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

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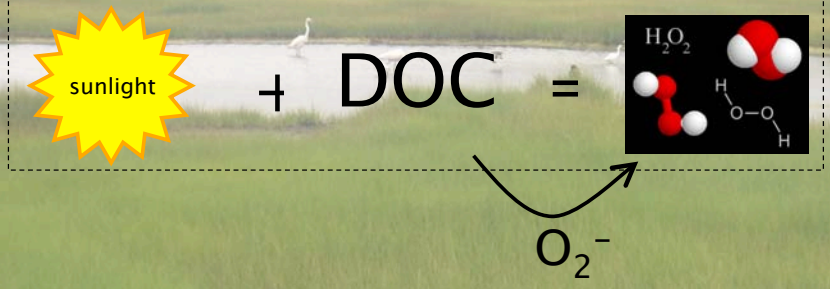
Introduction – Hydrogen Peroxide (H_2O_2) in Nature

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

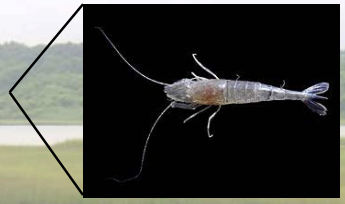


Introduction – Hydrogen Peroxide (H_2O_2) in Nature

- Common in intertidal areas and estuarine ecosystems
  - Experiences tidal submersions and emersions
  - Extreme conditions for marine life
    - Salinity, Temperature, pH and O_2 concentrations

Introduction – H_2O_2 Effects on Marine Biota

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



en.wikipedia.org

www.stageopantarctica.nl

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

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

<u>Temperature</u>

• Assessed Nematostella's survival across a temperature range (Unpublished, Reitzel et al.)

•Growth rate and regeneration rate increased at high temperatures

<u>Hydrogen Peroxide</u>

•Assessed Nematostella's survival/regenerative success rate in the presence of H_2O_2 (Unpublished, Sullivan, J)

•0.0005% H_2O_2 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:

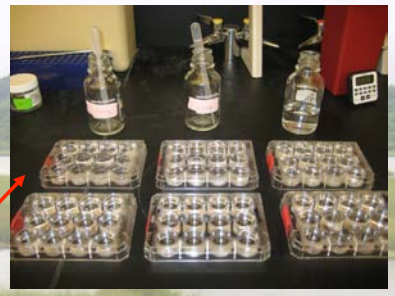
BEFORE

AFTER

Methods & Materials

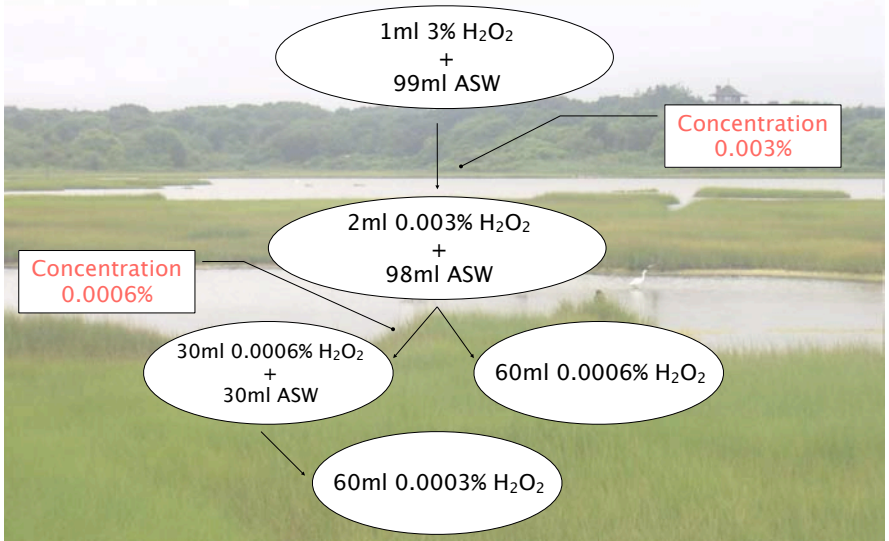
•Peroxide Treatments

- •0.0003% and 0.0006%
- •Control (0%)
- •Changed daily



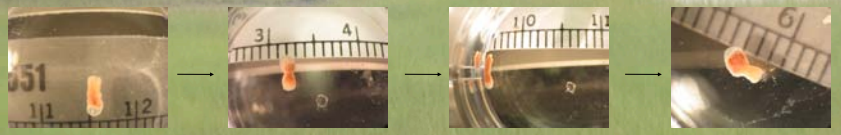
- •Corresponding heads placed in separate trays
- •Heads allowed to regenerate in artificial sea water

Methods & Materials – Serial Dilutions



Methods & Materials

- •Regeneration recorded at the same time everyday for 8 days
  - •Compared to earlier pictures
  - •Compared to corresponding head

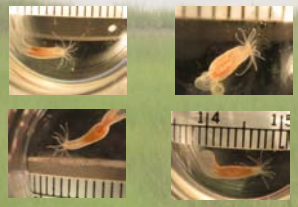


DAY 1 → DAY 2 → DAY 3 → DAY 4…

Results – Final Regeneration Count

Successful Regeneration

31 of the 32 anemones regenerated (most fully) and were capable of eating brine shrimp when fed after completion of regeneration analysis



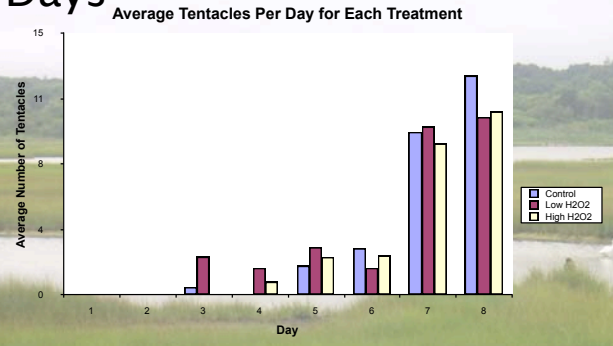
Failed Regeneration

1 of the 32 anemones analyzed in this regeneration experiment did not successfully regenerate. It was treated in a low concentration of hydrogen peroxide

Results – Final Regeneration Count

Control	9/9 Individuals Regenerated Successfully
Low Concentration H_2O_2 (0.0003%)	10/11 Individuals Regenerated Successfully
High Concentration H_2O_2 (0.0006%)	12/12 Individuals Regenerated Successfully

Results – Tentacle Count Over 8 Days



Between treatments:

p-value = 0.98

Within treatments:

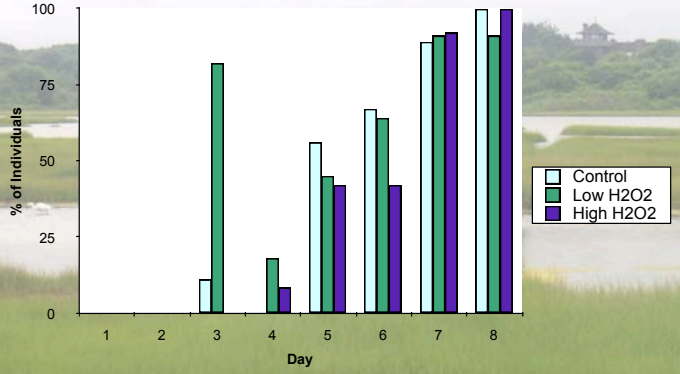
Control: p-value < 0.05

Low: p-value < 0.05

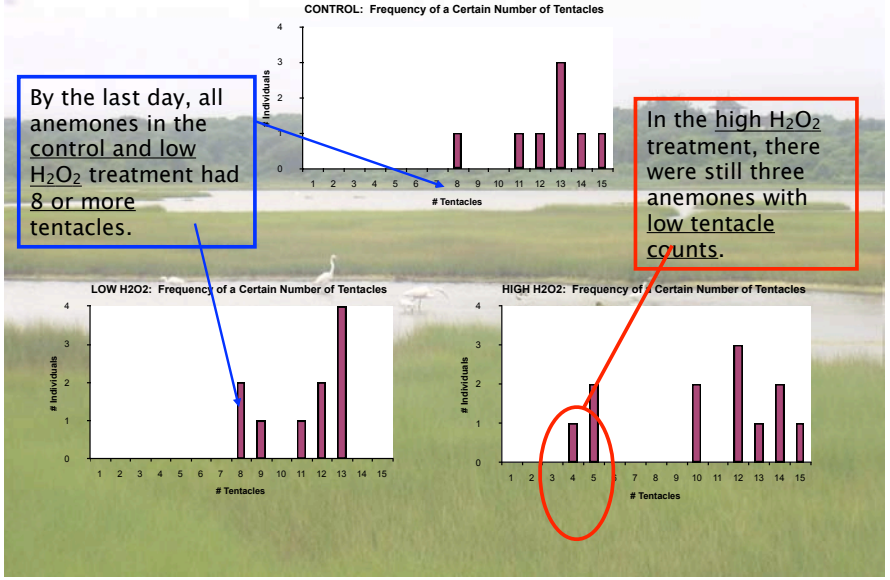
High: p-value < 0.05

- 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



Results – Final Tentacle Count



By the last day, all anemones in the control and low H_2O_2 treatment had 8 or more tentacles.

In the high H_2O_2 treatment, there were still three anemones with low tentacle counts.

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?

↓

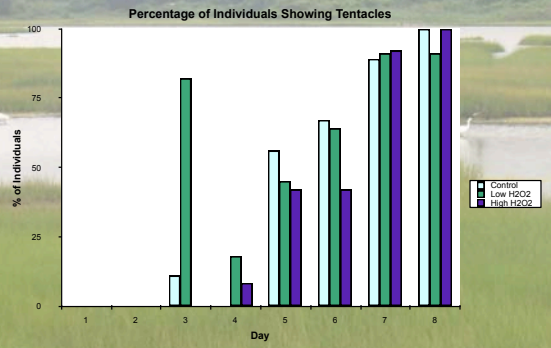
Differences between previous study and our study:

Blind Study vs. Known Gene Pool

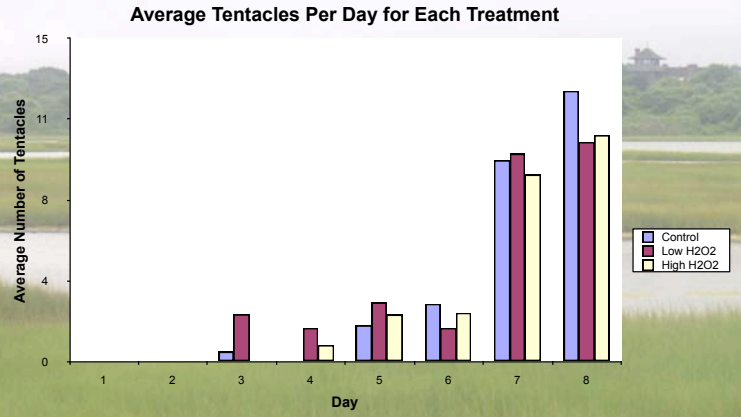
Changing Water Daily vs. Using Same Water

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

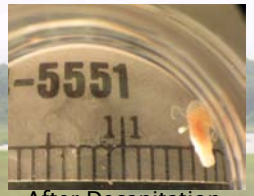


Discussion – Ambiguous Data



Discussion – Issues Encountered

Figure A1



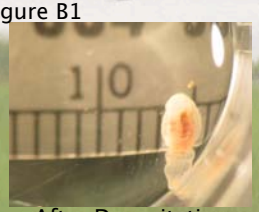
After Decapitation

Figure A2

Day 8

"HAIRCUT" REGENERATION!

Figure B1



After Decapitation

Figure B2

Day 8

GENUINE REGENERATION!

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 then were present the day before.

Day 3
3 Tentacles

Day 4
9 Tentacles

Day 5
2 Tentacles

Variable 0.0003, Individual A3

Discussion – Regeneration in Oxidative Environments

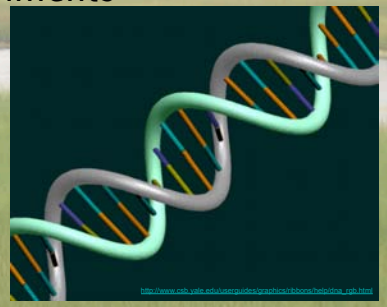
Data Supporting Hypothesis	Data Invalidating Hypothesis
•Some of the anemones in high concentrations of hydrogen peroxide grew less tentacles by day 8 than those at lower concentrations.	•12/12 anemones in the high concentration of hydrogen peroxide treatment regenerated successfully.
•One anemone subjected to low concentrations of hydrogen peroxide did not regenerate at all.	•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

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Literature Cited

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