

# *A.cer* in nutrients and heat stress

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## Contents

<b>Collection and maintenance</b>	<b>3</b>
July 24, 2017 . . . . .	3
July 25, 2017 . . . . .	3
July 26, 2017 . . . . .	4
July 31, 2017 . . . . .	4
Aug 1, 2017 . . . . .	4
Aug 11 and 12, 2017 . . . . .	4
<b>SAMPLING POINT TIME 1 (BASE LINE BEFORE EXPERIMENT STARTS)</b>	<b>5</b>
Aug 30, 2017 . . . . .	5
Aug 31, 2017 . . . . .	5
Sep 2-12, 2017 . . . . .	5
Sep 18, 2017 . . . . .	5
Sep 19, 2017 . . . . .	5
Oct 05, 2017 . . . . .	6
Oct 06, 2017 . . . . .	6
Oct 07, 2017 . . . . .	6
Oct 08, 2017 . . . . .	6
Oct 09, 2017 . . . . .	6
Oct 10, 2017 . . . . .	6
Oct 11, 2017 . . . . .	7
Oct 15, 2017 . . . . .	7
Oct 18, 2017 . . . . .	7
Oct 19, 2017 . . . . .	7
Oct 25, 2017 . . . . .	8
Oct 26, 2017 . . . . .	8
Oct 27, 2017 . . . . .	8
Oct 28, 2017 . . . . .	8
Nov 6, 2017 . . . . .	9
Nov 10, 2017 . . . . .	9
Nov 15, 2017 . . . . .	9
Nov 16, 2017 . . . . .	9
<b>STARTED NUTRIENT ADDITION!!!</b>	<b>10</b>
Nov 17, 2017 . . . . .	10
Nov 19, 2017 . . . . .	10
Nov 20, 2017 . . . . .	10
Nov 22, 2017 . . . . .	10
Nov 23, 2017 . . . . .	10
Nov 27, 2017 . . . . .	11
Nov 28, 2017 . . . . .	11
Nov 29, 2017 . . . . .	11
Dec 06, 2017 . . . . .	11
Dec 13, 2017 . . . . .	12
Dec 14, 2017 . . . . .	12
Dec 17, 2017 . . . . .	12
Dec 18, 2017 . . . . .	12

Dec 19, 2017	13
Dec 20, 2017	13
Dec 21, 2017	13
Dec 22, 2017	13
Dec 27, 2017	14
Jan 03, 2018	14
Jan 05, 2018	14
Jan 08, 2018	14
Jan 09, 2018	15
Jan 10, 2018	15
Jan 11, 2018	15
Jan 16, 2018	16
Jan 17, 2018	16
Jan 18, 2018	16
Jan 19, 2018	17
Jan 20, 2018	17
Jan 22, 2018	17
Jan 23, 2018	17
Jan 25, 2018	18
Jan 26, 2018	18
Jan 28, 2018	18
Jan 29, 2018	18
Jan 30, 2018	18
Feb 01, 2018	19

## **RAMPING UP 19**

Feb 02, 2018	19
Feb 03, 2018	19
Feb 05, 2018	19
Feb 06, 2018	20
Feb 07, 2018	20
Feb 08, 2018	20
Feb 09, 2018	20
Feb 11, 2016	21
Feb 12, 2016	21
Feb 13, 2018	21
Feb 14, 2018	21
Feb 15, 2018	22
Feb 18, 2018	22
Feb 19, 2018	22
Feb 20, 2018	23
Feb 21, 2018	23
Feb 22, 2018	24
Feb 23, 2018	24
Feb 25, 2018	24
Feb 26, 2018	25
Feb 27, 2018	25
Feb 28, 2018	25
Mar 01, 2018	26
Mar 02, 2018	26
Mar 03, 2018	26
Mar 05, 2018	27
Mar 06, 2018	27
Mar 07, 2018	27

Mar 08, 2018 . . . . .	28
Mar 12, 2018 . . . . .	28
Mar 13, 2018 . . . . .	29
Mar 14, 2018 . . . . .	29
Mar 15, 2018 . . . . .	29
Mar 16, 2018 . . . . .	29
Mar 19, 2018 . . . . .	30
Mar 20, 2018 . . . . .	30
Mar 21, 2018 . . . . .	30
Mar 22, 2018 . . . . .	30
Mar 23, 2018 . . . . .	31
Mar 25, 2018 . . . . .	31
Mar 26, 2018 . . . . .	31
Mar 28, 2018 . . . . .	31
Apr 11, 2018 . . . . .	31
Apr 12, 2018 . . . . .	32
Apr 22, 2018 . . . . .	32
Apr 26, 2018 . . . . .	32
Apr 27, 2018 . . . . .	33
Apr 28, 2018 . . . . .	33
Apr 29, 2018 . . . . .	34
Apr 30 - May 2, 2018 . . . . .	34
May 03, 2018 . . . . .	34
May 16, 2018 . . . . .	35
May 17, 2018 . . . . .	35
May 21, 2018 . . . . .	35
May 22, 2018 . . . . .	35
Jul 2, 2018 . . . . .	36
Jul 3, 2018 . . . . .	36

## Collection and maintenance

### July 24, 2017

#### Adapting *Acropora cervicornis* to laboratory conditions

- Corals were obtained from MOTE laboratory from various locations in the Florida Keys
- MOTE provided 6 tagged genotype
  - Yellow (30)
  - Orange (31)
  - Green and Orange (31)
  - Red and Yellow (31)
  - Green (30)
  - Red and Orange (30)
- corals were added to a tank with other *A. cervicornis* kept at 26-27°C with two pumps and build my LED light at 100% intensity

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### July 25, 2017

#### Epoxy

- Genotypes were epoxied together on clipboards and labelled to prevent the pumps from blowing the fragments into no man's land
- 

## July 26, 2017

### Tagging fragments

- Plastic numbered tags + glue + previous epoxy

### IPAM Time 0

- First IPAM data taken by CD and AP
    - The fu\*(&^% IPAM is not working in 1/4 of the field
    - Average YII values ~ 0.6, one genotype is slightly lower than 0.6
  - No pics taken
- 

## July 31, 2017

### Death

- Corals 185, 200, 275, 279
- 

## Aug 1, 2017

### New lights

- All the A.cer, including the old fragments were fed moved to tank 1A with the new lights.
- Same HOBO to follow temps

## Aug 11 and 12, 2017

### Death!

- Rapid tissue necrosis in many fragments

Genotype	Control	N	NP	Dead
Green	10	9	9	2
Green and Orange	10	10	9	2
Orange	6	6	6	13
Red and Orange	9	9	9	3
Red and Yellow	4	4	5	18
Yellow	3	3	3	21
Total	42	41	41	59

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## **SAMPLING POINT TIME 1 (BASE LINE BEFORE EXPERIMENT STARTS)**

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**Aug 30, 2017**

### **IPAM Time 1**

- Data IPAM taken by CD, AP and Becca using the lab (fixed) machine
- Pictures with color and size scale

### **Tissue samples Time 1**

- Razor blade samples by PR

### **Buoyant Weight Time 1**

- Data taken by CD and AP with CL scale
  - YSI data taken every 10 fragments
- 

**Aug 31, 2017**

### **Prok incubation of all samples T=1**

- 100uL aliquotes + 6uL of prok / 3 hours
- 

**Sep 2-12, 2017**

### **IRMA!!**

- The corals were not cleaned or fed until 12th when Phil could get into RSMAS
- 

**Sep 18, 2017**

- Cleaned and fed all the Acer, they look ok in general
- 

**Sep 19, 2017**

- Samples 136 and 145 had mortality on the base, they were cut and re-glued
  - Sample 192 fall and were re-glued
-

## **Oct 05, 2017**

- Organic extraction and 1st EtOH from sample 101 to 236 by CD
  - New 10 gallon aquaria and pumps set up
- 

## **Oct 06, 2017**

- 2nd EtOH from sample 101 to 236 by AP and PR
  - Organic extraction and 1st EtOH from sample 236 to 300 by AP and PR
- 

## **Oct 07, 2017**

- 2nd EtOH from sample 237 to 300 by AP
  - Make stock solutions and started the pumps.
  - Took water samples for nutrient analysis day 1
- 

## **Oct 08, 2017**

- Took water samples for nutrient analysis day 2
- 

## **Oct 09, 2017**

- EtOH wash from sample 101 to 300 by AP and BG
    - 101 to 165 (BG)
    - 106 to 300 (AP)
  - All the samples are in 100uL of 0.1X TE
  - Took water samples for nutrient analysis day 3
  - Cleaned all fragments
- 

## **Oct 10, 2017**

### **IPAM 2 for Acer and Pictures w color scale**

- Values are lower vs Sep 1st (IPAM 0)
-

**Oct 11, 2017**

**Preliminary qPCR (only symbionts)**

- Clades A, B, C and D were tested for 2 samples of each genotype
    - F: 136, 139
    - Green: 176, 179
    - GO: 116, 119
    - Orange: 149, 1152
    - RO: 162, 166
    - RY: 204, 102
    - Yellow: 143, 231
  - Results: Samples 162, 143, 139, 119 and 166 have one replicate with late D amplification, keep and eye on background, but all the corals are pretty much A
- 

**Oct 15, 2017**

**New Nutrient stocks**

- Made more Stock 1 and Stock 2 Nutrients
    - Stock 1 N: 4.011825g of  $\text{NH}_4\text{Cl}$  + 250mL of RO water
    - Stock 1 P: 1.034925g of  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  + 250mL of RO water
    - Stock 2 N: 21mL of Stock 1 + 987mL of RO water
    - Stock 2 NP: 21mL of N\_Stock 1 + 42mL of P\_Stock 1 + 945mL of RO water
  - New Stock 2 solutions were attached to the pumps at 4:06PM to follow volume delivery for 24hours
- 

**Oct 18, 2017**

**Tissue samples Time 3 for Acer**

- Samples taken by PR
- 
- SDS incubation

**Buoyant weight 3 for Acer**

- Data taken by CD, PR and AP
- 

**Oct 19, 2017**

**IPAM 3 for Acer**

- Values are higher than time 2, but still lower than time 0

### **ProK incubation samples Time 3**

- Prok by CD
- 

2017-10-27 ## Oct 23, 2017

**Corals moved inside aquaria, not nutrients added yet**

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**Oct 25, 2017**

**Organic Extraction of 77 samples by CD and PR**

- R1 and some from R2
- 

**Oct 26, 2017**

**2nd EtOH for 77 samples**

- R1 and some from R2
  - AP and AG
- 

**Oct 27, 2017**

**EtOH wash 77 samples**

- R1 and some from R2
- Tested some samples for other clades (contamination) with ER plate 2 SSID. Samples are contaminated with CD

**Organic Extraction**

- Last 57 Samples with new Prok, new CTAB and New NAOH. If there is contamination it is in the SDS...
- 

**Oct 28, 2017**

**EtOH wash 57 last samples**

- Samples labeled with red sharpie



## qPCR

- Acer 181, 232 checked in qPCR for clade C and D contamination
- 

**Nov 6, 2017**

## IPAM 4 for Acer

- Values are higher than 0.6!! and than time 0!
  - The corals look great! Ready to start the experiment!
- 

**Nov 10, 2017**

## Buoyant weight before sampling

- Data taken by CD and BG
- 

**Nov 15, 2017**

## Tissue samples T=5

- Acer by AP

## Pictures T=5

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**Nov 16, 2017**

## IPAM T=5

- Values are lower than time 4 :/ May be because they were sampled one day before?

## Buoyant weight after sampling

- Data taken by CD and AP
-

# STARTED NUTRIENT ADDITION!!!

**Nov 17, 2017**

- To start concentrations:
    - N: 1mL of  $\text{NH}_4\text{Cl}$  [Stock 1 = 300mM] in 30L filtered sea water
    - NP: 1mL of  $\text{NH}_4\text{Cl}$  [Stock 1 = 300mM] + 1mL  $\text{NaH}_2\text{PO}_4$  [Stock 1 = 30mM] in 30L filtered sea water
  - To keep concentrations
    - N: 24mL of  $\text{NH}_4\text{Cl}$  / day [Stock 2 = 6.25uM]
    - NP: 24mL of  $\text{NH}_4\text{Cl}$  +  $\text{NaH}_2\text{PO}_4$  / day [Stock 2 = 6.25uM + 1.25uM]
- 

**Nov 19, 2017**

**Fed Acer**

- Inside the aquariums with the larvae food
- 

**Nov 20, 2017**

**ProK incubation**

- All Acer samples by CD
- 

**Nov 22, 2017**

**Fed Acer**

- Inside the aquariums with the larvae food
- 

**Nov 23, 2017**

**IPAM time = 6**

- AP and CD
- Small increase in general

**Water change and horizontal rotation**

---

**Nov 27, 2017**

**Mesenterial filaments out in NP**

- Mesenterial filaments were out only in the NP treatment
  - The water was murky
  - Opened the new water income and let it run for a long time. Then water was removed and added more stock 1 nutrients
- 

**Nov 28, 2017**

**Fed Acer**

- Inside the aquariums with the larvae food
- 

**Nov 29, 2017**

**IPAM time = 7**

- AP and BG
- Small increase in general

**Water change and vertical rotation**

**Water sample for nutrient analysis**

\* ~ 50 mL from each aquarium

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**Dec 06, 2017**

**IPAM time = 8**

- PR and CD
- Constant values

**Water change and vertical rotation**

**Pictures with color scale**

- One coral was found broken
-

**Dec 13, 2017**

**IPAM time = 8**

- CD and AP
- Values look stable, control R2 is a bit lower

**Water change and horizontal rotation**

- Celia

**Buoyant weight before samples**

- BG and PR
- 

**Dec 14, 2017**

**Tissue samples + Picture with color scale**

- Taken by AP
  - Three polyps per coral
- 

**Dec 17, 2017**

**ProK incubation Acer R1 and begging R2**

- 100uL sample + 10uL Prok
- Control, N and NP - R1
- Control-R2, + samples N R2 (236-278)

**Water change and vertical rotation**

- AP

**New Stock 2 of nutrients**

- AP
- 

**Dec 18, 2017**

**Death**

- Fragment 237 in NP R2
-

**Dec 19, 2017**

**ProK incubation Acer R2**

- from 285 to 235 (38 samples) with R2 Ssid
- 

**Dec 20, 2017**

**Organic extraction last Acer R2 from Dec 14**

- from 285 to 235 (38 samples) with R2 Ssid
  - CTAB AP- 1st EtOH PR
- 

**Dec 21, 2017**

**Organic extraction Acer R1 and begging of R2 from Dec 14**

- from 137 to 154 (96 samples)
- AP

**Second EtOH last Acer R2 from Dec 14**

- from 285 to 235 (38 samples) with R2 Ssid
- AP

**Cleaned tanks**

- AP, corals left without nutrients
- 

**Dec 22, 2017**

**Second EtOH Acer R1 and begging of R2 from Dec 14**

- from 137 to 154 (96 samples)
- AP

**EtOH wash last Acer R2 from Dec 14**

- from 285 to 235 (38 samples) with R2 Ssid
- AP

### **Cleaned corals**

- AP, horizontal rotation
- 

**Dec 27, 2017**

**Fed corals + vertical rotation**

---

**Jan 03, 2018**

**Fed corals + horizontal rotation**

- With S.D

**IPAM time = 10**

- SD and AP
  - Values look stable, but control R2 declined more
- 

**Jan 05, 2018**

**New nutrient stock (1) and (2)**

- Replaced stock 2 in pumps
- 

**Jan 08, 2018**

**Water change with non filtered water**

- Added more stock 1

**Organic Extraction samples from 11-15**

- All R1 and begging of R2 (Control and few N)
  - Samples 137-187
  - With S.D
-

**Jan 09, 2018**

**Acer (Dec) qPCR with all 4 clades**

- CD
  - Samples in the left rows did not amplified ok
  - B positive did not amplified
- 

**Jan 10, 2018**

**2nd ETOH from 11-15**

- All R1 and begging of R2 (Control and few N)
- Samples 137-187
- With S.D

**Acer (Dec) qPCR with B, C and D clades**

- CD
- We figured that the MM was not mixed, maybe that was the problem?
- B did not amplified in any positive control

**Fed corals and stayed in extra tank for cleaning next day**

- CD and SD
- 

**Jan 11, 2018**

**ETOH wash from 11-15**

- All R1 and begging of R2 (Control and few N)
- Samples 137-187
- With S.D

**Acer (Dec) qPCR with A and Acer**

- CD

**Cleaned corals + vertical rotation**

- CD and SD
-

**Jan 16, 2018**

**Cleaned tanks + horizontal rotation**

- CD and SD

**Mesenterial filaments were out, specially in N and NP**

- We figured that evaporation may increase salinity??

**Acer buoyant weight**

- CD and SD
- We did not take BW data after sampling last time, so this will only be useful as initial values for the next time point

**Organic Extraction last batch of samples from 11-15**

- Most of N and all NP from R2
  - 39 Samples from 278 to 235 plus - control
  - S.D
- 

**Jan 17, 2018**

**Second EtOH, last batch of samples from 11-15**

- Most of N and all NP from R2 (S.D)
  - 39 Samples from 278 to 235 plus - control Se ### Second Pictures of N weird tissue lost in N
  - CD
- 

**Jan 18, 2018**

**EtOH wash last batch of samples from 11-15**

- Most of N and all NP from R2 (S.D)
- 39 Samples from 278 to 235 plus - control

**Water change**

- CD, the corals were left in holding tank to IPAM next day
  - Corals were fed
-



**Jan 19, 2018**

**ITS-2 PCR last batch of samples from 11-15**

- Most of N and all NP from R2 (S.D)
- 39 Samples from 278 to 235 plus - control

**IPAM T=11**

- CD and AP
- Values look stable, but control R2 does not recovered

**Corals back to treatments**

- Corals moved inside the tanks and nutrients added
  - Vertical rotation
  - We bleached the whole recirculation system
- 

**Jan 20, 2018**

**3 plates of Acer to test Pax-C and CAM primers**

- See R project

**Recirculation drained and added fresh water**

---

**Jan 22, 2018**

**last BW NP R2 data is bullshit**

---

**Jan 23, 2018**

**Water change by CD + horizontal rotation**

- Corals in control(both replicates) had mesenterial filaments out
- Another coral in Nitrogen R2 is showing signs of tissue loss

**Temperature and Salinity measurements taken using YSI from Langdon Lab**

- Control \*\* R1: 26.1, 37.2 R2: 26.3, 36.9
  - Nitrogen \*\* R1: 26.1, 36.9 R2: 26.2, 36.9
  - NP \*\* R1: 26.2, 37.0 R2: 26.2, 36.8
-

**Jan 25, 2018**

**IPAM Time=12**

- AP and CD
- 

**Jan 26, 2018**

**Nutrient Stock Replenished/HOBOs checked**

- Changed the bottles, but used stock made before.
- 

**Jan 28, 2018**

**Water change + vertical rotation**

- Fed corals
  - Corals (both replicates) had mesenterial filaments out
  - AP and CL
- 

**Jan 29, 2018**

**BW Before Samples->Sample-> BW after samples (Time = 13)**

- BW Before Samples by CD and AP
- 3 polyps samples by AP
- BW After Samples by CD and SD

**ProK incubation samples Time = 13**

- By SD and CD
- 

**Jan 30, 2018**

**IPAM T=13**

- CD and AP
-

**Feb 01, 2018**

**Organic extraction of Acer replicates 1 and 2 from Jan 29 sampling**

- CD
- 

## **RAMPING UP**

**Feb 02, 2018**

**Second EtOH precipitation of Acer (both replicates) from Jan 29**

- SD

**Thermal stress begins (26 → 27)**

- AP
- 

**Feb 03, 2018**

**EtOH wash and resuspension of Jan 29 samples**

- 1st 16 re-suspended in 0.1xTE (made by AP) and the 2nd 16 re-suspended in 1xTE (made by SD)
  - SD
- 

**Feb 05, 2018**

**Took samples for blasting and put them in -80**

- chose 1 sample per genotype (where available)

**Water changes and horizontal AND vertical rotation**

- done by AP and PR

**Took water samples after nutrients were added for analysis**

- done by PR

**Temperature increase from 27 to 28**

- corals were kept at 27 over the weekend
-

**Feb 06, 2018**

**Acer corals found to be in wrong treatments for ~24 hours**

- Control (R2) was in NP (R1)
- Control (R1) was in Control (R2)
- N (R1) was in Control (R1)
- NP (R1) was in N (R1)

**Temperature increase 29**

---

**Feb 07, 2018**

**IPAM Time=14**

- IPAM done by CD and AP
- Corals were at 29 degrees and temperature increased to 30 at the end of the day
- So far no decline in YII values for Acer corals in any treatment

**Water samples taken**

- CD
- 

**Feb 08, 2018**

**Water samples taken**

- SD

**qPCR from Dec samples**

- Plates 12 and 13 by CD
  - A-actin and Acer CaM SYBER assays
- 

**Feb 09, 2018**

**qPCR from Dec samples**

- Plates 14 to 17 by CD
  - A-actin and Acer CaM SYBER assays
-

**Feb 11, 2016**

**Temperature set to 31**

---

**Feb 12, 2016**

**IPAM + Pictures Time=15**

- IPAM done by CD and AP
- Corals in N and NP keep losing tissue. Two corals completely dead (164 and 135)

**Salinity and Temperature**

- Control 1: 40 - 30.6
- Control 2: 39.9 - 30.6
- N1: 40.3 - 30.7
- N2: 39.8 - 30.6
- NP1: 39.9 - 30.7
- NP2: 35.5 - 30.5

**Water change**

- Vertical and horizontal rotation

**Feeding + Cleaning**

- PR, SD, AP
- 

**Feb 13, 2018**

**Salinity and Temperature**

- Control 1: 34.4 - 30.6
  - Control 2: 34.5 - 30.7
  - N1: 34.6 - 30.6
  - N2: 34.4 - 30.7
  - NP1: 34.5 - 30.6
  - NP2: 34.6 - 30.8
- 

**Feb 14, 2018**

**Salinity and Temperature**

- Control 1: 34.8 - 31.4

- Control 2: 35.4 - 31.7
- N1: 35.3 - 31.4
- N2: 35.2 - 31.6
- NP1: 35.2 - 31.5
- NP2: 35.3 - 31.7

#### **BW corals at 31 degrees**

- weighted by CD and Celia
- 

#### **Feb 15, 2018**

##### **Salinity and Temperature**

- Control 1: 36.6 - 30.6
- Control 2: 36.7 - 30.6
- N1: 36.4 - 30.9
- N2: 36.2 - 30.6
- NP1: 36.3 - 30.9
- NP2: 36.5 - 30.7

##### **IPAM and pictures with color scale time=16**

- IPAM done by CD and AP

##### **Water changes**

- Vertical and horizontal shift of aquaria
- Done by AP and CD

##### **Stopped nutrient addition**

**Corals with tissue loss were flash frozen for future gene expression work**

---

#### **Feb 18, 2018**

- Added fresh water to aquaria

~ 100mL of RO water was added to each one

---

#### **Feb 19, 2018**

- Blasted 13 Acer fragments (CD, CL and AP)

### **Salinity and Temperature before WC**

- Control 1: 37.4 - 31.1
- Control 2: 38.1 - 31.5
- N1: 37.9 - 31.2
- N2: 38.2 - 31.5
- NP1: 38.2 - 31.2
- NP2: 38.0 - 31.5

### **IPAM and pictures with color scale time=17**

- IPAM done by CD and AP

### **Water changes + Feeding**

- Vertical and horizontal shift of aquaria
  - Done by AP and PR
- 

## **Feb 20, 2018**

### **Salinity and Temperature**

- Control 1: 35.1 - 31.5
  - Control 2: 35.5 - 31.8
  - N1: 35.3 - 31.6
  - N2: 35.4 - 31.8
  - NP1: 35.5 - 31.6
  - NP2: 35.4 - 31.7
- 

## **Feb 21, 2018**

### **Salinity and Temperature**

- Control 1: 36.1 - 31.5
  - Control 2: 36.0 - 31.7
  - N1: 36.0 - 31.7
  - N2: 36.1 - 31.8
  - NP1: 36.2 - 31.6
  - NP2: 36.1 - 31.7
  - RO water added to aquaria to reach target salinity (34.5-35.0)
-

**Feb 22, 2018**

**IPAM and pictures with color scale time=18**

- IPAM done by CD and AP

**Water changes and feeding**

- Vertical and horizontal rotation

**Got HOBOs data from Jan-Feb**

---

**Feb 23, 2018**

**Buoyant weight**

- Done by CD and AP

**Razor blade samples taken**

- Done by AP

**Salinity and Temperature**

- Control 1: 35.4 - 31.7
  - Control 2: 35.7 - 31.6
  - N1: 35.6 - 31.8
  - N2: 35.8 - 31.6
  - NP1: 35.9 - 31.8
  - NP2: 35.8 - 31.6
  - RO water added to aquaria to reach target salinity (34.0-34.3)
- 

**Feb 25, 2018**

- Added ~ 1 cup of RO water
-



**Feb 26, 2018**

**Salinity and Temperature**

- Control 1: 35.8 - 31.7
- Control 2: 35.8 - 31.6
- N1: 36.0 - 31.7
- N2: 36.2 - 31.6
- NP1: 36.4 - 31.7
- NP2: 36.3 - 31.6

**Blasted 15 Acer fragments**

- done by CD, AP, SD and CL

**IPAM and pictures with color scale time=19**

- done by CD, AP, and SD

**Water changes and feeding**

- Vertical and horizontal rotation
- 

**Feb 27, 2018**

**Salinity and Temperature**

- Control 1: 34.4 - 31.5
- Control 2: 34.6 - 31.6
- N1: 34.9 - 31.6
- N2: 34.8 - 31.7
- NP1: 35.0 - 31.6
- NP2: 34.8 - 31.7

**Chlorophyll for 15 blasted samples on Feb 26, 2018**

- Done by CD and AP
- 

**Feb 28, 2018**

**Salinity and Temperature**

- Control 1 35.5 - 31.6
- Control 2: 35.6 - 31.6
- N1: 35.8 - 31.6
- N2: 35.7 - 31.6

- NP1: 35.8 - 31.6
- NP2: 35.8 - 31.6

**CTAB incubation, organic extraction, and 1st EtOH precipitation of Acer samples taken on Feb 23, 2018**

- Done by CD and AP
- 

**Mar 01, 2018**

**Water changes and feeding**

- Vertical and horizontal rotation

**IPAM and Pics T=20**

- Done by CD and AP

**2nd EtOH precipitation and wash of Acer samples taken on Feb 23, 2018**

- Done by CD and SD
- 

**Mar 02, 2018**

**Sampled dying corals**

- Acer 282, 288, 221, 177, 123, and 178 were sampled and frozen
- 

**Mar 03, 2018**

**Salinity and Temperature (adjusted with ~ 2 cups of RO)**

- Control 1 36.2 - 31.3 (35.3)
- Control 2: 34.4 - 31.6 (35.4)
- N1: 35.8 - 31.7 (35.4)
- N2: 36.2 - 31.6 (35.4)
- NP1: 36.4 - 31.7 (35.3)
- NP2: 36.3 - 31.6 (35.4)

**Sampled dying corals**

- Acer 117, 288, 221 were sampled and frozen
-

## **Mar 05, 2018**

### **Water changes and feeding**

- Vertical and horizontal rotation

### **IPAM and Pics T=21**

- Done by CD and AP

### **ProK removed fragments**

- 117, 288 and 221
- 

## **Mar 06, 2018**

### **Tissue samples**

- Taken by AP, only controls and some NP are left
- SDS incubation

### **Organic Extraction (Initial blastate samples)**

- SD and CD
- 

## **Mar 07, 2018**

### **Acer initial cell counts diluted**

- 1st batch was already diluted in  $\sim 1/2$  by CD
- 1st and 2nd batch were further diluted in  $1/4$
- Dilutions by CL

### **Salinity and Temperature**

- Control 1: 37.0 - 31.5
- Control 2: 37.0 - 31.7
- N1: 37.0 - 31.5
- N2: 37.2 - 31.7
- NP1: 37.1 - 31.6
- NP2: 37.1 - 31.8

**ProK incubation 3/6/18 samples**

**2nd EtOH (Initial blastate samples)**

- SD
- 

**Mar 08, 2018**

**Female corals strike**

**Salinity and Temperature**

- Control 1: 35.9 - 31.4
- Control 2: 35.4 - 31.6
- N1: 35.6 - 31.3
- N2: 35.7 - 31.6
- NP1: 35.5 - 31.4
- NP2: 35.7 - 31.7

**Bouyant W before recovery**

- Controls R1 and R2, few NP2
- Corals have been sampled 2x since last BW, so we need to correct for this loss

**IPAM and pictures with color scale T=22**

- AP and CD

**Took fragments for blasting and put in -80**

- AP and CD
- 

**Mar 12, 2018**

**qPCR for Acer samples taken on 2/23/2018**

- Acer - SYBR, A - SYBR
  - Quant Studio 3 machine
  - Run by CD
-

**Mar 13, 2018**

**qPCR for Acer samples taken on 2/23/18 and blastate samples**

- Acer - SYBR, A - SYBR
- Quant Studio 3 machine
- Run by CD

**CTAB incubation, Organic extraction, and 1st EtOH for Acer samples taken on 3/6/18**

- Done by CD
- 

**Mar 14, 2018**

**2nd ETOH for Acer samples taken on 3/6/18**

- Done by SD and CD
- 

**Mar 15, 2018**

**Corals in recovery moved outside the aquaria and put on recirculating system**

- Ssid and Ofav dip in insecticide

**Lights with calibrated lightmeter**

- Control 1: Right top: 321
  - Control 2: Right bottom: 420
  - N1: Left, top: 350
  - N2: Left, bottom: 365
  - NP1: Center, top: 345
  - NP2: Center, bottom: 435
- 

**Mar 16, 2018**

**qPCR run for Acer blastate and sick samples**

- Run by CD
-

**Mar 19, 2018**

**EtOH wash and resuspension of DNA samples taken on 3/6/18**

- Done by CD
- 

**Mar 20, 2018**

**Blasted all Acer fragments collected on Mar 08**

- done by CD, AP, SD

**Blasted all Ofav fragments collected on Mar 08 (bleaching)**

- done by CD, AP, SD
- Samples taken from the -80
- Took aliquots for:
  - Cell counts (0.5mL + 50uL lugols)
  - DNA (0.5mL + 1/2(500DNAB + 83uL of 10% SDS)) SDS incubation for 1:30 at 65C
  - Chlorophyll A (~ 3-4mL filtered + 4mL of Methanol)
  - Lipids (~ 3-4mL filtered + moved back to -80), some samples did not have enough blastate for lipids

**SDS incubation of blastate samples**

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**Mar 21, 2018**

**Chl-a for Acer fragments collected on Mar 08**

- done by CD, AP

**PCR ITS-2 selected samples to sequence symbionts**

**\* 2 fragments per genotype**

- Done by SD
- 

**Mar 22, 2018**

**qPCR for Acer Nutrients Plates 23 and 24**

**ProK incubation of Acer blastate taken on Mar 08, 2018 (bleaching)**

- Done by SD
-

**Mar 23, 2018**

**qPCR for Acer Nutrients Plates 25 and 26**

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**Mar 25, 2018**

**Last IPAm before babaies come back to the ocean (T=23)**

- CD and AP
- 

**Mar 26, 2018**

**They took them back to the nursery :/**

- Good bye babies... Giraffe will be watching you!
- Make your mom's proud!!!!

:'(

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**Mar 28, 2018**

**qPCR plates 27 and 28 for Acer Nutrients**

- Sybr Green for Acer/A
- 

**Apr 11, 2018**

**1:8 dilutions for Acer cell count aliquots BEFORE BLEACHING**

- Old dilutions were thrown out
- 20uL of cells, 130uL DNAB, 10uL of lugols
- done by CD

**Cell counts for Acer before bleaching**

- CL
-

**Apr 12, 2018**

**Water samples taken from experiment tank and cleaning tank (5B) to measure DIC and TA for insight to what CO2 levels are in system**

- Samples taken from incoming water, recirculating system, aquarium with pumps, and aquarium with pump and bubbler
  - 8 samples taken total (1 replication)
  - Done by Emma Pontes and new lab manager in Langdon Lab
- 

**Apr 22, 2018**

**1:5 dilutions for Acer cell count aliquots AFTER BLEACHING**

- 20 uL of cell, 75 uL DNAB, 5 uL of lugols
  - Done by CD
- 

**Apr 26, 2018**

**Copy number estimation for Symbiodinium type A using 2 strategies:**

- PCR A, B, C and D standards at CR2-60 (PR) using actin primers. ... was supposed to use the M13 primers to amplify the plasmids :(

**1. Using DNA already extracted and estimating the number of cells/ul**

Spp	Colony	Treatment	Replicate	Fragment	CELLS/ul archive	CELLS/ul DNA	cells/10uL for 8000	Take 10uL DNA and add water
A. cer	Green	NP	R2	181	1734.56	1656.51	20.71	10.71
A. cer	Green	Control	R2	290	1432.25	1367.80	17.10	7.10
A. cer	Orange	N	R1	158	1513.72	1445.60	18.07	8.07
A. cer	Green and Orange	Control	R1	129	969.13	925.51	11.57	1.57
A. cer	Green	Control	R1	189	896.23	855.90	10.70	0.70
A. cer	Red and Yellow	NP	R2	110	866.21	827.23	10.34	0.34

- Found the DNA samples and caculated the amount of DNA to be diluted with water to get a target of 800 cells/uL using a DNA extraction efficiency of 95%

**2. Extracting DNA from balstate of known of cells/ul**



Spp	Colony	Treatment	Replicate	Fragment	CELLS/ul archive	Aliquote for 100.000 cells	Add SDS	Volumen of extraction
A. cer	Green	NP	R2	181	1734.56	57.65	142.35	100000 cells in 100TE
A. cer	Green	Control	R2	290	1432.25	69.82	130.18	100000 cells in 100TE
A. cer	Orange	N	R1	158	1513.72	66.06	133.94	100000 cells in 100TE
A. cer	Green and Orange	Control	R1	129	969.13	103.19	96.81	100000 cells in 100TE
A. cer	Green	Control	R1	189	896.23	111.58	88.42	100000 cells in 100TE
A. cer	Red and Yellow	NP	R2	110	866.21	115.45	84.55	100000 cells in 100TE

- Got 5 replicate aliquots (equivalent to 100.000 cells) of each blastate and ProK

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**Apr 27, 2018**

**Copy number estimation for Symbiodinium type A using 2 strategies**

- Standards (actin) gel check (A, B, D worked, rePCR C)
- PCR A, B, C and D standards at CR2-57 with M13 primers (SD)

**1. Using DNA already extracted and estimating the number of cells/ul**

- Dilute DNA to get target concentration (SD)

**2. Extracting DNA from balstate of known of cells/ul**

- Organic extraction adding SDS to get a 200uL archive volume (SD)

---

**Apr 28, 2018**

**Copy number estimation for Symbiodinium type A using 2 strategies**

- Standards (actin) gel check (A, B, D worked, rePCR C)
  - Nanodrop and calculate initial dilution for D, but do not have A, B amplicon lenght or C PCR product :/
- Standards (M13) gel check (A, B, C, D worked, for almost all the samples)
  - Samples 5, 6 7,8 worked better in general, however, A2 did not worked at all
  - Samples A1, B1 and B2, C1-4 abd D1, D3, D4 had very weak amplification.

**2. Extracting DNA from balstate of known of cells/ul**

- 2nd EtOh and EtOh wash (AP)
-

**Apr 29, 2018**

### Copy number estimation for Symbiodinium type A strategy #2

- PCR clean up all samples (SD)
- Nanodrop (3 times) the A standards (SD)
  - Sample A4 had a very variable concentration, discarded

Standard Sample	ng/uL	Vector+Amplicon (Da/copy]	Da/copyng/copy	Copy/uL	Copy/uL *10 <sup>10</sup>	TE Added	Final Volume of 10 <sup>10</sup> /uL
A1 (22-3)Big	43.63	860	567600	9.43E-10	46294238598.8373	36.29	46.29
A2 (22-4)Big	48.00	860	567600	9.43E-10	50927199069.7609	40.93	50.93
A3 (22-3)Sm	45.97	860	567600	9.43E-10	48769866331.3058	38.77	48.77
A4 (22-4)Sm	Fucked	860	567600	9.43E-10	??	#VALUE!	#VALUE!

- Bring the samples to 10<sup>10</sup> concentration and make serial dilutions
  - 10<sup>8</sup>: 5uL of 10<sup>10</sup> + 495 0.1XTE
  - 10<sup>6</sup>: 5uL of 10<sup>8</sup> + 495 0.1XTE
  - 10<sup>4</sup>: 5uL of 10<sup>6</sup> + 495 0.1XTE
  - 10<sup>2</sup>: 5uL of 10<sup>4</sup> + 495 0.1XTE
  - 10<sup>1</sup>: 5uL of 10<sup>2</sup> + 45 0.1XTE
- SYBER plate with the Standards and samples extracted by duplicate
- Copy number estimation ~ 9, but high SE

**Apr 30 - May 2, 2018**

### Recount for high SE Acer cell counts at all timepoints

- Done by CD

### qPCR for Ssid Nutrients Plates 32-35

- done by CD

**May 03, 2018**

### qPCR of multiple times to compare fluorescence

\*Fragments 184, 152, 160 abd 265

**May 16, 2018**

**New positive (+) control made for qPCR with A,B,C,D symbionts**

- 20 uL of OfavSC (Ofav Plate 16 columns 7-12) qPCR product
  - 10 uL of A1 standard actin cleaned PCR product
  - 10 uL of A2 standard actin cleaned PCR product
  - 10 uL of B actin standard #3 PCR product
  - 10 uL of B actin standard #4 PCR product
  - 10 uL of D actin standard #3 PCR product
  - 10 uL of D actin standard #4 PCR product
  - 20 uL of Acer CaM (Acer Plate 31 rows BCD column 1)
  - 10 uL of C standard #3 M13 PCR product
  - 10 uL of C standard #4 M13 PCR product
  - Total volume of 100 uL then cleaned using Wizard SV Gel and Clean-up System protocols
  - Done by CD and AP
- 

**May 17, 2018**

**qPCR for Acer Nutrients from 03/06/18**

- Check for A,B,C,D symbionts post bleaching and to rule out contamination
  - Master mix: Actin primers and probes and EMM
- 

**May 21, 2018**

**PCR stock made**

- Done by CD

**1:10 dilutions of the super positive made on 05/16/18**

- 10 uL of (+) and 90 uL of UP water
- 

**May 22, 2018**

**PCR for Ssid, Ofav, and Acer Nutrient using Ssid PaxC, OfavSC and AcerCaM primers respectively**

- this will be used for copy #, fluorometry corrections, and specialized/species super positive qPCR sample

- Done by CD
- 

**Jul 2, 2018**

**PCR a subset of samples to look at the bacterial community**

- PCR + gel. All the samples worked, there is not - control Samples: 284, 217, 102, 231, 187, 120, 232, 234, 188, 128, 104, 233
- 

**Jul 3, 2018**

**PCR cleanup and qubit**