Nematostella vectensis & Edwardsiella lineata:

A comparison of Planula

Congrats on a very polished looking presentation.

"A comparison" is pretty generic. Can you think of a title that more specifically describes what you did?

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Nematostella vectensis

http://people.bu.edu/jrf3/BI547/

Photos of planulae would have been a nice touch here.

Edwardsiella lineata

http://www.sciencemadecool.com/2008/05/friday-parasite.html

Introduction

- distribution of marine species is of huge impact
  - fisheries
  - tourism
- adult success depends on their larvae
- what do larvae sense?
- how do larvae behave?
- marine management areas

http://bibii-littleworld.blogspot.com/2009/08/dream-of-snorkeling.html

Background

- *N. vectensis* and *E. lineata* are closely related sea anemones
- *E. lineata* has a recently evolved parasitic life stage
- an organism must have a predisposition to parasitism
- *E. lineata* and *N. vectensis* have common histories with new adaptations

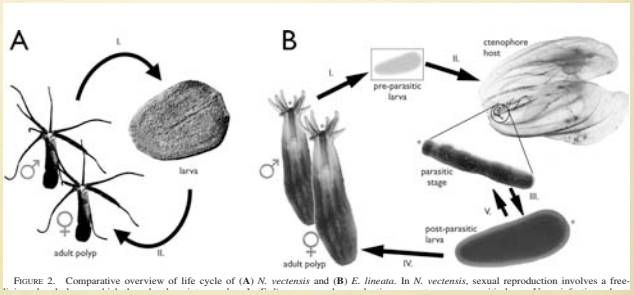


Figure 2. Comparative overview of life cycle of (**A**) *N. vectensis* and (**B**) *E. lineata*. In *N. vectensis*, sexual reproduction involves a free-

Poulin,R.2007.Evolutionary Ecology of Parasites, 2ed.Princeton University Press,Princeton, New Jersey, 342p.

Reitzel, Daly, Sullivan, Finnerty. Journal of Parisitology, 2009.

Why Choose E. lineata & N. vectensis?

- closely related species with different life cycles.
- can see their different ecological niches early on in development.
- Their shared history with current evolved changes presents the opportunity for an interesting larval comparison.

Good general introduction. One more way to think of it: Nv and El have very different habitat distributions, but their adults actually have similar forms and habits (so the behavior of adults can't be responsible for the habitat differences).

Main Hypothesis

- Although *N. vectensis* and *E. lineata* are related sea anemones, *E. lineata* has an additional parasitic life stage which presents different requirements and implications. Therefore, we predict that *N. vectensis* and *E. lineata* planula will display different sense capabilities and responses as well as different behavior and movement patterns.

More info on differences bewteen species with respect to where they live here?: emphasize the epipelagic niche of the parasite and the infaunal estuarine niche of Nematostella.

Planula Experiments

- Ctenophore Recognition
- Settlement
- Geotaxis
- Phototaxis

More accurately, this page could be titled "Obtaining Specimens" or something tho that effect.

General Methods

- *Mnemiopsis leidyi* was collected from Woods Hole and Sippewissett during September 2009.
- *E. lineata* parasites were excised from the collected ctenophores and were allowed 2-4 days to mature into planula before use in experiments.
- *E. lineata* trials were always executed in full strength sea water.
- *N. vectensis* planula were spawned from laboratory culture polyps.
- *N. vectensis* trials were always executed in 1/3 strength sea water.

Ctenophore Recognition

- Can planula sense ctenophores?
- Does only *E. lineata* have this ability or is it shared with *N. vectensis*?
- How are planula able to recognize their host?
- Do parasites recognize the health of their host?

- http://www.volkskrant.nl/multimedia/archive/00073/kwal500_73596a.jpg

Hypothesis

- During early observations, after removing *E. lineata* from *M. Leidyi*, we noticed parasites attempting to infect small pieces of jelly left over from the ctenophores. Therefore we think that *E. lineata* planula have the the ability to sense when their host *M. leidyi* is near and will attempt to infect the ctenophore regardless of its health.
- *N. vectensis* planula should not have this capability because it does not have a parasitic life stage.

Recognition Trials

- (1) Can *E. lineata* and *N. vectensis* planula detect and infect a small piece of *M. leidyi*?
- (2) Will *E. lineata* planula swim towards *M. leidyi* even when they are separated by a filter?

Ctenophore Infection (1) methods

- placed 4 *E. lineata* and 4 *N. vectensis* in a petri dish with a piece of *M. leidyi*
- observed organism location every 5 minutes for 20 minutes
- 5 samples

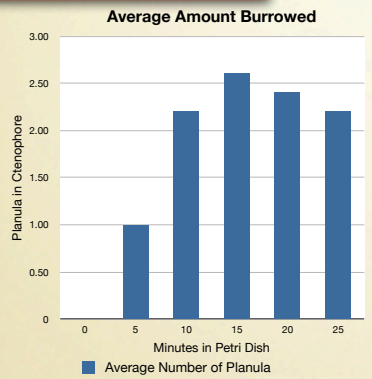
Time Lapse

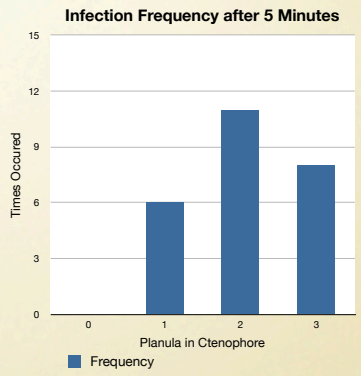
The time lapse is great. Very convincing...very compelling.

Ctenophore Infection (1) results

I would present data from Nematostella in graphical form too. You could use a stacked column format with ("outside ctenophore" stacked on "inside ctenophore").

What does (1) refer to?

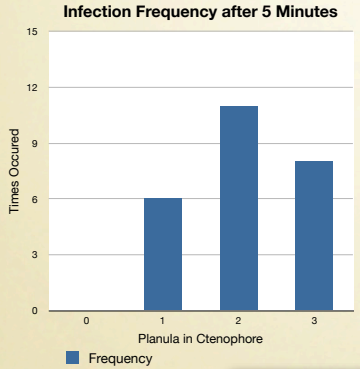




Results from *E. lineata*, there was no response

Titles of graphs don't make it immediately clear how the graphs differ. Graph 1 could be titled "Average number of ctenophores burrowing into Mnemiopsis at 5 minute intervals" Graph 2 could be titled "Average number of ctenophores burrowed into Mnemopsis over the first five minutes"

Ctenophore Infection (1) discussion



- 1/100 chance that 1 planula will be in a ctenophore piece at any given time
- 1/10,000 chance that 2 planula . . .
- 1/1,000,000 chance that 3 planula . . .

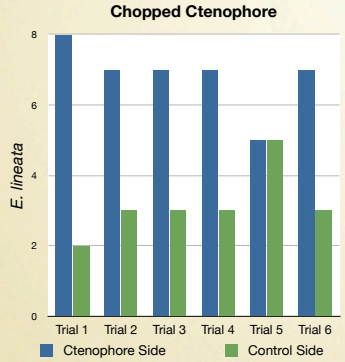
Make it clear that ctenophore fits in a single square of a grid containing 100 squares. Then, based on random chance, there is a 0.01 probability that a planula is in the square occupied by the ctenophore.

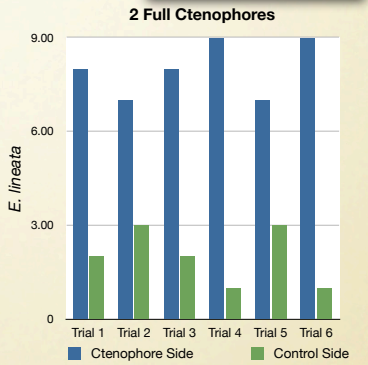
Swim Direction (2) Methods

- PVC pipe with 3 sections separated by filters (ctenophore, planula, empty)
- added 10 *E. lineata* planula for 3 minutes and recorded their end location

Swim Direction (2) Results

"live" instead of "full"





- average planula amount on ctenophore side is 6.83

"On average, 6.83 / 10 planulae are in the sector closest to the ctenophore."

- average planula amount on ctenophore side is 8

Swim Direction (2) Discussion

<u>*M. Leidyi* Pieces</u>

- average planula amount on ctenophore side is 6.83
- average amount swimming against the filter is 3.6
- chi squared is 8.06

<u>2 Whole *M. leidyi*</u>

- average planula amount on ctenophore side is 8
- average amount swimming against the filter is 5.5
- chi squared is 21.6

I think this could all fit on the previous slide.

Do either of these findings represent a significant departure from the 50/50 expectation? Make clear what your chi-squared test was testing: that there is a preference for swimming in the sector closer to the ctenophore.

I like the way you've organized the talk into specific questions / hypotheses

Settlement

- Where do planula go to settle when their larval stage is over?
- Do planula show a preference for certain substrates?
- Do *N. vectensis* and *E. lineata* have similar or different preferences?

http://www.uibk.ac.at/public-relations/presse/medienservice/images/2005/jan_05/prof._hobmayer/nematostella.jpg

Hypothesis

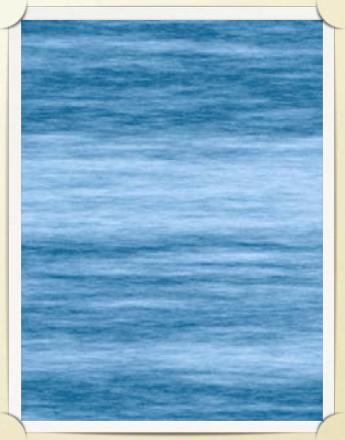
- During collections we observed *N. vectensis* around shallow muddy areas and *M. leidyi*, the host for *E. lineata*, around sandy coastal areas. Therefore we predict that if we set up a system where planula can choose the sediment in which they settle, a greater amount of *N. vectensis* will settle in mud, while a greater amount of *E. lineata* will settle in sand.

Methods

- set up a temporary partition in a shallow bowl
- added clean mud and sand to each side
- removed the partition, then added water and organisms
- let bowl sit for at least a week

Geotaxis

- Where do planula prefer to be in the water column?
- Do *E. lineata* planula and *N. vectensis* planula show different swimming patterns?
- Do environmental changes alter the swimming patterns of planula?



http://www.heathrowe.com/tuts/oceanmistimages/6.jpg

Avoid anthropomorphic terms like "like." Also, I think I would put it differently. A challenge for E. lineata is to find a epipelagic host, so they should be high in the water column because that's where the host is.

Hypothesis

- *E. lineata*'s parasitic stage suggests that the planula likes to be dispersed over large distances. Therefore we believe that the planula will prefer to be higher up in the water column where there is a greater chance for dispersal.
- Studies have shown that *N. vectensis* usually colonizes locally*. Therefore we believe that *N. vectensis* planula will stay lower in the water column to avoid dispersal, unless water conditions are unfavorable.

*Reitzel, Darling, Sullivan, Finnerty. Biological Invasions, 2008.

I think I would have separated the aversion behavior into a separate hypothesis.

Methods

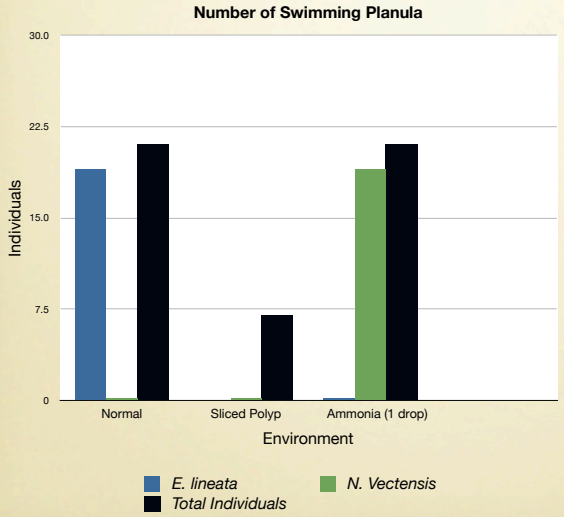
- added 3 different solutions to 10 mL graduated cylinders and observed swimming behavior and depth
  - normal salinity (*N. vectensis* and *E. lineata*)
  - solution from a sliced planula (*N. vectensis*)
  - diluted ammonia (*N. vectensis*)

Avoid anthropomorphic terms to describe the animal's motivation (like "like"). Also, I think I would put it differently. A challenge for E. lineata is to find an epipelagic host, so they should be high in the water column because that's where the host is.

Results

- *E. Lineata* show a preference for being near the surface, and exhibit considerable swimming capabilities (90.5% swam).
- *N. Vectensis* do not swim under normal conditions. They move horizontally on the bottom when exposed to a sliced polyp solution. And they generally keep themselves on the surface of the water in unfavorable conditions (90.5%).

Number of swimming planula in each environment



19 out of 21 *E. lineata* swam

0 out of 7 *N. Vectensis*

19 out of 21 *N. Vectensis*

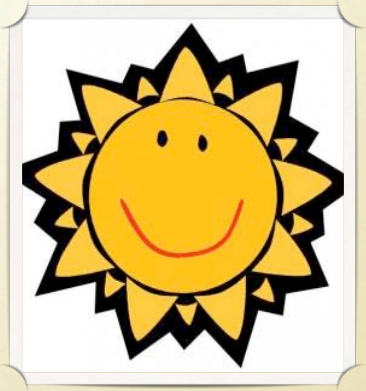
Chi-squared was significant (38)

Cool video!

Ed the Swimmer

Phototaxis

- Can planula sense light?
- Does it affect their behavior or not?
- Will they move towards or away from light?



Hypothesis

- While collecting in Woods Hole we noticed a large number of *M. leidyi* near the surface of the water, therefore we predict that *E. lineata* will have a preference for epipelagic environments and furthermore, well lit environments.

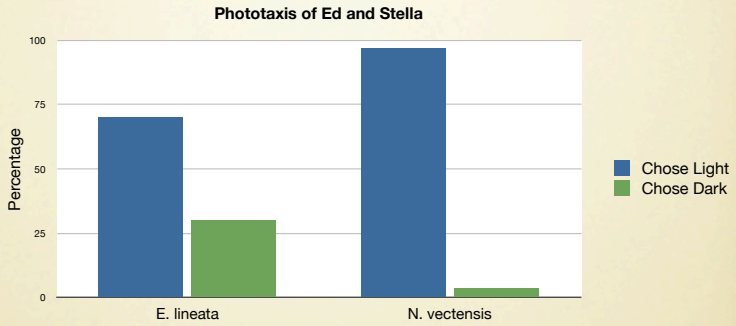
- *N. vectensis* live in shallow tidal areas, therefore we predict that *N. vectensis* planula will also show a preference

Methods

- We set up a piece of PVC pipe so that half was covered by a piece of black plastic and the other half was lit by a lamp.
- We then added both *N. vectensis* and *E. lineata* to the center and allowed them to swim to either side.

Both anemones in the same salinity? ...at the same time? These things aren't clear from your slide.

Results



- *E. lineata* showed a small preference for the lit side of the flume and *N. vectensis* showed a large preference for the light.
- The Chi-squared value was significant (11.04)

Provide P values.

Edwardsiella lineata Discussion

- We found that *E. lineata* prefer to be high in the water column, they show some preference for well lit areas, and they have the sensory capabilities to detect and seek out their hosts, *M. leidyi*.

Nematostella vectensis Discussion

- *N. vectensis* shows a preference for benthic environments unless those benthic environments are unfavorable, prefers light over dark, and doesn't have the ability to sense or seek out *M. leidyi*.

Conclusion

- Our results and statistical analyses support our hypothesis that while E. lineata and N. vectensis are closely related anemones, they exhibit very different behavioral patterns and life styles because E. lineata is a parasite and N. vectensis is not.

N. vectensis is highly derived in its own right. It is the only member of its family that has invaded the estuarine environment (and the only member of its family that lays its eggs in a negatively buoyant egg mass---Is this connected to its derived estuarine existence? I think so.)

Future Ideas

- Try to dye an *N. vectensis* planula and then plant it inside a *M. leidyi* to see if it has the capability to survive inside without being digested.
- Repeat our experiments but on a much larger scale to yield more accurate and average results.
- Do more test to see how capable *E. lineata* is at distinguishing it's host (*M. leidyi*) from other ctenophores, and from jelly substitutes (i.e. cteno agar).

The End

AJ

Pam

Notice our bilateral symmetry!

Special Thanks

- Special thanks to Adam Reitzel for his helpful ideas and sharing information, Professor Finnerty for always finding the supplies we needed, tanks that we didn't end up using and labs that we ended up abandoning, Derek Stefanik for helping us spawn stella so we could use the planula, and Tristan Lubinski for getting our video camera working.