Investigating the effects of environmental stress on the host-parasite relationship between a ctenophore and a sea anemone

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Specifications? e.g., The effects of variable temperature and salinity on....

Outline

- Introduction
  - Invasive Species
  - Parasitism
- Materials and Methods
- Preliminary findings
- Future Research

Invasive Species

- According to the United States DOA, an invasive species is defined as:
  1. non-native (or alien) to the ecosystem under consideration and
  2. whose introduction causes or is likely to cause economic or environmental harm or harm to human health

http://www.invasivespeciesinfo.gov/whatis.shtml

Invasive Species

- Cane Toad (Bufo marinus)
  - Introduced to Australia in 1935 to combat beetle infestation
  - 102 specimens
  - Couldn’t eat the beetles
  - 200 million toads now, government working on eradication

http://en.wikipedia.org/wiki/File:Bufo_marinus_from_Australia.JPG
Portrait of an invasive species: *Mnemiopsis leidyi*

- Lobate ctenophore, "comb jelly"
- Four rows of ciliated combs used for locomotion
- Diploblastic, has muscle and nerve cells
- Carnivorous planktonic predator
- Native to Western Atlantic waters
- Tolerant of a wide range of salinity and temperature
  - 2-38‰ salinity
  - 2-32°C

Hansson, 2006

Portrait of an invasive species: *Mnemiopsis leidyi*

- Introduced in the Black Sea in the 1980’s
- Within 10 years, biomass reached an estimated 1 billion metric tons
- Anchovy catch plummeted
- The biological controls considered to include *E. lineata*

Kube et al., 2007.

The sea anemone *Edwardsiella lineata* is a parasite of *M. leidyi*

Reitzel et al., 2007

Characteristics of *E. lineata* that may make it a disruptive non-native species

"Non-specific" is another key term here—in addition to competing with other sessile benthic critters for suitable substrate, it might also cause decline in whatever it preys upon as an adult.

Adult anemones form dense mats

Sea bathers eruption

Worst case of sea-bathers eruption I ever saw!

Reitzel et al., 2007

Freudenthal, 1993
The significance of parasitism

Parasites far outnumber free-living organisms

Zimmer cites this approximate figure in *Parasite Rex*.

John Finnerty, Personal Communication

Parasitism has evolved in many animal lineages

- **Cnidaria** (Sea anemones, Jellyfish)
- **Deuterostomia** (mice, zebrafish)
- **Lophotrochozoa** (earthworm, squid)
- **Ecdysozoa** (fruit fly, nematode)

C. elegans is actually a free-living nematode. It lives in the soil and eats bacteria.

Evidence that parasites can sense host viability…?

- While not parasitic, the relationship between coral and zooxanthellae is a good example of symbiont behavior affected by environmental stress
- Zooxanthellae perform photosynthesis and provide the coral polyp with food
- When stressed (ie temperature too high), corals expel zooxanthellae, leading to “bleaching”

http://www.estrellamountain.edu/beauty/farabee/biobk/01-zooxanthellae.jpg

http://www.osdpd.noaa.gov/ml/ocean/cb/stage.jpg

Not entirely apt comparison because in this case, the host expels the symbiont. It’s not a case of the symbiont choosing to jump ship.


Hypothesis

If parasitic sea anemones can sense host ctenophore viability and or stress, then the parasite will respond with physiological and or behavioral changes.

Predictions stemming from this hypothesis:
1) Parasites will exit their host under environmental stresses, such as salinity, temperature and starvation
2) Parasites will respond to unhealthy environmental conditions by reducing in size

Is shrinking really a “response,” or just an inevitable outcome of not having enough food.
Methods

Headquarters: Dr. Karen Warkentin’s Lab, BRB 526

What’s that dark stripe under Conor’s nose?

Collecting in Woods Hole, MA

- collected parasitized *Mnemiopsis leidyi* with our peers off the coast of Woods Hole, MA

Preparing The Water and Acclimation

- Obtained 9 *Mnemiopsis leidyi* form the BUMP Laboratory holding tanks (each infected by only 1 parasite)
- Prepared salt water varying at 3 different salinities (25‰, 30‰, & 35‰) using Instant Ocean Salt, Salt water and Fresh water.
- To prevent salinity shock to the ctenophores, we acclimated them to their new salinities by dripping the water into a chamber with their original water over the course of hours.
- 3 ctenophores would be kept at one of the salinities and all 9 would each have their own chamber.

Photographing

- Before beginning the trials, we photographed each individual and gave it its own name (A-I, 1-9, etc.), with a clear image of their parasite to track the *E. lineata*’s progress
  - Have they grown or shrunk?
  - Have they left the host?
  - Have they died?
- Each individual was photographed every 24 hours over the span of 4 days

How did you “compare” the multiple photographs taken of each individual on each day? Did calculate the area of each ctenophore in each photo using ImageJ and quantify the variance? What was the variance? Isn’t it possible that the parasite shrunk over a given day?
Incubation

• 3 incubators: 15°C, 22°C, 29°C
• 1 ctenophore from each salinity was placed in each of the incubators
  ○ Creating 9 different combinations of salinities and temperature.

Here's a good place to tell the audience that n=6 Mnemiopsis per treatment, each harboring 1 parasite.

Maintenance

• Salinity & Water level check
• Changing water
  ○ Avoiding temperature shock
• Pictures
• Minimal Feeding

Methods: Starvation-induced stress

Results
Parasites exited or remained in the host at similar frequencies.

I would have broken off the parasite from ctenophores that died into a separate category.

- Total of 54 infected Mnemiopsis

Taking a closer look...

**Reason for Parasite Departure**

<table>
<thead>
<tr>
<th>Type of Departure</th>
<th>Percent of Parasites that Exited (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mnemiopsis Death Induced Exit</td>
<td>n=13</td>
</tr>
<tr>
<td>Non-Mnemiopsis Death Induced Exit</td>
<td>n=16</td>
</tr>
</tbody>
</table>

- Total number = 28 parasites (only ones that exited their host)
- 12 exited presumably due to the Mnemiopsis death
- 16 exited while Mnemiopsis still alive (autoexcision)

**Variation of autoexcision across 9 stress categories is not statistically significant**

<table>
<thead>
<tr>
<th>Autoexcised Parasites Based on Temperature and Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent of Autoexcision (%)</td>
</tr>
<tr>
<td>0-5° C/‰: n=3</td>
</tr>
<tr>
<td>5-10° C/‰: n=2</td>
</tr>
<tr>
<td>10-15° C/‰: n=2</td>
</tr>
<tr>
<td>15-20° C/‰: n=2</td>
</tr>
<tr>
<td>20-25° C/‰: n=2</td>
</tr>
<tr>
<td>25-30° C/‰: n=2</td>
</tr>
<tr>
<td>30-35° C/‰: n=2</td>
</tr>
<tr>
<td>35-40° C/‰: n=2</td>
</tr>
<tr>
<td>40-45° C/‰: n=1</td>
</tr>
</tbody>
</table>

**Autoexcision varies significantly with respect to temperature and salinity independently**

- Total of 16 parasites that autoexcised
- There is no statistical significance in the parasites' autoexcision in different combination of temperature and salinity

\[ X^2 = 9.65, \text{df} = 8 \]

\[ X^2 = 52.38, \text{df} = 2 \]

Hard to believe the chi-squared value is this high when the numbers are almost identical. Also, what is the P value?

\[ X^2 = 40.00, \text{df} = 2 \]

What is the P value?
No statistical significance in ctenophore mortality across experimental conditions

\[ X^2 = 7.878, \text{ df} = 8 \]

What is the P value?

Mnemiopsis mortality varies significantly with respect to temperature

\[ X^2 = 6.50, \text{ df} = 2 \]

I think it may have been preferable to discuss ctenophore mortality prior to parasite excision.

Statistically insignificant difference in percent change of parasite length across temperatures

What statistical test was performed here?

15° vs. 22°C
\[ \text{df} = 4 \]
\[ p = 0.2598 \]

22° vs. 29°C
\[ \text{df} = 4 \]
\[ p = 0.5327 \]

15° vs. 29°C
\[ \text{df} = 4 \]
\[ p = 0.3002 \]

Statistically significant difference in percent change of parasite length across temperatures

15° vs 22°C
\[ \text{df} = 4 \]
\[ p = 0.05 \]

22° vs 29°C
\[ \text{df} = 4 \]
\[ p = 0.0604 \]

15° vs 29°C
\[ \text{df} = 4 \]
\[ p = 0.0813 \]
**Hypothesis**: If parasitic sea anemones can sense host ctenophore viability and or stress, then the parasite will respond with physiological and or behavioral changes.

**Conclusions**

- While autoexcision of parasites was not affected by temperature, it was affected by extreme salinities.
- Mnemiopsis death was most frequent in high temperatures (29°C).
- At the extreme salinities, parasite size decreased regardless of temperature, where at 30‰, the temperature caused a statistically significant difference in parasite size.
- Starvation may not be a feasible assay to assess parasite behavior.
Moving Forward

• To Improve This Study
  ◦ Control of ammonia and nitrate levels
  ◦ Parasites of all the same initial size and more accurate measurements of their lengths
  ◦ Recording parasite movement within the Mnemiopsis
  ◦ Better preventing Mnemiopsis death
  ◦ Larger sample sizes

• Future Studies
  ◦ Test environmental extremes on parasites after exiting the host
  ◦ Larger varying ranges of both salinity and temperature
  ◦ Test this along with uninfected Mnemiopsis to determine parasites role as a stress

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