

Examination of fitness and health consequences of parasitism of the ctenophore *Mnemiopsis leidyi* by the lined sea anemone, *Edwardsiella lineata*, as demonstrated by environmental shock, regeneration, and fecundity studies

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Photo retrieved from http://people.bu.edu/jrf3/BIS47/IMAGE_CTENOPHORA_Mnemiopsis.jpg

Presentation Outline

- I. General Introduction
- II. Specimen Collection Methods
- III. Specific Tests
 1. Salinity shock
 2. Heat shock
 3. Regeneration
 4. Spawning
- IV. General Conclusions



<http://web2.uwindsor.ca/courses/biology/macisaac/pages/mnemiopsis.htm>

Background Information

Mnemiopsis leidyi

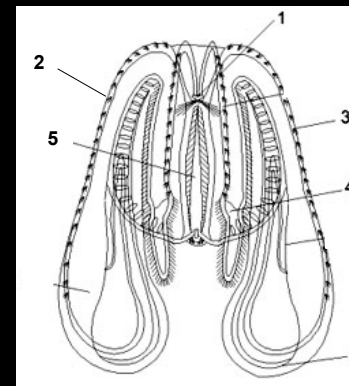
- Member of Ctenophora phylum:
 - biradial symmetry, oral-aboral axis, thick mesoglea, 8 comb rows of interconnected cilia used for locomotion (Pang and Martindale 2008)
- *M. leidyi* is a lobate ctenophore characterized by oral feeding lobes (Pang and Martindale 2008)
- Adults are voracious carnivores
- Natural habitat: Atlantic coast/estuarine areas of North/South America (Pang and Martindale 2008)
- Found in temperatures of 2-32°C and salinities of 2-38 parts per thousand (Purcell et al 2001)
- Form cydippid larva (Pang and Martindale 2008)



Background Information

Morphology

I think the anatomical detail is unnecessary here.



1. Apical organ
2. Radial (meridional) canals
3. Comb (ctene) rows
4. Auricles
5. Pharynx
6. Oral lobes

Later, when you talk about body movements, it is important to identify ctene rows, oral lobes, and auricles.

<http://www.caspianenvironment.org/mnemiopsis/mnemmenu5.htm>



Background Information

Edwardsiella lineata

- Lined sea anemone that parasitizes *M. leidyi* (Reitzel et al 2007)
- Only anemone in family Edwardsiidae with parasitic larval stage (Reitzel et al 2007)
- Stages of Development:
 1. Sexual reproduction produces pre-parasitic larva, which enters ctenophore host **Metamorphoses into worm-like body plan.**
 2. Exits host and becomes post-parasitic larva (planula)
 3. Transforms into polyp
 4. Adult anemones burrow in soft sediment (Reitzel et al 2007)



Background Information

Ecological Issues

- Introduction to non-native habitats via ship ballast water
- Consumption of planktonic fish larva and subsequent decline of commercially important fisheries particularly when introduced to the Black Sea in the late 1980s.
- *M. leidyi* is the preferred host of *E. lineata*
- If presence of anemone parasite proves to be disadvantageous to health of *Mnemiopsis*, *E. lineata* could be useful biological control of invasive *M. leidyi* (Reitzel et al 2007)



Photo retrieved from <http://barelyimaginedbeings.blogspot.com/2009/08/different-kind-of-ghost.html>

Background Information

Objectives

"were examined"

Health and fitness consequences of *E. lineata* parasite on *M. leidyi* host are to be examined by comparing responses of non-infected and infected ctenophores to rapid temperature and salinity changes, as well as by monitoring the differences in the rate of wound healing and fecundity of non-infected and infected hosts.



Photo retrieved from sciencemadecool.com



Effects of Salinity on *M. leidyi*: recovery from rapid change in salinity

Background

- ❖ *M. leidyi* have been collected in 2-38 salinities (Purcell et al. 2001)
- ❖ *M. leidyi* avoids predation and competition by entering estuarine waters (Purcell 2005)
- ❖ Limited freshwater input lead to high salinity waters in Black Sea (Purcell 2005, Purcell et al 2001)
- ❖ *M. leidyi* also abundant invasive in Baltic Sea (Kube et al. 2007)
- ❖ Methods based on 2005 study by Ma and Purcell in which effects of salinity were tested on cnidarian medusa *Moerisia lyonsi*

Why grey text in body of slide? Less contrast with background than white text used on other slides.

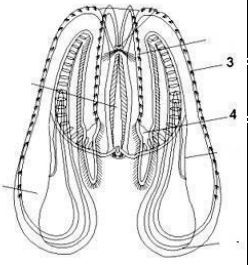
Methods

- Following collection kept in circulating tanks of 34 salinity and 19 °C
- Aboral to oral length measured to ensure approximately equal size (mean = 3.8 cm) **Information about size never provided.**
- Categories of *M. leidyi*: uninfected, 1-5 infections, >5 infections
- Salinities: 5, 10, 15, 20, 25, 30, 40, 45 **Units?**
- Four *M. leidyi* of each category at each salinity
- Air temperature held at 13-14 °C, water temperature at 18 °C
- 100ml of water in each container **Water temp didn't equilibrate to air temp?**
- Fed *Artemia* twice daily
- Observed every hour for first 6 hours, at 18 and 24 hours.

Experimental Setup



It's generally a good idea to edit out extraneous objects from the background (e.g., salt-encrusted outlet).



3 → Cten row
4 → Auricles

You should justify why different movements merit different scores, and ideally, you would discuss how the scoring system impacts the apparent statistical significance of your results.

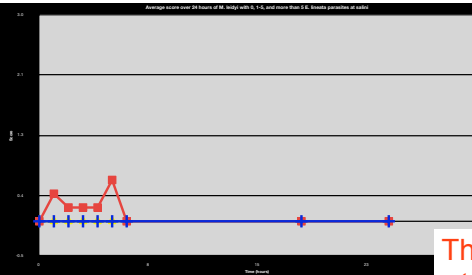
<http://www.caspianenvironment.org/mnemiopsis/mnmemenu5.htm>

Observation	Points
No movement	0
Auricles movement	1
Comb row movement	2
Muscle Movement	1
Swimming	3

t Tests

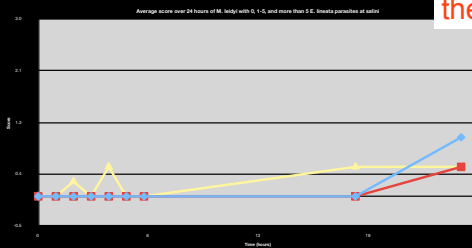
- Difference between two means/variance
- Two-tailed: either group can have larger mean
- P value = probability that difference in means are result of chance
- $\alpha = 0.05$

There is no need to describe how a t-test works.

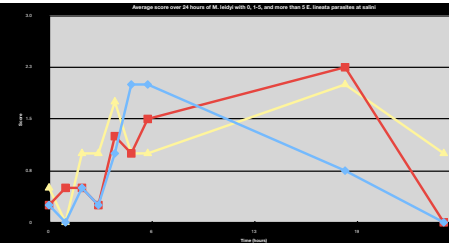


At 5 hours
 $p = 0.2254$

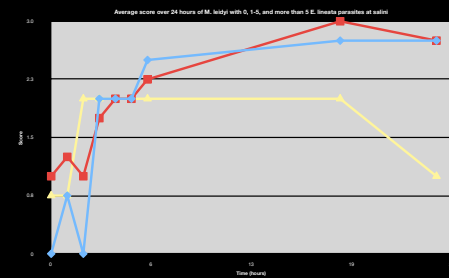
The labels are illegible
--try one graph per page, and increase the size of the text.



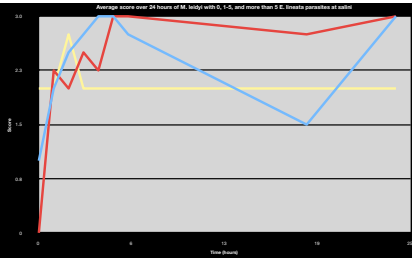
At 24 hours
 $p = 0.3901$



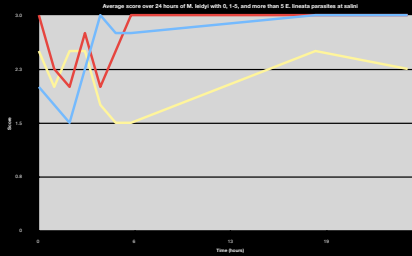
At 18 hours
 $p = 0.0138$ for 1-5 infections;
 $p = 0.0796$ for >5 infections



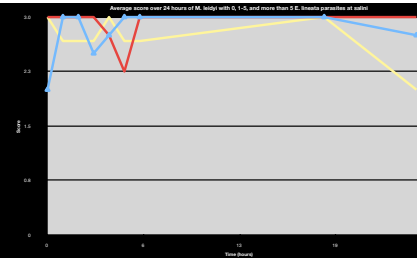
At 24 hours
 $p = 0.0577$



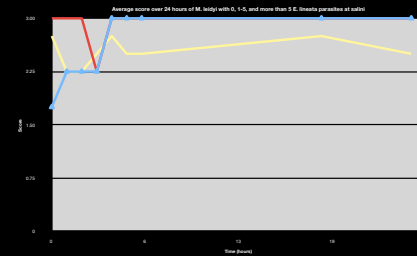
At 18 hours
 $p = 0.0917$
 for 1-5
 infections;
 $p = 0.3910$
 for >5
 infections



At 24 hours
 $p = 0.0577$



At 24 hours
 $p = 0.3910$
 for 1-5
 infections;
 $p = 0.1835$
 for >5
 infections



At 24 hours
 $p = 0.3910$

Salinity	Significance of Average Score	P value
5	Not significant	0.2254
10	Not significant	0.3910
15	1-5 infections significantly higher score than 0 infections >5 infections nearly significantly higher than 0 infections	0.0138 0.0796
20	>5 infections nearly significantly lower score than 1-5 and 0 infections	0.0577
25	1-5 infections nearly significantly higher score than 0 infections >5 infections not significant	0.0917 0.3910
30	>5 infections nearly significantly lower score than 1-5 and 0 infections	0.0577
40	Not significant	0.3910 0.1835
45	Not significant	0.3910

What does "no significant results mean?" State clearly what you were testing: whether parasite load impacts ctenophore movements at different salinities.

Discussion

- 1-5 infections being higher or the same as 0 infections contradicts hypothesis
- >5 infections being lower than 1-5 and 0 infections is consistent with hypothesis
- Overall inconclusive

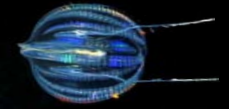
Factors to consider:

- Scoring is somewhat subjective
- Small increases in salinity overnight (especially low)
- Small sample size

Future Projects

- Extend time period of study (48-72 hours)
- Observe more frequently
- Effect of salinity on *E. lineata*
- Use data and reevaluate as we learn more on function of nervous system

Heat Shock



- Heat shock was used to test whether the *Edwardsiella* parasite has an effect on the fitness of the ctenophore, *M. leidyi*
- The ctenophores were shocked to a temperature higher than their normal temperature range (2°-32°C) to see if the parasitic ctenophores died at a lower temperature than the uninfected ctenophores. (Purcell, 2001)
- It is expected that the ctenophores with more infections will die at a lower temperature than those without infections

You were directly testing the effect of parasite load on heat tolerance (not fitness) of *Mnemiopsis*.

Edit out unnecessary clauses (such as "the experimental design consisted of.")
You can start with "ctenophores placed in 100 ml of artificial sea water.."

Methods

- The experimental design consisted of placing the ctenophores in 100 milliliters of water from the tanks (20°-24°C) after measuring the length and recording the number of parasites **Length of host or parasite?**
- Between 65-100 milliliters of 75°C water was added to each container to bring the temperature to a temperature in the range of 34°-45°C
- The ctenophores were then observed for 10 minutes, recording whether they were alive or dead at 5 minute intervals.

Methodology seems imprecise--did you use any volume between 65-100?

Ctenos were only observed twice during a 10 period--that doesn't seem sufficient to evaluate their tolerance to heat shock.



It's generally a good idea to edit out extraneous objects from the background (e.g., flasks, dirty paper towel).



Results

Results



Results



What was mean number of parasites in 1-5 category versus >5 category? How different were these categories really?

- As expected, the uninfected ctenophores died at a higher temperature than those with parasites
- Surprisingly there was no difference between ctenophores with 1-5 parasites and >5 parasites
- The fact that there was no difference between the two different parasitic groups disagrees with the hypothesis which is based on the fact that the number of parasites is indirectly proportional to the fitness of the ctenophore.

It's generally a good idea to refrain from qualifiers that imply investigator bias in the text on the slide, e.g., "as expected" and "surprisingly."

Awkward wording....the uninfected parasites proved more tolerant of high temps.

Chi Square Test

Chi Square Test the Amount of Individuals Healed in 6 Hours

	healed	didn't heal	1 degree of freedom
uninfected (expected)	10	5	
1-5 parasites (observed)	2	13	
Deviation (d)	-8	8	
Deviation ² (d ²)	64	64	significant
D ² /e	6.4	12.8	
chi square	19.2		
P value is less than 0.0001			
	healed	didn't heal	1 degree of freedom
uninfected (expected)	10	5	
>5 parasites (observed)	1	14	
Deviation (d)	-9	9	
Deviation ² (d ²)	81	81	significant
D ² /e	8.1	16.2	
chi square	24.3		
P value is less than 0.0001			



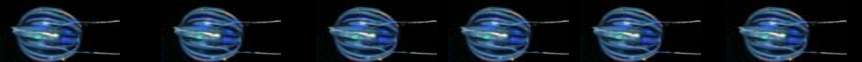
Not necessary. This table is widely available.

Degrees of Freedom (df)	Probability (p)										
	0.95	0.90	0.80	0.70	0.50	0.30	0.20	0.10	0.05	0.01	0.001
1	0.004	0.02	0.06	0.15	0.46	1.07	1.64	2.71	3.84	6.64	10.83
2	0.10	0.21	0.45	0.71	1.39	2.41	3.22	4.60	5.99	9.21	13.82
3	0.35	0.58	1.01	1.42	2.37	3.66	4.64	6.25	7.82	11.34	16.27
4	0.71	1.06	1.65	2.20	3.36	4.88	5.99	7.78	9.49	13.28	18.47
5	1.14	1.61	2.34	3.00	4.35	6.06	7.29	9.24	11.07	15.09	20.52
6	1.63	2.20	3.07	3.83	5.35	7.23	8.56	10.64	12.59	16.81	22.46
7	2.17	2.83	3.82	4.67	6.35	8.38	9.80	12.02	14.07	18.48	24.32
8	2.73	3.49	4.59	5.53	7.34	9.52	11.03	13.36	15.51	20.09	26.12
9	3.32	4.17	5.38	6.39	8.34	10.66	12.24	14.68	16.92	21.67	27.88
10	3.94	4.86	6.18	7.27	9.34	11.78	13.44	15.99	18.31	23.21	29.59

Nonsignificant Significant

Chi Square Analysis

1-5 Infected vs. Uninfected				
Temp	Chi Square	df	P Value	Significant
38	3516.162	1	0.0001	Yes
39	81	1	0.0001	Yes
40	308.717	1	0.0001	Yes
41	239.413	1	0.0001	Yes
42	263.014	1	0.0001	Yes
43	1533.511	1	0.0001	Yes





Chi Square Analysis

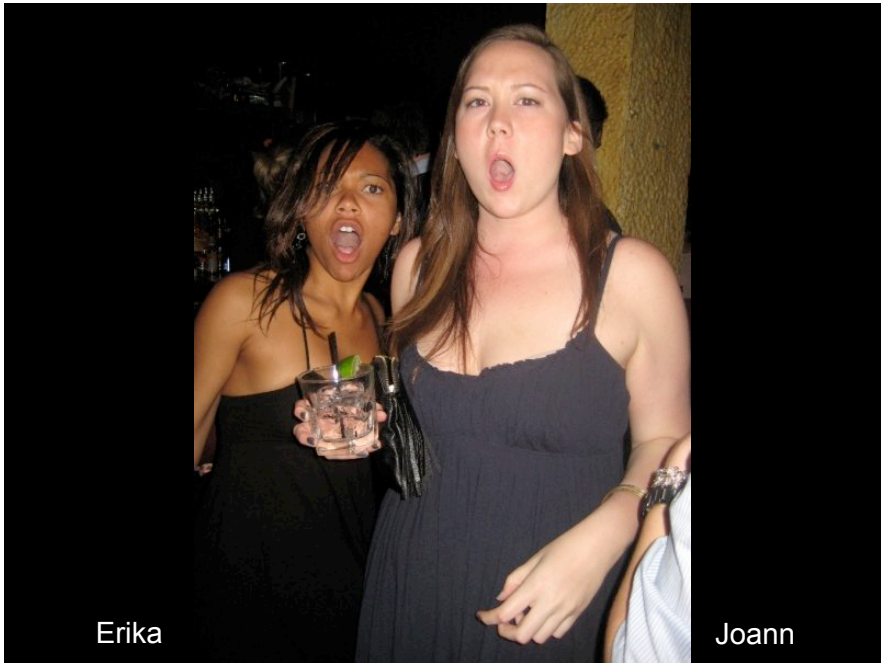
>5 Infected vs. Uninfected				
Temp	Chi Square	df	P Value	Significant
36	64.646	1	0.0001	Yes
37	581.818	1	0.0001	Yes
38	1034.343	1	0.0001	Yes
39	18.063	1	0.0001	Yes
40	347.271	1	0.0001	Yes
41	246.613	1	0.0001	Yes
42	34.297	1	0.0001	Yes
43	4.433	1	0.0353	Yes



- Because it is difficult to regulate the change in temperature when using water, the sample size for each temperature was not equal. This may have affected the overall results.
- If a future study was conducted, it would be recommended to somehow regulate the temperature more closely so that the sample sizes are equal.
- Also, categories in which 100% died or lived could not be chi squared because it would put 0% in the expected column, which is impossible to calculate.

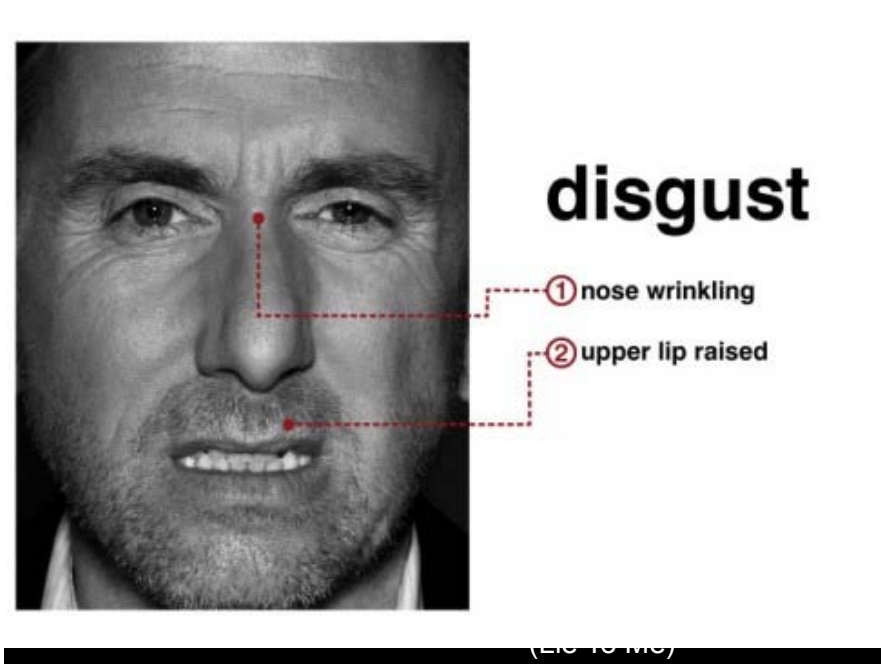
Purcell, J.E. et al. "The ctenophore *Mnemiopsis* in native and exotic habitats U.S. versus the Black Sea basin."
Hydrobiologia 451(2001): 145–176.





Erika

Joann

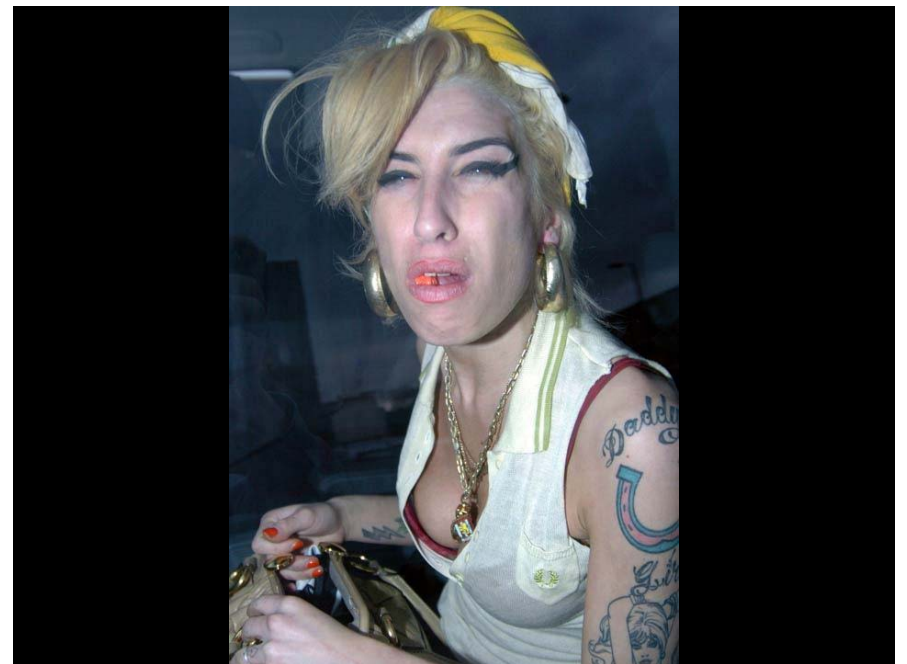


disgust

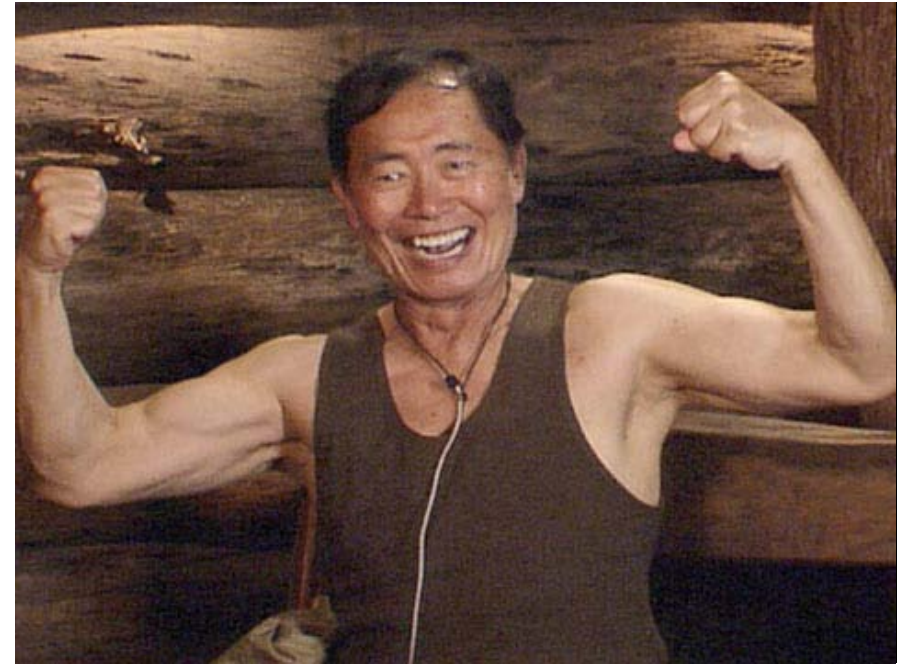
① nose wrinkling

② upper lip raised

(LIS TO ME)

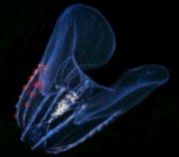






Inspired by John Finnerty

Background



- *Mnemiopsis leidyi* have been seen to regenerate lost parts in 8 -12 days (Coonfield, B. R. 1937)
- When wounded 3mm wide by 3mm *M. leidyi* can heal virtually scar-less in 30 minutes (Dobson, Matt. Moss, Anthony 2000)
- Wound Closure in 5 hours, completed in 18, ctenes regenerated in 20. (Coonfield, B. R. 1937)
- Wound healing is important for *M. leidyi* escape because they can usually get damaged or lose a body part (which they regenerate with in a few days) (Kreps, Purcell, Heidelberg 1997) within

awkward wording...how about "they incur frequent injuries, including the loss of a body part"

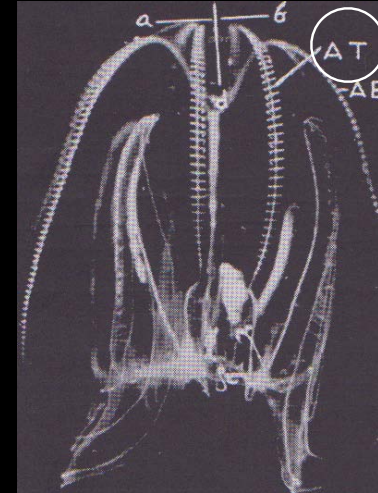
Methods

- 1) Ctenophores were measured and their parasites were counted Information about ctenophore size?
- 2) They were placed individually into petri dishes
- 3) A cut was made in their aboral end that cut through two ctene rows and the intermediate jelly
- 4) Their petri dishes were filled with a salt water solution
- 5) They were observed every hour under a microscope
- 6) Observations were accompanied by a water change
- 7) They were fed 5 hours after being cut

"salt water solution"--be more specific.
Artificial sea water / salinity.



Wound Cut (AT)



B.R. Coonfield

Experimental Set Up



It's generally a good idea to edit out extraneous objects from the background (e.g., scalpel wrapper).

Wound healing stages

- There was no set progression for the healing of a wound
- Wounds did not always heal

Can you give examples of progressions you did observe--i.e., give an idea of what is variable and what doesn't vary from case to case. Presumably, fusion of ctene rows would never precede wound closure.



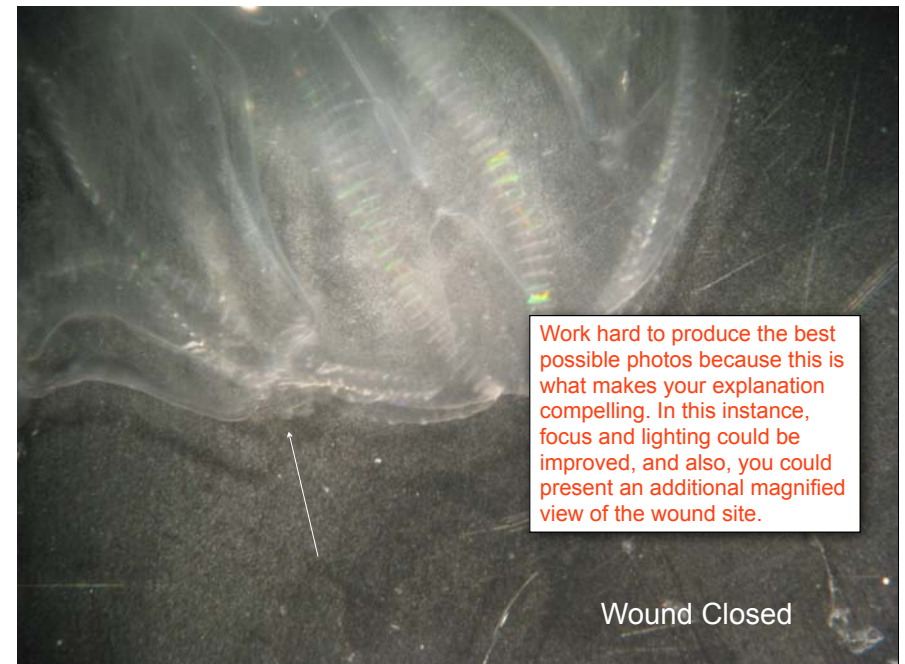
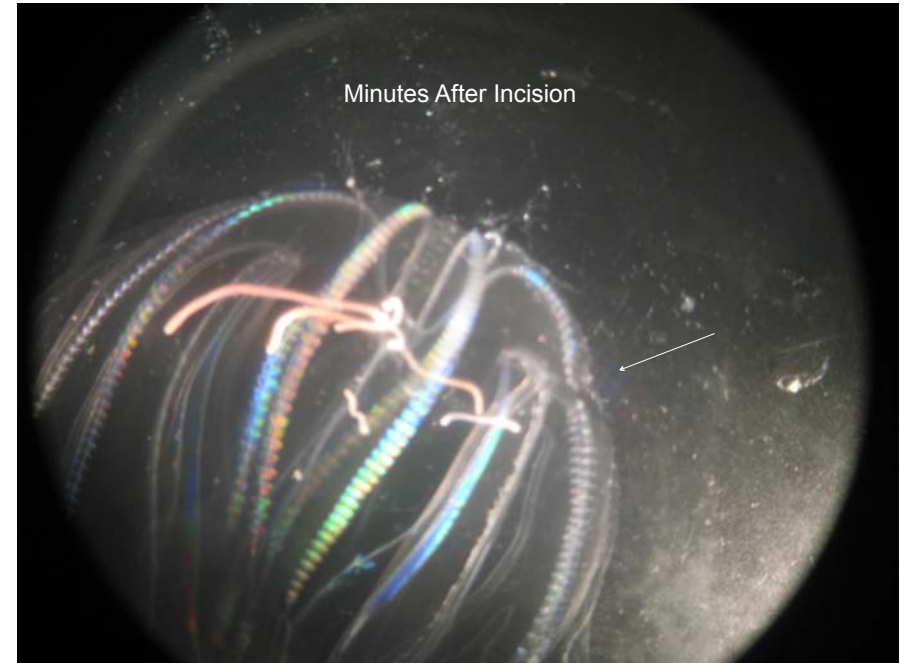
How about the title: "Recognizable events during wound healing"

Wound stages

- Event 1: No oozing
- Event 2: Wound closed
- Event 3: One ctene row closed
- Event 4: Both ctene rows closed
- Event 5: One ctene row synced
- Event 6: Both ctene rows synced (healed)
- Event 7: Wound Spread
- Event 8: Ctenes healed around spread wound (healed)
- Event 9: Ctenophore died



The numbering makes it seem like they are stages. Also, it may have been less confusing if you listed the events for successful healing separately from failed healing, so it would be clear that 7 doesn't occur after 6.






Outcomes

- There were three outcomes that could occur from cutting the *Mnemiopsis leidyi*
 - 1) Normal healing: The wound closed and the ctenes rows healed over in and synced
 - 2) Abnormal healing: The wound spread and the ctenes rows healed around each half
 - 3) Death

It's hard to visualize #2. Perhaps a diagram would have made this more clear.



This slide discusses the three possible outcomes of cutting a Mnemiopsis leidyi. It lists normal healing, abnormal healing, and death. A red text box notes that the abnormal healing outcome is difficult to visualize and suggests a diagram. An illustration of a Mnemiopsis leidyi is shown at the bottom right, with a red line indicating the location of a cut.



Chi Square Test Results for 1-5 Parasites Death

Chi Square:	1.071
Percentage:	0.3006
Significance:	Insignificant

Chi Square Test Results for >5 Parasites Death

Chi Square:	26.786
Percentage:	<0.0001
Significance:	Significant

Chi Square Test Results for Abnormal Healing of 1-5 Parasites Vs >5 Parasites

Chi Square:	4.286
Percentage:	0.0384
Significance:	Significant

It's not correct to use the uninfected values as the expected values, because you're testing whether the infected and uninfected have different rates of healing.

Chi Square Test Results for 1-5 Parasites Normally Healed

Chi Square:	4.286
Percentage:	0.0384
Significance:	Significant

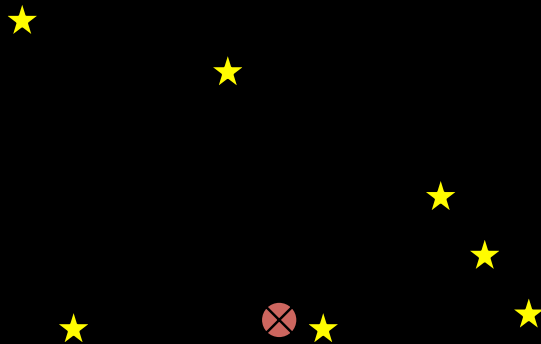
Chi Square Test Results for >5 Parasites Normally Healed

Chi Square:	52.5
Percentage:	<0.0001
Significance:	Significant

Does "Percentage" mean P-value?

Healing

Three of the next four slides didn't reproduce on my electronic copy of the powerpoint file.



Amount Healed Normally

Time past (hours)



Chi Square Test Results for 1-5 Parasites (one degree of freedom)

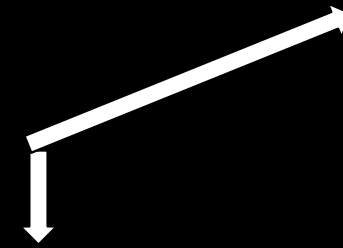
	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours	9 hours
Chi Square Value	2.308	2.308	13.125	19.2	21.818	9.643	4.286
P Value	0.1287	0.1287	0.0003	<0.0001	<0.0001	0.0019	0.0384
Significance	Insignificant	Insignificant	Significant	Significant	Significant	Significant	Significant

As a slide title, use the question you're asking (e.g., "Did Parasitized Ctenophores Fully Heal as Often as Unparasitized?"), not "Chi Square Test."

Chi Square Test Results for >5 Parasites (one degree of freedom)

	3 hours	4 hours	5 hours	6 hours	7 hours	8 hours	9 hours
Chi Square Value	2.308	2.308	13.125	24.3	34.091	129.643	52.5
P Value	0.1287	0.1287	0.0003	<0.0001	<0.0001	<0.0001	<0.0001
Significance	Insignificant	Insignificant	Significant	Significant	Significant	Significant	Significant

Amount Healed Normally



Time past (hours)

Discussion



- Normal healing occurred sooner in uninfected ctenophores.
- More uninfected Mnemiopsis healed normally than infected ones.
- More Mnemiopsis infected with 1-5 parasites healed more quickly than those infected with >5 parasites.
- Fewer uninfected Mnemiopsis died than infected.
- Fewer Mnemiopsis infected with 1-5 parasites died than those with >5 parasites.
- No abnormal healing occurred in uninfected Mnemiopsis.
- More abnormal healing occurred in those infected with >5 parasites than those infected with 1-5.

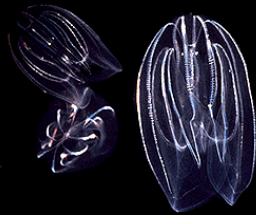
Errors and Future Studies



- Mnemiopsis wounds not identical.
- Mobile desk possibly led to deaths.
- Water went from cool aquarium temp (18) to room temp (21) every hour.
- Size variation led to variation in mobility in the petri dishes.
- Future studies could include puncture wound recovery, ctenes row regeneration, and apical organ regeneration.

Conclusion

Uninfected Mnemiopsis healed more quickly and more completely than those with infections. Those with more than 5 infections did not heal as well as those with 1-5 infections.

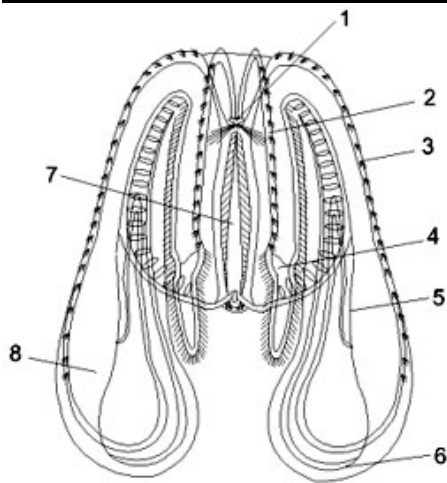


Redundant with 2 slides earlier.

Effects of *E. lineata* parasite on *M. leidyi* fecundity



Effects of *E. lineata* parasite on *M. leidyi* fecundity



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<http://www.caspianenvironment.org/mnemiopsis/mnemenu5.htm>

<http://jellynews.blogspot.com/2009/08/internal-self-fecundation-in-pelagic.html>

Effects of *E. lineata* on *M. leidyi* fecundity

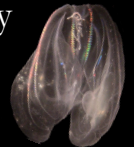
Background

– Bumann and Puls study (1996)

- Growth rates of non-infested animals were greater than infested animals
- Egg production over 1 day was not significantly different for infested and non-infested
- Use 1 day of data to extrapolate a weeks data to conclude the infested population produced fewer and fewer eggs than non-infested

Project Objectives

- Spawn ctenophores for several days in order to determine if indeed parasites cause lower fecundity of their hosts
- Fecundity is the most direct measure of fitness, directly measure fitness effects of *E. lineata* on host



Effects of *E. lineata* on *M. leidy* fecundity

Methods

Published protocols:

-Baker and Reeve 1974, Bumann and Puls 1996, Martindale 1987, Pang and Martindale 2008, Strathmann 1987

- Dark-light cycle to induce spawning
- Fresh caught animals
- Feed/change water 1 to 3 times daily



Effects of *E. lineata* on *M. leidy* fecundity

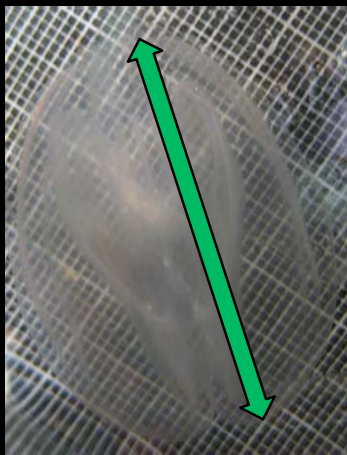
Methods

Experiment: **9 trials**

- Conditions: 10 *M. leidy* uninfected, 10 infected
 - 250 ml salt water in 266 ml container
 - Fluorescent light with timer (Pang and Martindale 2008)
 - Water changed at least 2 times a day
 - Feed *Artemia*, failed collection of plankton from field
 - Plan to measure before and after spawning
 - Plan to use aid of filters/ dissecting scope to count eggs

Important methodological point: did you infect the ctenophores yourself? Did Bumann & Puls? What are the advantages/ disadvantages of using field caught parasitized animals versus animals you parasitized yourself?

Effects of *E. lineata* on *M. leidy* fecundity



Effects of *E. lineata* on *M. leidy* fecundity

Methods

- Variations/alterations during experiment
 - Dark-time length
 - Temperature range: room temperature to 13 degrees C (Pang and Martindale 2008)
 - Old specimens to fresh (tried new specimens upon each collection day)
 - Addition of estradiol hormone to 5 infected, 5 uninfected, (Tarant 2005)



Effects of *E. lineata* on *M. leidy* fecundity



Results...

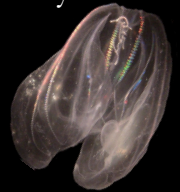
- Eggs should be abundant, visible to naked eye (200 μ m in diameter) (Pang and Martindale 2008), sink to bottom of container (Pang and Martindale 2008), form fine haze of tiny particles (Baker and Reeve 1974)



Spawning!

Effects of *E. lineata* on *M. leidy* fecundity

Discussion



-Possible reasons for lack of spawning

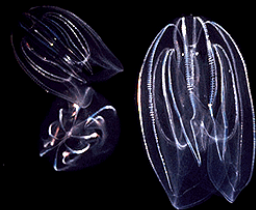
- Lab conditions
 - *Artemia* insufficient? (Martindale 1987)
 - Too much temperature fluctuation? (Pang and Martindale 2008)
- Seasonality of reproduction?
 - *Mnemiopsis* most abundant in Woods Hole in late summer and early fall (Reitzel et al 2007)
 - Indicative of recent high levels of reproduction?
 - What seasonal/physiological cues responsible for decreased ability to spawn
 - Dumann and Puls 1996 study, successful spawning of September-caught specimens

Effects of *E. lineata* on *M. leidy* fecundity

Discussion

• Future Studies

- Lab conditions
- Seasonal differences
- Hormone studies
 - Estrogens leeching into marine environments from sewage (Atkinson et al 2003)
 - Possible that environmental chemicals may disrupt gametogenesis and spawning in cnidarians (Tarrant 2007)
 - Increase in ovarian content/oocyte growth upon estradiol injection in echinoderms (Roepke et al 2005)



Group conclusions

Salinity:

- Although there was some evidence that *E. lineata* had an adverse effect on *M. leidy*, there was also some evidence to suggest the opposite.
- Overall, results are inconclusive

Temperature:

- The fact that there was no difference between the two different parasitic groups disagrees with the hypothesis which is based on the fact that the number of parasites is indirectly proportional to the fitness of the ctenophore.

Wound Healing:

- Uninfected *Mnemiopsis* healed more quickly and more completely than those with infections.
- Those with more than 5 infections did not heal as well as those with 1-5 infections.

Fecundity: inconclusive

- In order to determine most direct effect of *E. lineata* on fitness, spawning experiments must be implemented
- Understanding of spawning/reproduction may have yield knowledge of blooms/invasions, help with biological controls

General Conclusion

There is only evidence to support the hypothesis in relation to wound healing. *M. leidy* with more *E. lineata* parasites healed more slowly and less completely. The other tests were inconclusive and therefore beget further study to determine the complete effect of *E. lineata* on its host *M. leidy*.

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