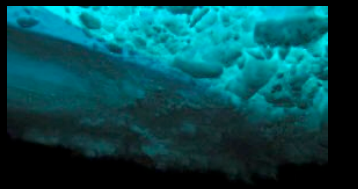
More specific? e.g., The effects of variable temperature and salinty on.....

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

Ross Hill, Tyler Isaac, M. Conor McManus & Derek Stefanik

Outline

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



www.xray-mag.com

Good set-up in this and the next three slides.

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

http://en.wikipedia.org/wiki/File:Bufo_marinus_from_Australia.JPG

- 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://www.imb.uq.edu.au/index.html?page=48437

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

people.bu.edu/jrf/BI547

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

njscuba.net

Sea bathers eruption

seafriends.org.nz

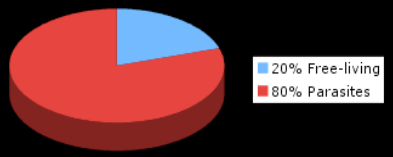
Worst case of sea-bathers eruption I ever saw!

Freudenthal, 1993

The significance of parasitism

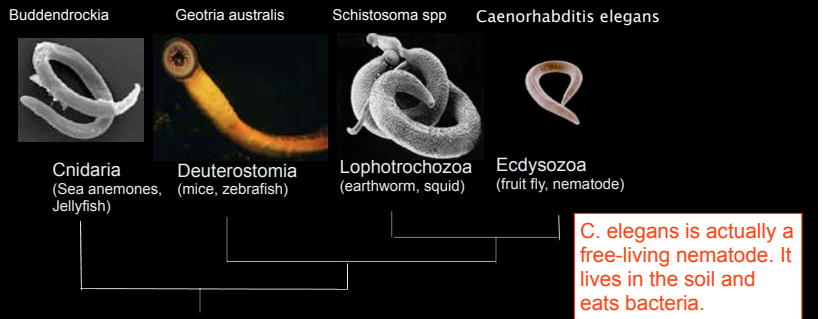
Parasites far outnumber free-living organisms

Zimmer cites this approximate figure in *Parasite Rex*.



John Finnerty, Personal Communication

Parasitism has evolved in many animal lineages

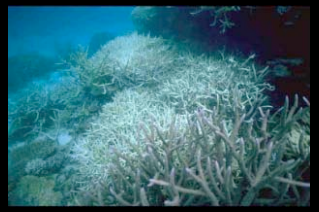


C. elegans is actually a free-living nematode. It lives in the soil and eats bacteria.

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/faculty/farabee/biobk/01-zooxanthellae.jpg



http://www.osdpd.noaa.gov/ml/ocean/cb/stags.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.

Dove, SG & O Hoegh-Guldberg. 2006.

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

http://commons.wikimedia.org/wiki/File:MarthasVineyard.map.png

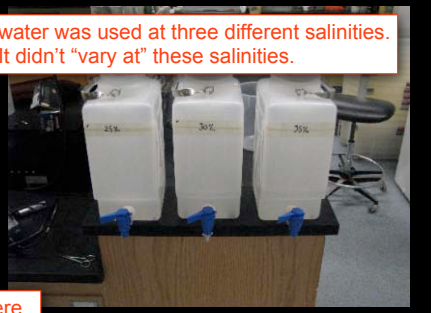
- collected parasitized *Mnemiopsis leidyi* with our peers off the coast of Woods Hole, MA

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

spelling

water was used at three different salinities. It didn't "vary at" these salinities.



awkward phrasing here.

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?
    - Measured on the grid sheet placed below
  - 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.

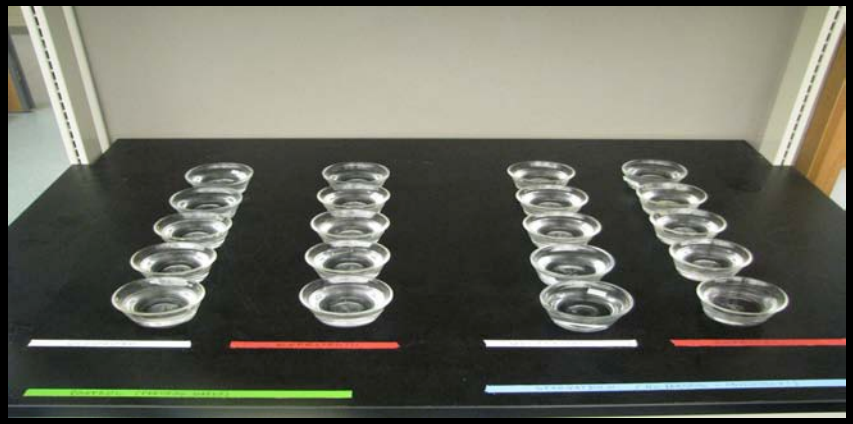
Here's a good place to tell the audience that n=6 Mnemiopsis per treatment, each harboring 1 parasite.

Maintenance

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

Good explanations of precautions you took to avoid confounding variation (e.g., evaporation; rotting food). Use labels to show the conditions in each bowl as in the next slide instead of having to explain everything verbally.

Methods: Starvation-induced stress

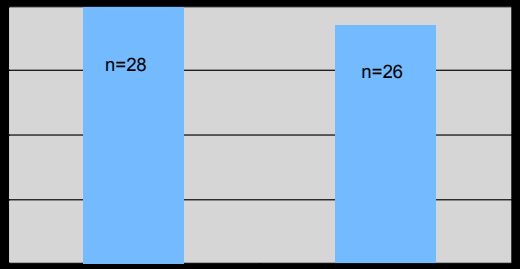


Uninfected Infected Uninfected Infected

Fed Starvation

Results

Parasites exited or remained in the host at similar frequencies

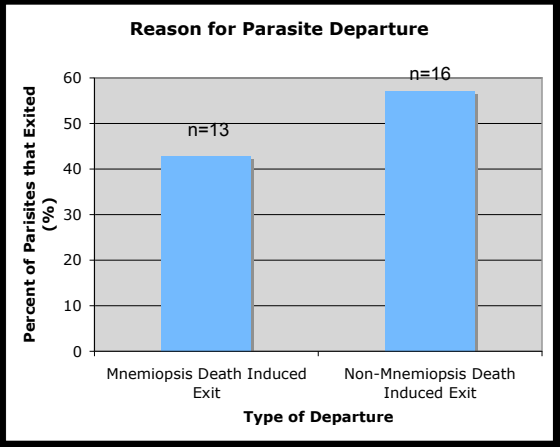


I would have broken off the parasite from ctenophores that died into a separate category.

- Total of 54 infected Mnemiopsis

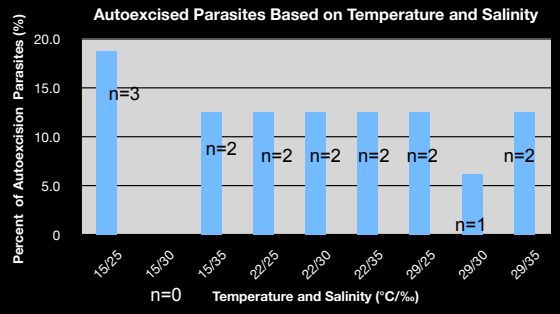
Taking a closer look…

If ctenophore disintegrated around parasite, is it really fair to say the parasite "exited" the ctenophore.



- 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)

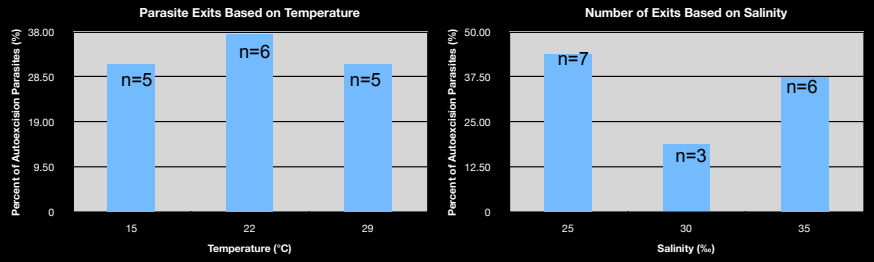
Variation of autoexcision across 9 stress categories is not statistically significant



$X^2 = 9.65$, df = 8

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

Autoexcision varies significantly with respect to temperature and salinity independently



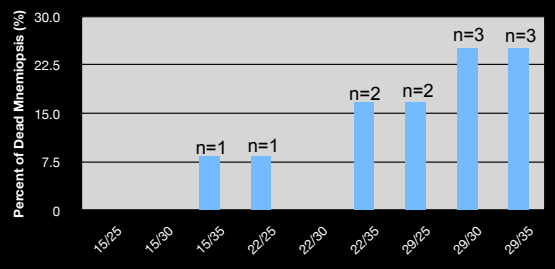
$X^2 = 52.38$, df = 2

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

$X^2 = 40.00$, df = 2

What is the P value?

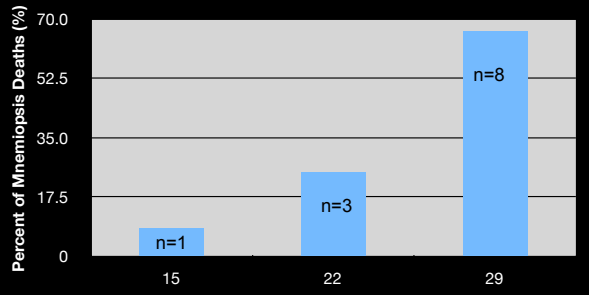
No statistical significance in ctenophore mortality across experimental conditions



$X^2 = 7.878$, df = 8

What is the P value?

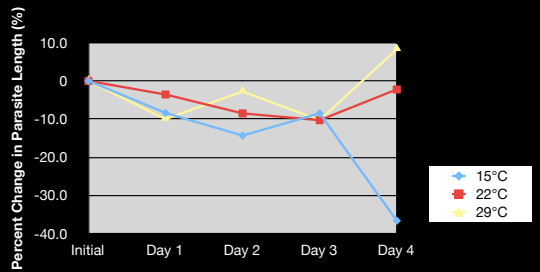
Mnemiopsis mortality varies significantly with respect to temperature



$X^2 = 6.50$, df = 2

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

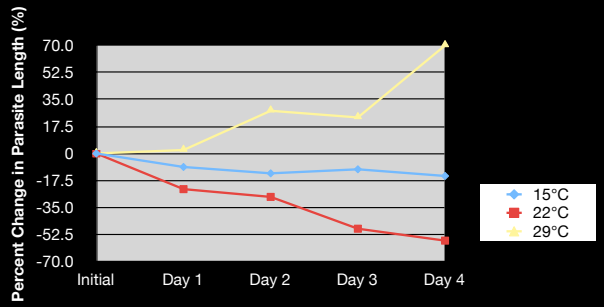
Statistically insignificant difference in percent change of parasite length across temperatures



What statistical test was performed here?

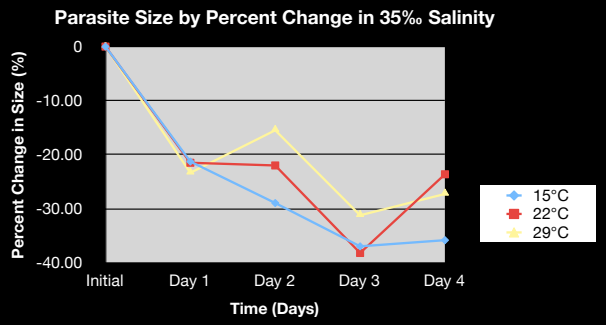
15º vs. 22ºC	22º vs. 29ºC	15º vs. 29ºC
df = 4	df = 4	df = 4
p = 0.2598	p = 0.5327	p = 0.3002

Statistically significant difference in percent change of parasite length across temperatures



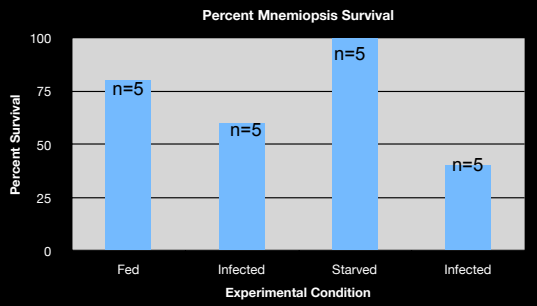
15º vs 22ºC	22º vs 29ºC	15º vs 29ºC
df = 4	df = 4	df = 4
p = 0.05	p = 0.0604	p = 0.0813

Statistically insignificant difference in percent change of parasite length across temperatures



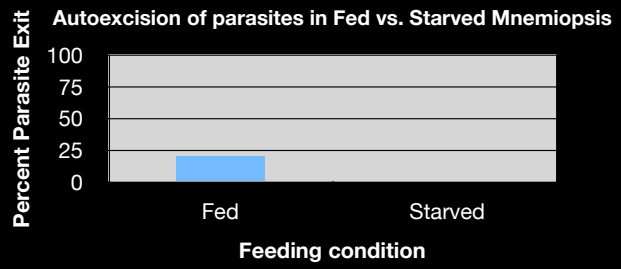
15º vs. 22ºC	22º vs. 29ºC	15º vs. 29ºC
df = 4	df = 4	df = 4
p = 0.2461	p = 0.5046	p = 0.1434

Mnemiopsis survival does not vary significantly between experimental conditions



Fisher's Exact Test: 2 - tailed p = 1.00

Only 1 parasite exited its host…



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

Moving Forward

- 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

Acknowledgments

- Dr. John Finnerty and Tristan Lubinski
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- Dr. Warkentin
- Our peers in BI 547