**NEMATOSTELLA VECTENIS BEHAVIOURAL RESPONSE TO ADDED CHEMICAL CUES**

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Marine Invertebrates: Professor J. Finnerty
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**Questions and Concerns**

- Can *Nematostella vectensis* respond to different environmental cues?
  - physical indicators (vibrations) eliminated
- Are there different responses to cues indicative of threats and opportunities in their environment?
  - repertoires?

**HISTORICAL VIEW ON SEA ANEMONES**

- Ortie de mer
- French for “Sea-nettle” or “Sea-flower”
- Represents interpretation of animals as that which move and, plants assessile and protruding from the earth.

Slides 3-7 present very interesting and effective background material.

**RÉNÉ ANTOINE FERCHAULT DE RÉAUMUR**

- 1710 Réaumur was put in charge by King Henry XVI to report on the industry and natural resources of France.
- Improved metal forging techniques
- Isolated gastric juices to investigate digestion
- Discovered the phenomenon of lost appendage regeneration in Cray fish
- 1710 published memoires demonstrating the classification of sea-anemones as animals and not marine flowers.
**Nematostella Phylogenetic Position**

- Genetic Conservation
- Genome closer to that of vertebrates than other “model” organisms, nematode and fly (drosophila)
- Both Sexual and Asexual forms of reproduction
- Repeatably spawning cycle
- Low cost

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**RECENT RECOGNITION AS MODEL SYSTEM**

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**SENSORY BIOLOGY OF NEMATOSTELLA**

- Nearly 300 years after Réaumur identified Sea anemones as animals, little has changed in the view of anemones as sessile organisms waiting for prey to fall into their tentacles.

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**Nematostella sampling**

- 12 samples were chosen from 8 separate populations
  - Sippewissett 2
  - Sippewissett 3C
  - Sippewissett 16A
  - Sippewissett 1.1A
  - Sippewissett 4
  - NJ 3
  - KNG 2.1
  - Baruch 3.1B

- A separate population of Nematostella (Spurwink, ME), was utilized for testing purposes.
  - Kept in separate 12-chamber cells labeled by population

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“For testing purposes” is too vague. Because you’ve singled them out, you should make clear the advantage of the Maine samples.
**Nematostella Upkeep**

- Before each new testing day:
  - replace water in each chamber with clean saltwater
  - Artificial saltwater (1/3 concentration)
  - feed each specimen brine shrimp, *Artemia*

- Before each new test/testing day:
  - replace water in each chamber with clean saltwater

**Nematostella Testing**

- Reactions of each specimen were observed and recorded:
  - Tentacle
    - Movement
    - Contraction
  - Body
    - Movement
    - Bending
    - Contraction
    - Segmentation
  - Control
    - Introduce one pipet drop of distilled water
    - Repeat at end of testing
  - Feeding:
    - Introduce one pipet drop of brine shrimp
  - Feeding Cue:
    - Brine shrimp (frozen)
  - Distress Cue:
    - Cut specimens from each population
    - Run same experiment using specimen from separate population (Spurwink, ME)

**Nematostella Recording**

- Written observations
- Video recordings

- Distress Cue:
  - Record on video for five minutes a previously untested *Nematostella* specimen under a microscope.
  - At the surface level of each chamber, introduce one pipet drop of water from the corresponding population's cut specimen.
  - Observe and record on video for an additional five minutes.

**Results**

Behavioural observations were assigned numerical values. Wilcoxon Signed Rank Test was used to test for significance.

- non-parametric
- for populations that can not be assumed to be normal

Put scoring system on slide so audience can follow along more easily.

More details about test than are required. But, make clear what you are testing—namely that there is more or less activity in animals given food or distress cue than in control animals.

- http://udel.edu/~mcdonald/statsignedrank.html
- uses the residuals to test for a significant difference
- n (population size) does not include individuals whose tests results in a residual of zero
Results Cont.: Control vs. Water infused with brine scent

Use “Artemia water” instead of “brine water” because brine just means salty water.

\[ n = 68 \]
\[ W^+ = 195 \]
\[ W^- = 2152 \]
\[ p \text{-value} < 0.001 \]
\[ p = 2.339 \times 10^{-9} \]

I think it would have been good to make the upper graph larger so the relationship between paired points would be more evident.

Results Cont.: Control vs. Cut from own population

\[ n = 94 \]
\[ W^+ = 4464 \]
\[ W^- = 0 \]
\[ p \text{-value} < 0.001 \]
\[ p = 3.918 \times 10^{-17} \]

Results Cont.: Control vs. Cut from other population

\[ n = 57 \]
\[ W^+ = 572 \]
\[ W^- = 1081 \]
\[ 0.02 < p \text{-value} < 0.05 \]
\[ p = 0.04358 \]

Very interesting evidence that Nematostella has the ability to discriminate self from non-self.

Results Cont.: Cut from own population vs. Cut from another

\[ n = 90 \]
\[ W^+ = 4095 \]
\[ W^- = 0 \]
\[ p \text{-value} < 0.001 \]
\[ p = 1.793 \times 10^{-16} \]
Results Cont.: Control Prior vs. Control Post

\[ n = 28 \]
\[ W^+ = 304 \]
\[ W^- = 102 \]
\[ 0.02 < p\text{-value} < 0.05 \]
\[ p = 1.793 \times 10^{-16} \]

Conclusions

- Statistically significant decrease in reaction/sensitivity between the first and second control tests.
  - Indicative of possible learned behavior/desensitization
- Significance of the reactions toward the filtered brine water, when compared with pure salt water (Control 1), indicates that *N. vectensis* responds to cues that represent a possible feeding opportunity.
- Statistically significant reactions towards the introduction of water from cut specimens from both the specimen’s own population as well as outside populations (Spurwink, ME).
  - Indicative of *N. vectensis* responding to a cue related to starlet sea anemone stress.

Limitations and Further Research

- Time of study
- Lack of background information
- More specific/consistent character response classifications:
  - Separate rankings for opportunistic versus threat-induced reactions
  - Image-based quantification of data
- Cataloging specific movement repertoires of *Nematostella vectensis*
- Video analysis using software
- Analysis of receptor sites
- Field-based comparison

I would add “blind scoring of videos”

Thank You!