Should use italics for genus/species names throughout talk.

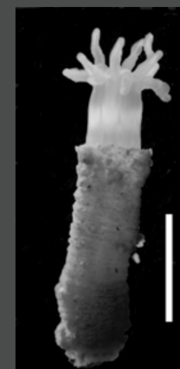
THE EFFECTS OF SALINITY ON THE METAMORPHOSIS AND REGENERATION RATES OF EDWARDSIELLA LINEATA AND NEMATOSTELLA VECTENSIS

A presentation by Brianne Cuffe

Introduction

- Like Brother and Sister:

  Edwardsiidae Family



Edwardsiella lineata

Nematostella vectensis

Eukaryota > Metazoa > Eumetazoa >Cnidaria > Anthozoa > Hexacorallia > Actiniaria > Edwardsiidae

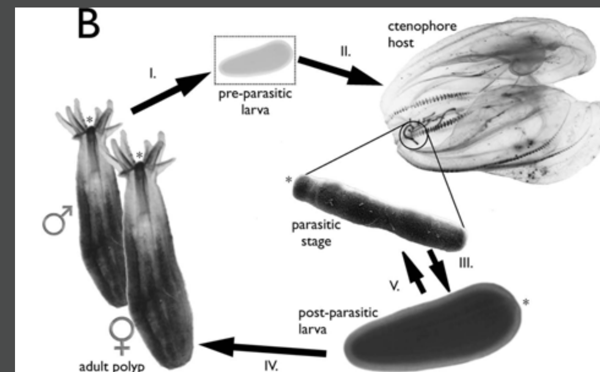
- Edwardsiidae
  - Edwardsia
    - Edwardsia andresi
    - Edwardsia elegans
    - Edwardsia gilbertensis
    - Edwardsia sipunculoides
    - Edwardsia sp.
    - Edwardsia tuberculata
  - Edwardsianthus
    - Edwardsianthus gilbertensis
  - Edwardsiella
    - Edwardsiella lineata
  - Nematostella
    - environmental samples
      - uncultured Nematostella sp.
    - Nematostella sp. JVK-2006
    - Nematostella vectensis (starlet sea anemone)
  - unclassified Edwardsiidae
    - Edwardsiidae sp. BAR
    - Edwardsiidae sp. CUR

- Edwardsiella and Nematostella may be part of the same clade according to Daly's 2002 combined analysis.
- The two species are often used in comparisons due to their close phylogenetic relationship and similar body plan (Reitzel et al 2006).

Differences

Metamorphosis

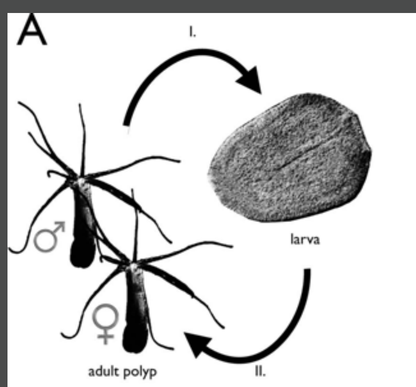
- Edwardsiella lineata:



Picture provided by Reitzel et al 2009

Metamorphosis

- Nematostella vectensis:



Picture provided by Reitzel et al 2009

Living Arrangements

- Edwardsiella lineata:

Woods Hole Mass

Shallow Ocean Water 30‰-50‰

- Edwardsiella lives as a parasite within the ctenophore Mnemiopsis.
- Mnemiopsis lives in habitats ranging from 3‰ to 38‰ (Dumont and Shiganova).
- Edwardsiella should therefore be best adapted to 30‰ to 38‰

Living Arrangements

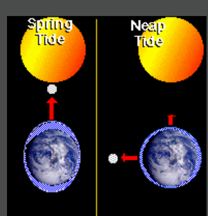
- Nematostella vectensis:

Sippewissett Mass

Salt Marsh
15‰-30‰

Nematostella has been observed in waters ranging from 8‰ to 38‰. (Inouye, S. 1976)

- Salinity readily changes in estuarine habitats due to fluctuations in freshwater and saltwater inputs.
  - Freshwater inputs:
    - Groundwater
    - Rainfall
  - Saltwater inputs:
    - Changing tides



Mention evaporation.

- Nematostella should be better adapted to varying salinities due to these fluctuations.
- Edwardsiella at all stages of life should be better adapted to salinities in the 30s.

Might point out some of the ramifications, e.g, if Edwardsiella is not tolerant of low salinities, it may not be able to tolerate the same range of salinities as its host ctenophore.

Research Objectives

- Investigate the salinity tolerance of Edwardsiella lineata and Nematostella vectensis.
- Investigate the effect of salinity on the metamorphosis of Edwardsiella lineata.

Methods

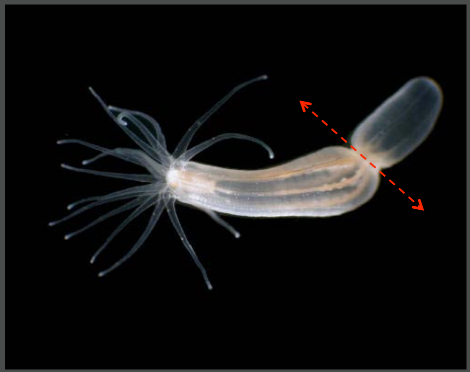
- Animal Collection:
  - Parasites were harvested from the ctenophore Mnemiopsis leidyi.
  - These ctenophores were collected at Woods Hole, Massachusetts by gently scooping in nets and carrying in buckets to be placed in the BUMP aquarium.
  - Nemastostella were collected from Sippewisset, Massachusetts by collecting and sifting through the mud.
  - Edwardsiella at all stages originated from parasites in Mnemiopsis collected at Woods Hole.

- Regeneration Rate:
  - Solutions: 1‰, 11‰, 21‰, 31‰, 41‰, 51‰
  - 5 Nematostella vectensis at each salinity
  - 3 Edwardsiella lineata at each salinity (minus 21‰)
  - Sea anemones were brought up or down step-wise to the appropriate salinity with steps at the 1s and 6s and an hour to adjust at each step.
  - 31‰ was used as the control for Edwardsiella.
  - 21‰ was used as the control for Nematostella.

Unclear. What are the abbreviations "1s" and "6s?"

Diagram showing salinity acclimation scheme would be very useful here.

- Regeneration Rate:
  - Foot of each organism was cut off



- Regeneration Rate:
  - Pictures were taken under an Olympus SZX9 microscope coupled with a Cannon Power Shot S51S camera against a 1mm^2 grid.
  - Size was measured in terms of 2-dimesional area using Motic Images Plus.
  - Sea anemones were feed every other day with artemia.
  - The water was changed to clean water of the appropriate salinity every day.

- Metamorphosis of Edwardsiella lineata:
  - Parasite→Planula
    - Solutions: 1‰, 11‰, 21‰, 31‰, 41‰, 51‰
    - Cut parasites out of ctenophores
    - Parasites were brought up or down step-wise to the appropriate salinity with steps at the 1s and 6s and an hour to adjust at each step.
    - 5 parasites at each salinity
    - The date of conversion was observed.
    - The water was changed every day to clean water of the appropriate salinity.

Perhaps remind viewer of the parasite->planula->polyp metamorphosis here?

How did you define conversion? How do we recognize parasite from planula?

- Metamorphosis of Edwardsiella lineata
  - planula→polyp
    - Solutions: 1‰, 11‰, 21‰, 31‰, 41‰, 51‰
    - Cut parasites out of ctenophores
    - All parasites were placed in 31‰ salinity.
    - Waited till they became planula.
    - Planula were brought up or down step-wise to the appropriate salinity with steps at the 1s and 6s and an hour to adjust at each step.
    - 5 planula at each salinity
    - The date of conversion was observed.
    - The water was changed every day to clean water of the appropriate salinity.

How did you define conversion? How do we recognize planula from polyp?

- Metamorphosis of Edwardsiella lineata
  - planula→parasite in presence of ctenophore
    - Solutions: 1‰, 11‰, 21‰, 31‰, 41‰, 51‰
    - Cut parasites out of ctenophores
    - All parasites were placed in 31‰ salinity.
    - Waited till they became planula.
    - Planula and uninfected ctenophores were brought up or down step-wise in separate containers to the appropriate salinity with steps at the 1s and 6s and 1hour to adjust at each step.
    - 5 planula and uninfected ctenophores at each salinity
    - 1 planula was paired with 1 uninfected ctenophore
    - Observations were made every 10minutes and time of infection for each pair was noted.

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Unclear. What are the abbreviations “1s” and “6s?”

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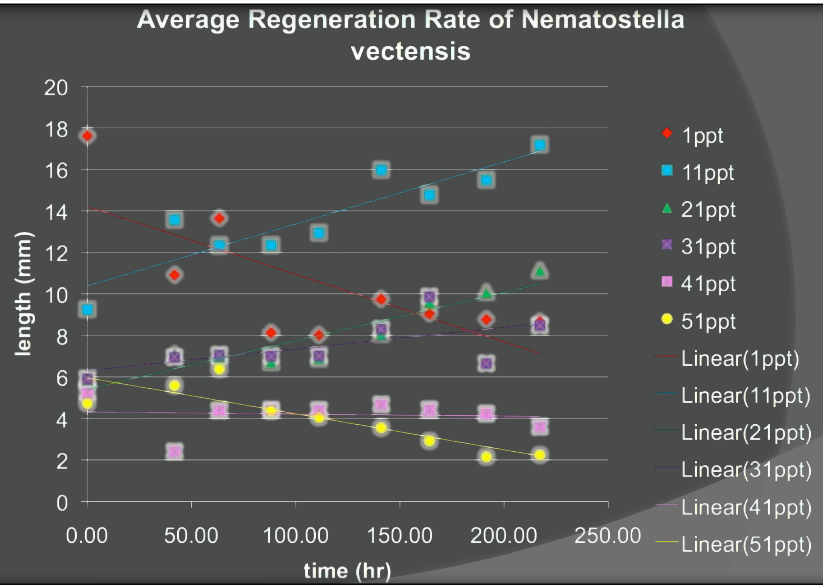
Results

This is more accurately described as “growth rate of regenerating physal fragment”



You can remove the “Linear(##ppt)”s from the legend. This is self-evident.

Regression Statistics	1‰
Multiple R	0.735163
R Square	0.540465
Adjusted R Square	0.474817
Standard Error	2.313933
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	44.08067	44.08067	8.232779	0.024017
Residual	7	37.48001	5.354287		
Total	8	81.56068			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	14.2076	1.501145	9.46451	3.07E-05	10.65796	17.75725	10.65796	17.75725
X Variable 1	-0.03266	0.011382	-2.86928	0.024017	-0.05957	-0.00574	-0.05957	-0.00574

Instead of presenting the table, I would show the 1% regression alone, and then show the correlation coefficient and the P-value. The same goes for each of the following graphs & associated tables. Also, remind your audience what the regression is testing: whether the animal grew, or shrunk, or stayed the same (whether the slope of the line relating body size to time is significantly positive or negative)

I think it would have been helpful to have a slide summarizing the results, e.g., “At the following salinities, Nematostella exhibited significant growth...at the following salinities, Nemaostella exhibited significant shrinkage...etc.”

Regression Statistics 11‰	
Multiple R	0.899306
R Square	0.808751
Adjusted R Square	0.781429
Standard Error	1.116906
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	36.92716	36.92716	29.60143	0.000966
Residual	7	8.732353	1.247479		
Total	8	45.65951			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	10.3865	0.724583	14.33445	1.91E-06	8.673137	12.09987	8.673137	12.09987
X Variable 1	0.02989	0.005494	5.440719	0.000966	0.016899	0.042881	0.016899	0.042881

Regression Statistics 21‰	
Multiple R	0.929528
R Square	0.864022
Adjusted R Square	0.844596
Standard Error	0.711036
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	22.48725	22.48725	44.47881	0.000285
Residual	7	3.539005	0.505572		
Total	8	26.02625			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	5.409737	0.461279	11.7277	7.42E-06	4.318987	6.500488	4.318987	6.500488
X Variable 1	0.023325	0.003497	6.669244	0.000285	0.015055	0.031595	0.015055	0.031595

Regression Statistics 31‰	
Multiple R	0.629215
R Square	0.395911
Adjusted R Square	0.309613
Standard Error	0.992549
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	4.519588	4.519588	4.587699	0.06944
Residual	7	6.896075	0.985154		
Total	8	11.41566			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	6.291552	0.643908	9.770887	2.49E-05	4.768952	7.814152	4.768952	7.814152
X Variable 1	0.010457	0.004882	2.141891	0.06944	-0.00109	0.022001	-0.00109	0.022001

Regression Statistics 41‰	
Multiple R	0.089647
R Square	0.008037
Adjusted R Square	-0.13367
Standard Error	0.84868
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.040847	0.040847	0.056712	0.818591
Residual	7	5.041799	0.720257		
Total	8	5.082646			

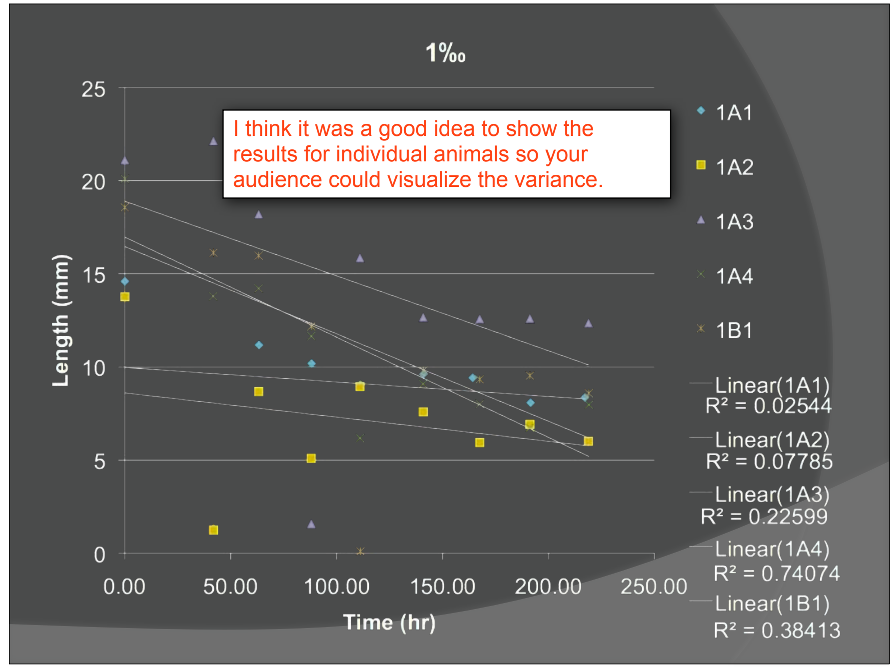
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.310382	0.550574	7.82889	0.000105	3.008482	5.612282	3.008482	5.612282
X Variable 1	-0.00099	0.004174	-0.23814	0.818591	-0.01087	0.008877	-0.01087	0.008877

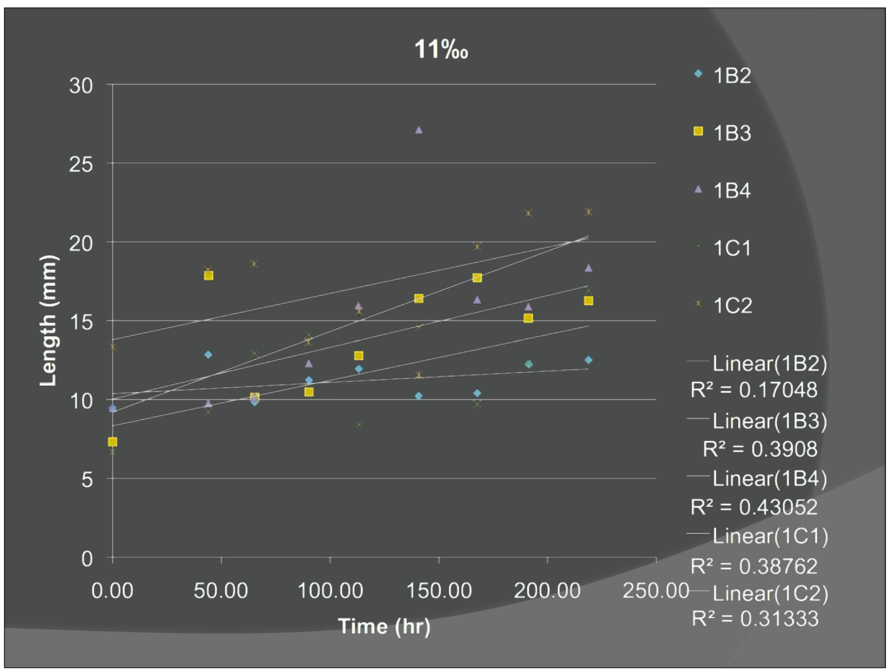
Regression Statistics 51‰	
Multiple R	0.864139
R Square	0.746736
Adjusted R Square	0.710556
Standard Error	0.777879
Observations	9

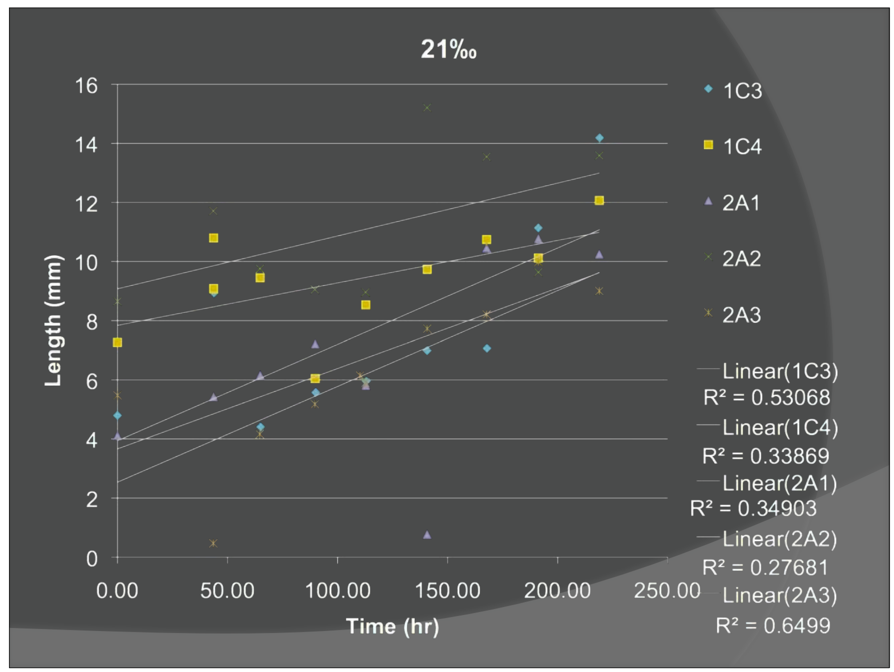
ANOVA

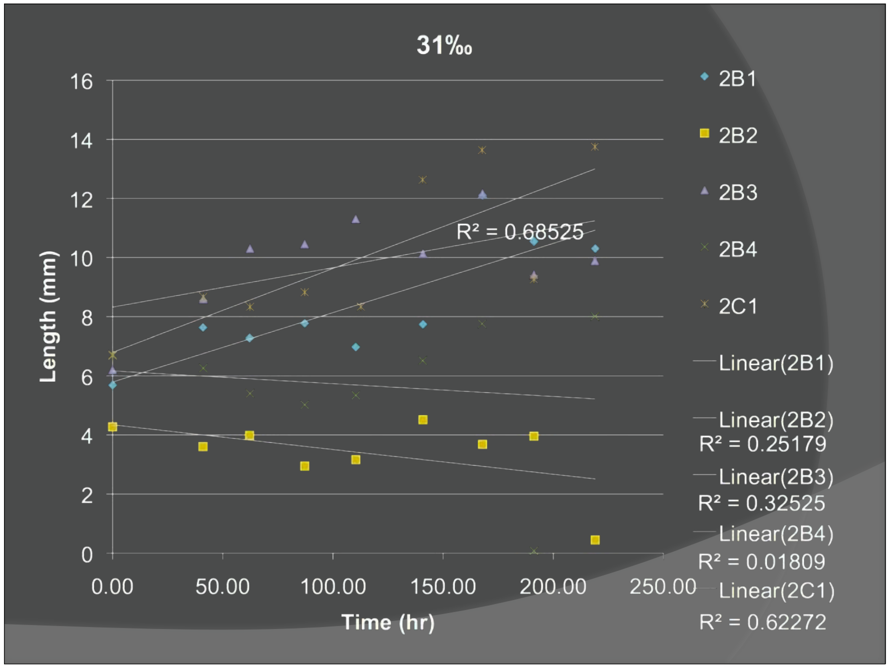
	df	SS	MS	F	Significance F
Regression	1	12.48869	12.48869	20.63919	0.002658
Residual	7	4.235671	0.605096		
Total	8	16.72436			

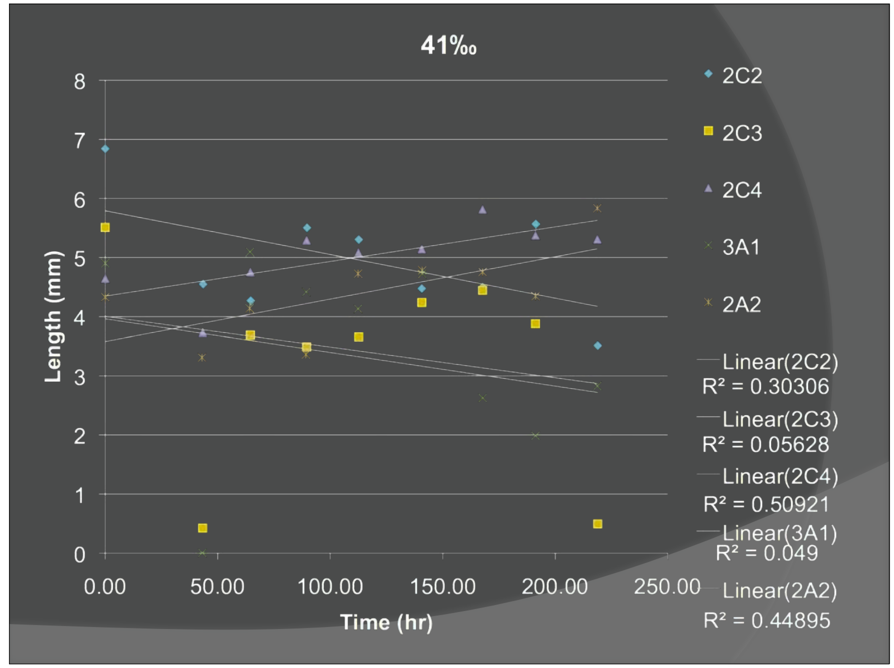
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	5.962831	0.504643	11.81595	7.05E-06	4.769541	7.156121	4.769541	7.156121
X Variable 1	-0.01738	0.003826	-4.54304	0.002658	-0.02643	-0.00834	-0.02643	-0.00834

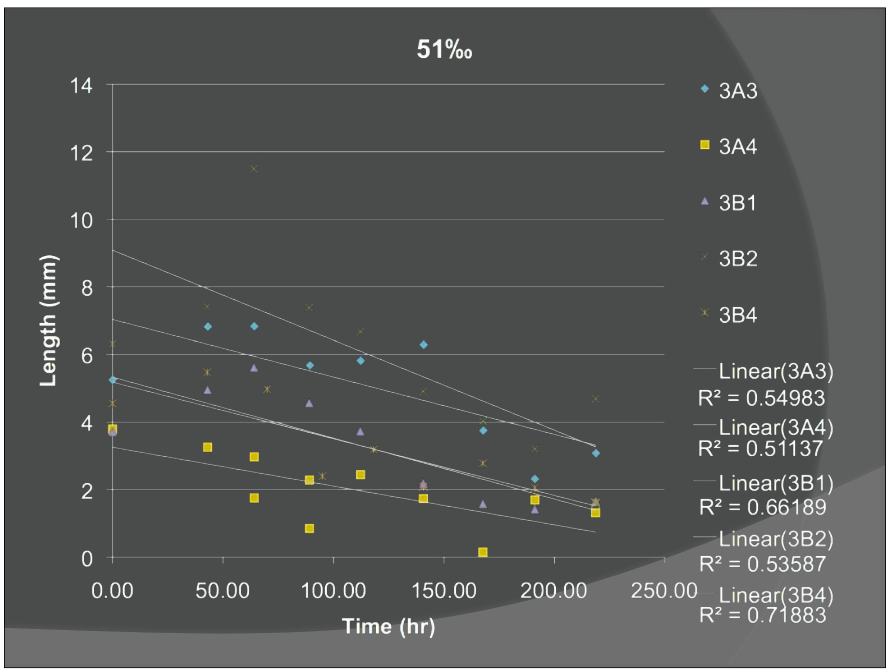


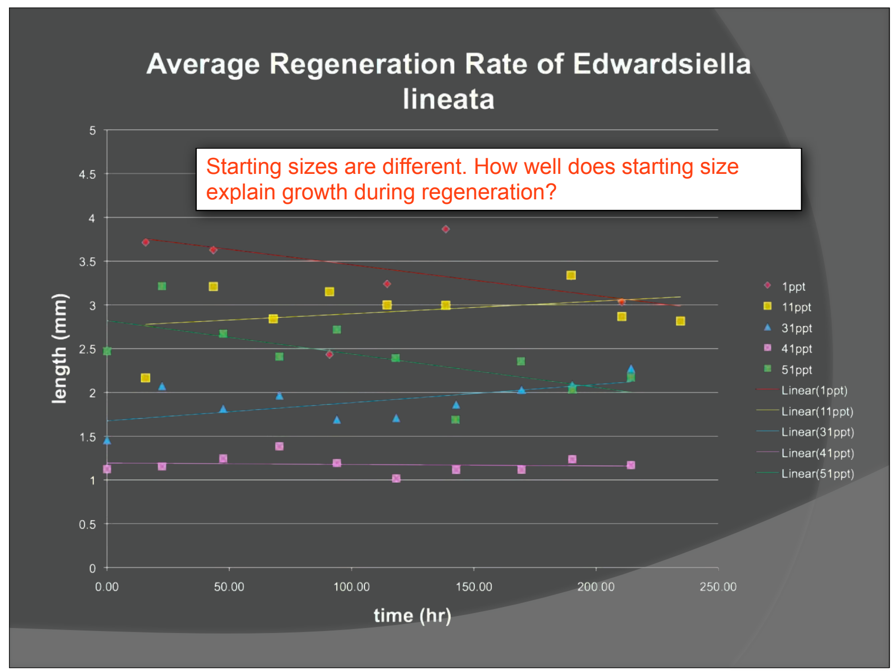





31‰
Length (mm)
Time (hr)
R² = 0.68525
2B1
2B2
2B3
2B4
2C1
Linear(2B1)
Linear(2B2)
R² = 0.25179
Linear(2B3)
R² = 0.32525
Linear(2B4)
R² = 0.01809
Linear(2C1)
R² = 0.62272


41‰
Length (mm)
Time (hr)
2C2
2C3
2C4
3A1
2A2
Linear(2C2)
R² = 0.30306
Linear(2C3)
R² = 0.05628
Linear(2C4)
R² = 0.50921
Linear(3A1)
R² = 0.049
Linear(2A2)
R² = 0.44895


51‰
Length (mm)
Time (hr)
3A3
3A4
3B1
3B2
3B4
Linear(3A3)
R² = 0.54983
Linear(3A4)
R² = 0.51137
Linear(3B1)
R² = 0.66189
Linear(3B2)
R² = 0.53587
Linear(3B4)
R² = 0.71883


Average Regeneration Rate of Edwardsiella lineata
Starting sizes are different. How well does starting size explain growth during regeneration?
length (mm)
time (hr)
1ppt
11ppt
31ppt
41ppt
51ppt
Linear(1ppt)
Linear(11ppt)
Linear(31ppt)
Linear(41ppt)
Linear(51ppt)

Regression Statistics 1‰	
Multiple R	0.466894
R Square	0.21799
Adjusted R Square	0.106274
Standard Error	0.544905
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.579381	0.579381	1.951292	0.205136
Residual	7	2.078453	0.296922		
Total	8	2.657834			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	3.809841	0.359097	10.6095	1.45E-05	2.960711	4.658971	2.960711	4.658971
X Variable 1	-0.00352	0.002521	-1.39689	0.205136	-0.00948	0.002439	-0.00948	0.002439

Regression Statistics 11‰	
Multiple R	0.324384
R Square	0.105225
Adjusted R Square	-0.0226
Standard Error	0.341489
Observations	9

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.095997	0.095997	0.823197	0.394407
Residual	7	0.816303	0.116615		
Total	8	0.912299			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.755718	0.225044	12.24524	5.55E-06	2.223574	3.287863	2.223574	3.287863
X Variable 1	0.001433	0.00158	0.907302	0.394407	-0.0023	0.005169	-0.0023	0.005169

Regression Statistics 31‰	
Multiple R	0.632675
R Square	0.400278
Adjusted R Square	0.325313
Standard Error	0.196009
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.205142	0.205142	5.33952	0.049632
Residual	8	0.307356	0.03842		
Total	9	0.512498			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.675119	0.114534	14.62549	4.69E-07	1.411002	1.939235	1.411002	1.939235
X Variable 1	0.002081	0.000901	2.31074	0.049632	4.26E-06	0.004158	4.26E-06	0.004158

Regression Statistics 41‰	
Multiple R	0.121895
R Square	0.014858
Adjusted R Square	-0.10828
Standard Error	0.103963
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.001304	0.001304	0.12066	0.737281
Residual	8	0.086466	0.010808		
Total	9	0.087771			

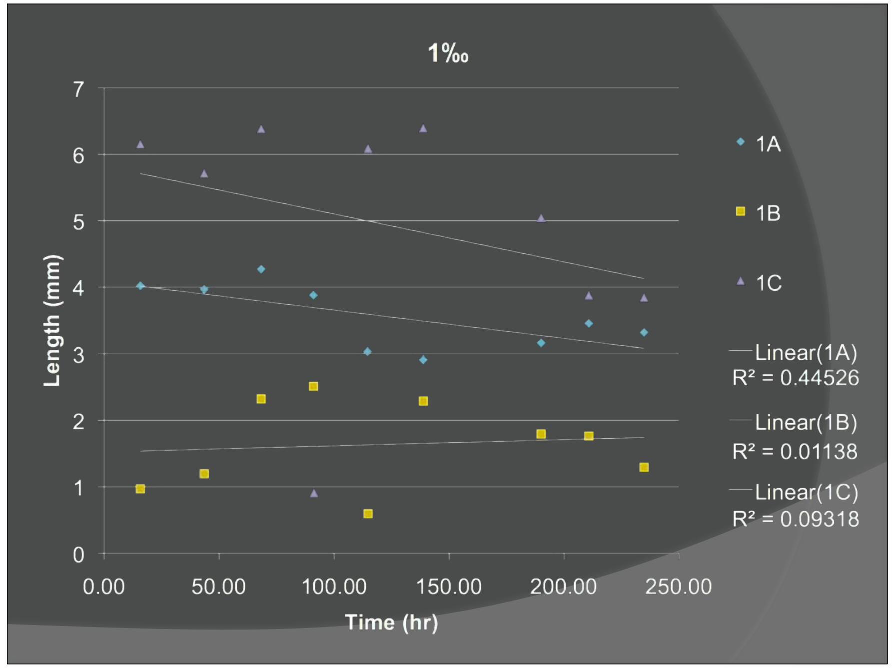
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	1.193888	0.060749	19.65285	4.67E-08	1.053801	1.333975	1.053801	1.333975
X Variable 1	-0.00017	0.000478	-0.34736	0.737281	-0.00127	0.000936	-0.00127	0.000936

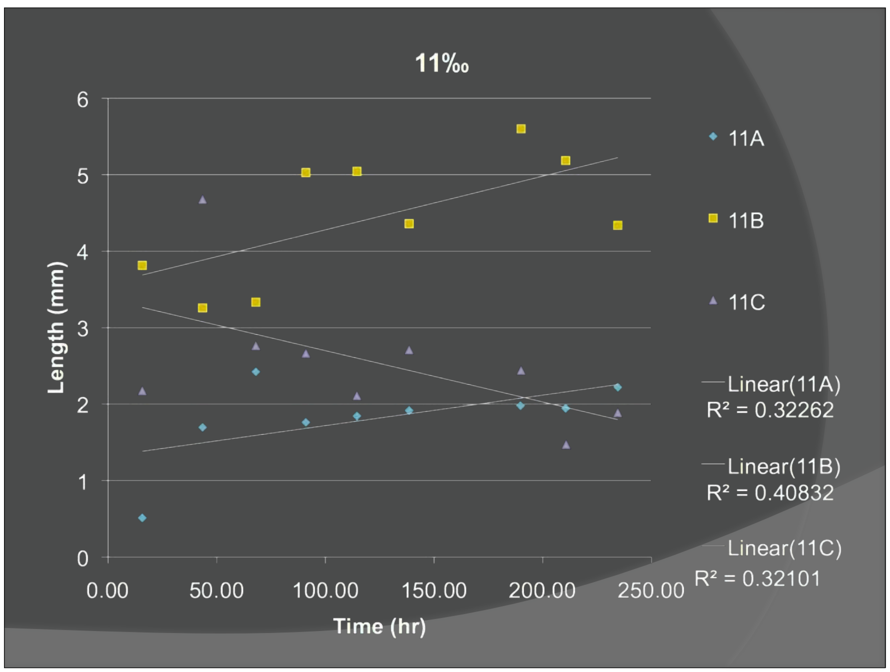
Regression Statistics 51‰	
Multiple R	0.668845
R Square	0.447353
Adjusted R Square	0.378272
Standard Error	0.32595
Observations	10

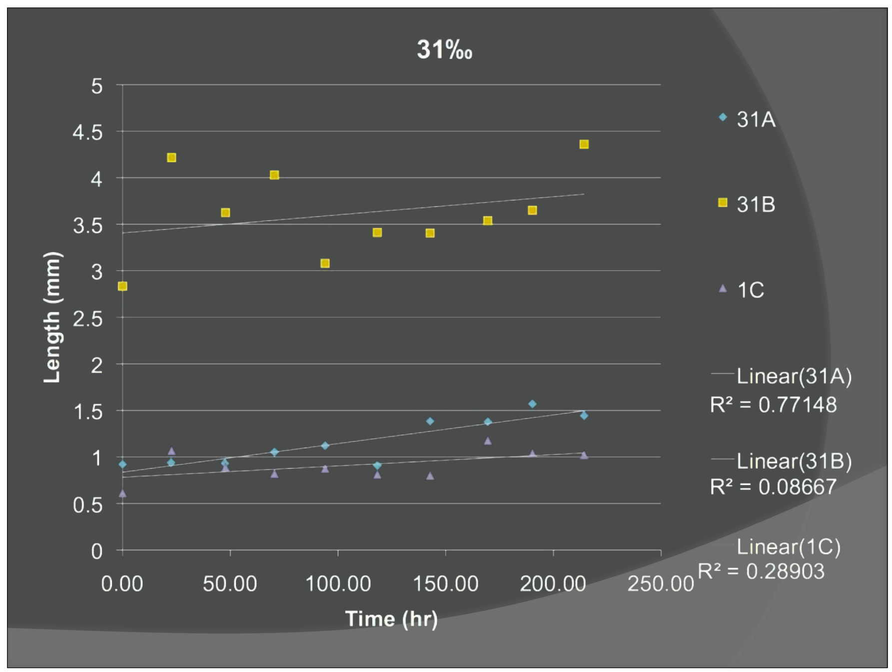
ANOVA

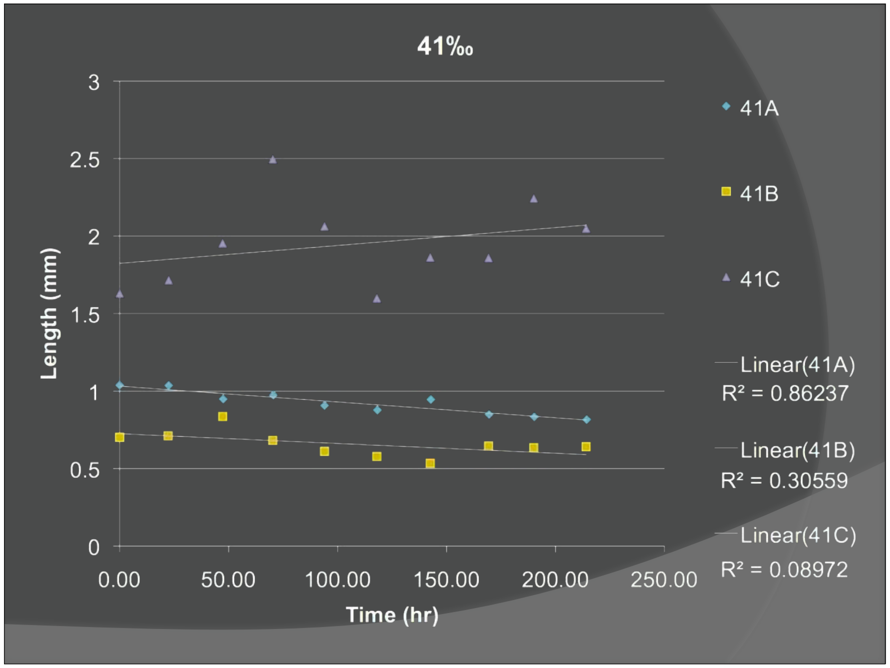
	df	SS	MS	F	Significance F
Regression	1	0.688011	0.688011	6.475789	0.034455
Residual	8	0.849948	0.106244		
Total	9	1.537959			

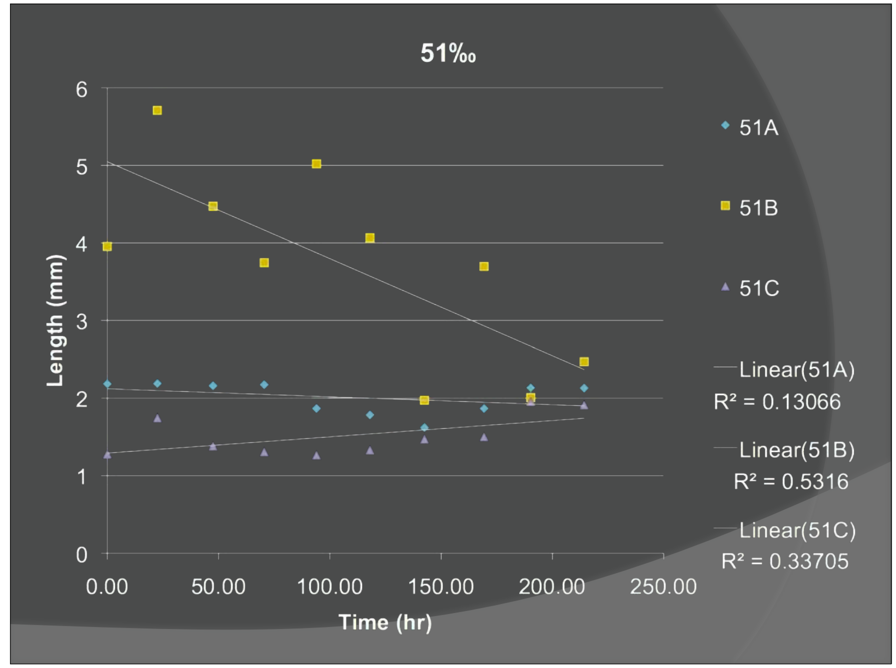
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 95.0%	Upper 95.0%
Intercept	2.820447	0.190446	14.80968	4.25E-07	2.381277	3.259617	2.381277	3.259617
X Variable 1	-0.00381	0.001498	-2.54476	0.034455	-0.00727	-0.00036	-0.00727	-0.00036

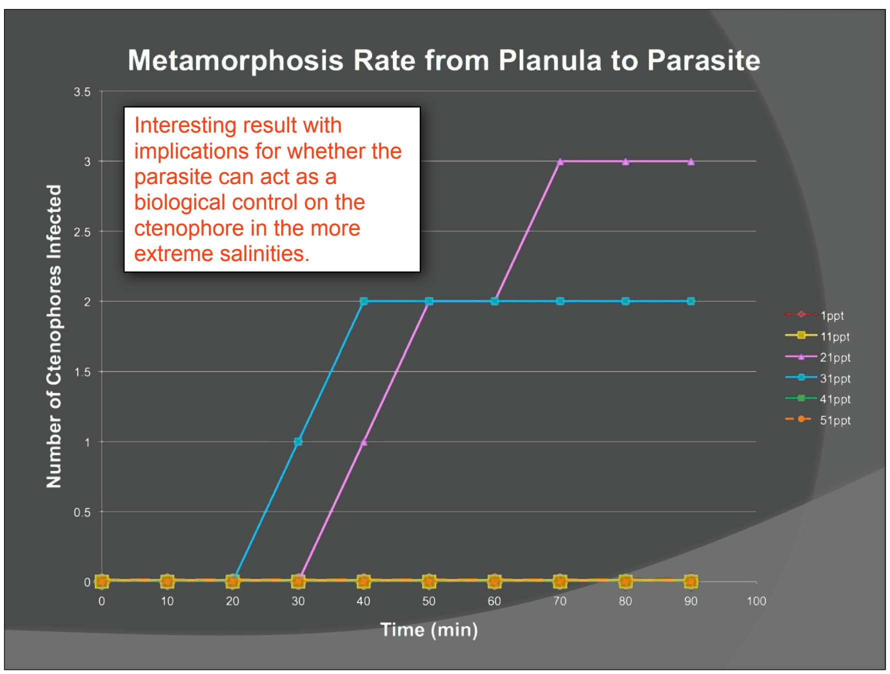


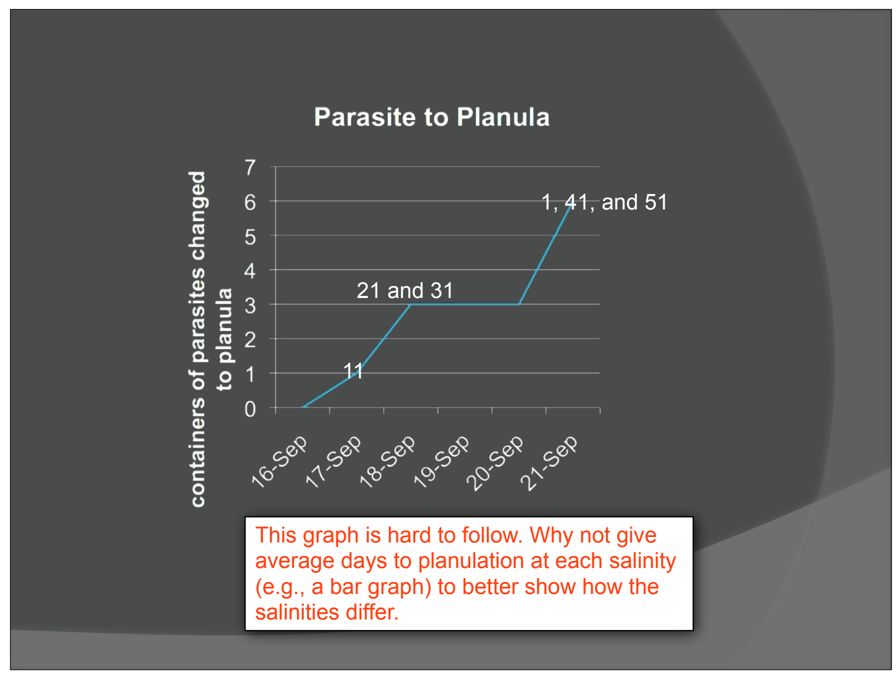


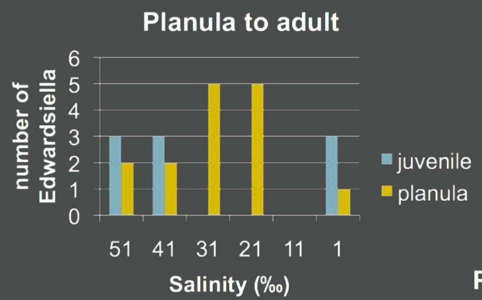



41‰
Length (mm)
Time (hr)
0
0.5
1
1.5
2
2.5
3
0.00
50.00
100.00
150.00
200.00
250.00
41A
41B
41C
Linear(41A)
R² = 0.86237
Linear(41B)
R² = 0.30559
Linear(41C)
R² = 0.08972

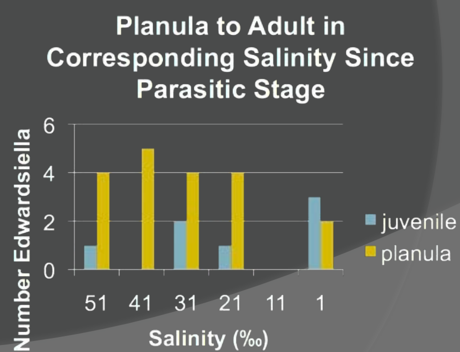

51‰
Length (mm)
Time (hr)
0
1
2
3
4
5
6
0.00
50.00
100.00
150.00
200.00
250.00
51A
51B
51C
Linear(51A)
R² = 0.13066
Linear(51B)
R² = 0.5316
Linear(51C)
R² = 0.33705


Metamorphosis Rate from Planula to Parasite
Interesting result with implications for whether the parasite can act as a biological control on the ctenophore in the more extreme salinities.
Number of Ctenophores Infected
Time (min)
0
0.5
1
1.5
2
2.5
3
3.5
0
10
20
30
40
50
60
70
80
90
100
1ppt
11ppt
21ppt
31ppt
41ppt
51ppt


Parasite to Planula
containers of parasites changed to planula
0
1
2
3
4
5
6
7
16-Sep
17-Sep
18-Sep
19-Sep
20-Sep
21-Sep
11
21 and 31
1, 41, and 51
This graph is hard to follow. Why not give average days to planulation at each salinity (e.g., a bar graph) to better show how the salinities differ.



9/22 to 9/25
3days
no changes after 9/25



What Happened to 11‰?

Conclusions

- Edwardsiella did not exhibit significant regeneration at any of the tested salinities.
- Nematostella exhibits significant regeneration at 11‰ and 21‰.
- This is consistent with the theory that Nematostella is better adapted to varrying salinities.

spelling

- Sea anemones exhibit better regeneration when they are larger in size to begin with (Reitzel et al).
- The adult Edwardsiella used in regeneration were small to begin with and thus the lack of any significant regeneration could be affected in large part by this.
- Also, the sample size of adult Edwardsiella in regeneration was smaller due to lack of resources.

- Data points for 9/17 were lost for all species and can only be speculated by trend lines which for most cases did not fit well.

Future Direction

- Regeneration of Edwardsiella under varying salinities should be repeated with larger animals and a larger sample set.
- The metamorphosis of Nematostella planula under varying salinities should be compared with that of Edwardsiella.
- Infection of ctenophores should be repeated with much smaller increments between steps.

Sea anemones in good health can hopefully be used to further research in the field.

We'll do our best to carry the torch!

Edwardsiella metamorphosis 21‰ from parasite to beginning of adult.