ability to survive and reproduce in an environment with pH extremes

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:

QuickTime Movie

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

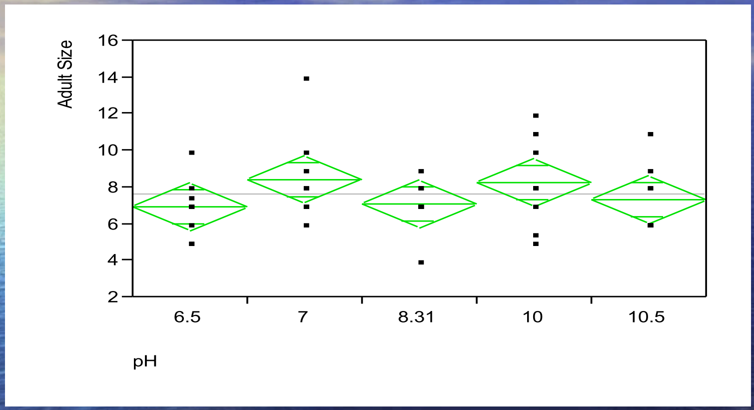
- Removed from the wash solution, and placed in a well-plate
- Anemones from different pools were kept separate
- 4 or 5 anemones were placed in solution at each pH value(6.5, 7.0, 8.30 (control), 10.0 and 10.5)

Methods: Regeneration

Methods: Regeneration

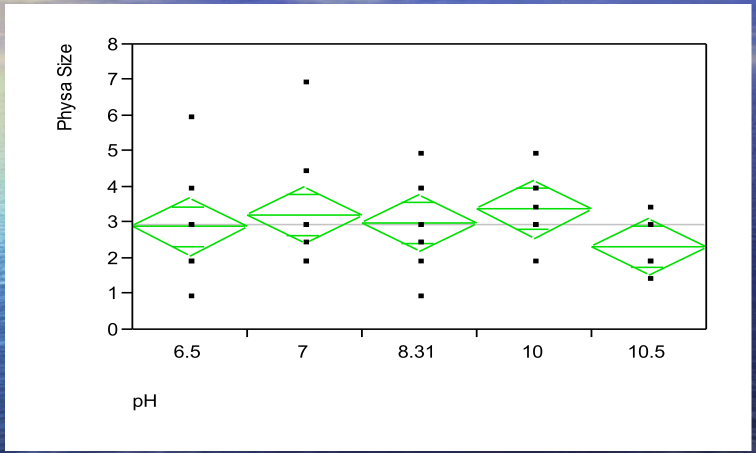
- Anemones were photographed throughout regeneration process
- Anemones were kept in solution and observed for 6 days
- The presence of a mouth, pharynx, and functioning tentacles were indicators of a successful regeneration

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



F=.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.

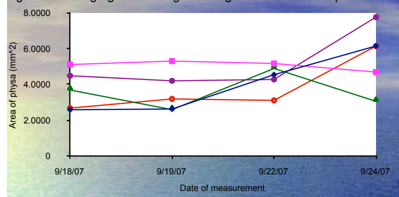


Figure 4: Average growth of regenerating Nematostella in a pH solution of 7.0.

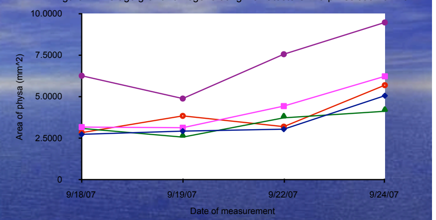


Figure 5: Average growth of regenerating Nematostella in a pH solution of 10.0.

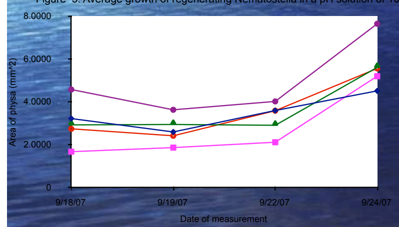


Figure 6: Average growth of regenerating Nematostella in a pH solution of 10.5.

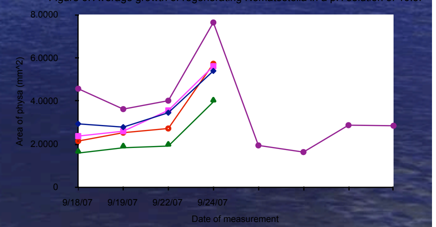
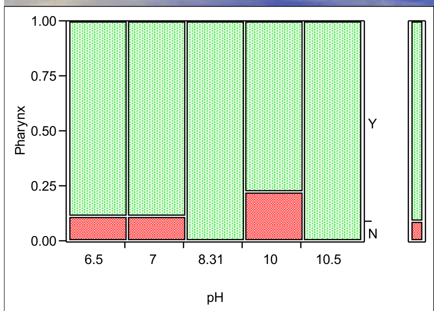


Figure 7: Contingency analysis of the appearance of a pharynx by pH level on 9/22/2007



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

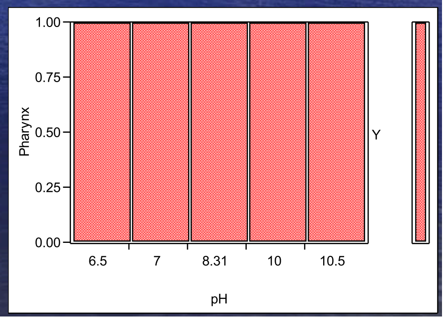
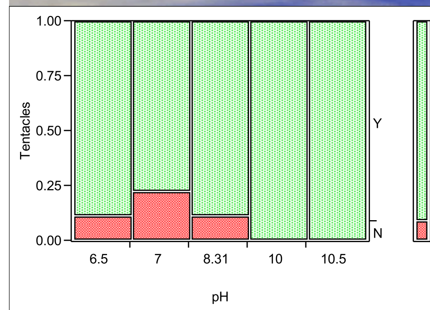
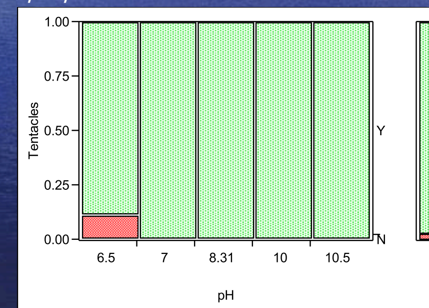


Figure 9: Contingency analysis of the appearance of tentacles by pH level on 9/22/2007



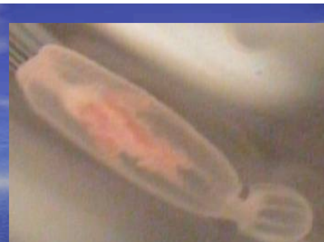
ChiSquare: 4.904
DF: 4
R-square: 0.1816

Figure 10: Contingency analysis of the appearance of tentacles by pH level on 9/24/2007



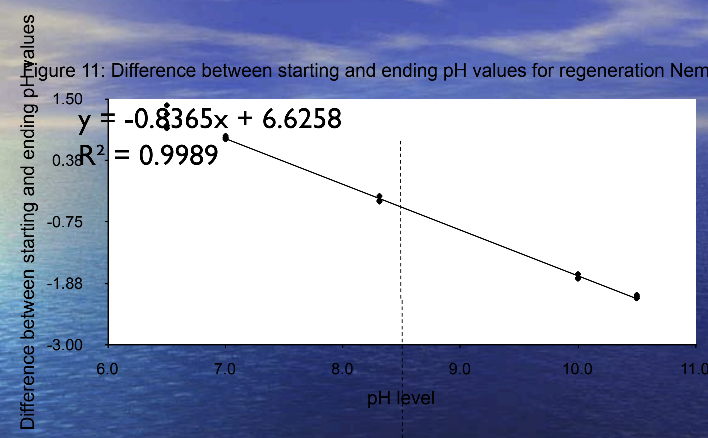
ChiSquare: 3.312
DF: 4
R-square: 0.3453

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

Figure 11: Difference between starting and ending pH values for regeneration Nematostella



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

Conclusions

- Why does the pH level not inhibit or alter regeneration?
- Why does the pH level change from the start to the end of the experiment?
- Could Nematostella be changing the pH of its surroundings?

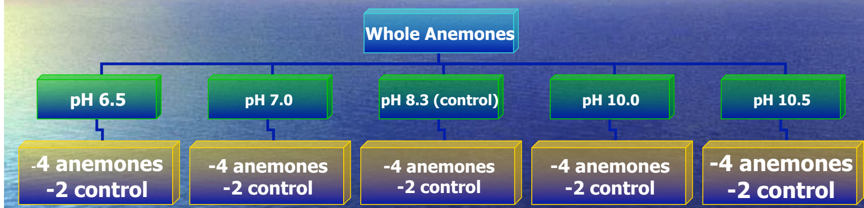
<u>Hypothesis</u>:

- Nematostella is able to regulate the pH of its environment.
  - <u>Possible Outcomes</u>
    - Nematostella is able to regulate the pH of its environment.
    - Nematostella is unable to regulate the pH of its environment.
    - <u>Null Hypothesis</u>-
      - Nematostella has no affect on the pH of its environment.

<u>Methods</u>: 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
    - **<u>Control</u>-**
      - 2 wells at each pH contained water only

Methods: pH Regulation

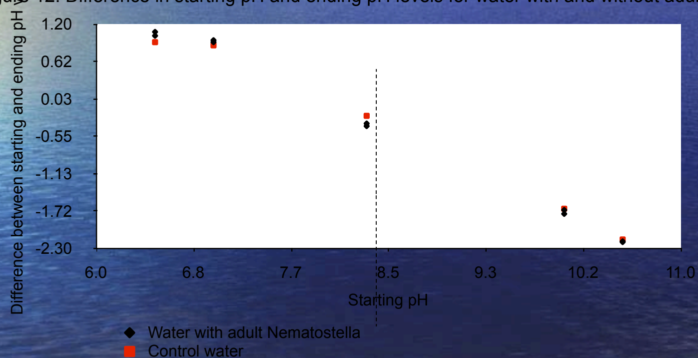


Methods: pH Regulation

- the pH of the solutions were recorded, and the anemones were removed
  - 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 Nematoste



re 13: Difference in starting pH and ending pH levels for water without adult Nematostella prese

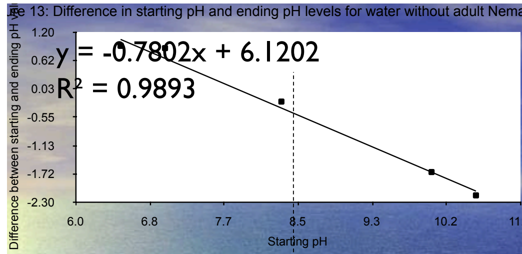
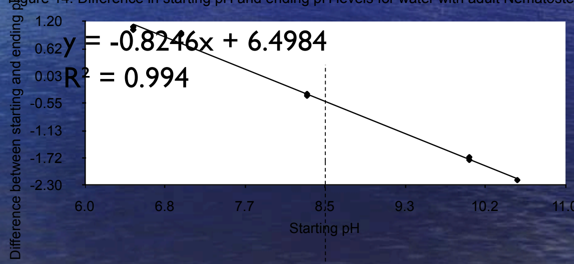


Figure 14: Difference in starting pH and ending pH levels for water with adult Nematostella prese



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

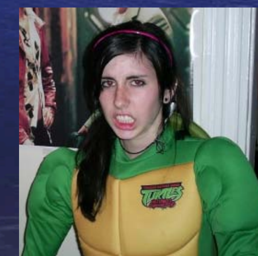
Coote et al. 2000.

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

Acknowledgements

- Prof. John Finnerty
- Jim Sullivan
- Boston University and BUMP
- The people of Sippewissett
- BI547 class



References

- Coote, P et al. Loss of Cmk1 Ca2+-calmodulin-dependent protein kinase in yeast results in constitutive weak organic acid resistance, associated with a post-transcriptional activation of the Pdr12 ATP-binding cassette transporter. Molecular Microbiology 37:595-605. 2000.
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