Orbicella faveolata and *Siderastrea siderea* cores in nutrients and heat stress

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Contents

Collection and maintenance

Aug 31, 2017

Cleaned and organized all the Ssid cores

- Master file updated with the cores in the tank

Sep 1, 2017

Ssid IPAM T= 0 and Pictures with color scale

- Colony 20 by Becca and AP
- Picture # 2 has more cores than the spread sheet, check numbers :/
- Colony 22, 23, 24, 27, 28 and 30 by PR and AP

Ssid Tissue samples T= 0

- Selected cores from colony 20 by Becca and AP

- Selected cores from colony 22, 23, 24, 28 and by PR and AP
- Colonies 27 and 30 were NOT sampled!!

Ssid SDS incubation

- All the samples were incubated in 1% SDS for 1:30 hours

Sep 2-12, 2017

IRMA!!

- The corals were not cleaned or fed until 13th when AB and Maddie could get into RSMAS and clean them

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Sep 18-19, 2017

- Cleaned and fed
- Lots of mortality

Sep 21, 2017

Ssid Tissue samples T= 0 for colonies Ssid 27

- Tissue samples taken by Carly Dennison
- SDS incubation

Sep 22, 2017

Ssid Tissue samples T= 0 for colonies Ssid 30

- Tissue samples taken by CD
- SDS incubation

Ofav new cores from colony Ofav "33"

- O.fav 33
  - This colony has been kept in the wet lab for a long time, one side is pale/bleach since It never got light
  - The side facing up had some tissue loss beacause it was out of the water

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Sep 26, 2017

Ofav 33 colony moved to tank 5A

- Colony cleaned with Bayer and moved to tank 5A

Sep 29, 2017

Ssid cores (8 per genotype) were selected to be used in the nutrient experiment

- Selection of cores
- Made new 7*8 eggcrates for them ———-

Oct 07, 2017

- Make stock solutions and started the pums.
- Took water samples for nutrient analysis day 1

Oct 08, 2017

- Took water samples for nutrient analysis day 2

Oct 09, 2017

- Took water samples for nutrient analysis day 3
- Cleaned all Ssid cores

Oct 10, 2017

IPAM 1 for Ssid and Pictures w color scale

- All the Ssid cores were ok
- Values are lower vs Sep 1st (IPAM 0)

Oct 15, 2017

New Nutrient stocks

- Made more Stock 1 and Stock 2 Nutrients
  - Stock 1 N: 4.011825g of NH4Cl + 250mL of RO water
  - Stock 1 P: 1.034925g of NaH2PO4.H2O + 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

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Oct 18, 2017

IPAM 2a for Ssid

- Data taken by AP
- Values are lower in the replicate 2 vs 1! , do not look good to start the experiment

Tissue samples 2 for Ssid

- Samples taken by AP
- SDS incubation
- Core 28-19 was not sampled... looks dead?

Buoyant weight 2 for Ssid

- Replicate 1 by CD and PR
- Replicate 2 by CD and AP

Oct 19, 2017

IPAM 2b for Ssid

- Data was taken again since yesterdas values were so low
- Less standard variation this time, but mean values did not improve

ProK incubation samples Time 2

- Prok by CD

Oct 20, 2017

Organic Extraction

- All Ssid R1 and almost all R2
- Started by BG, finished by PR

Oct 23, 2017

2nd EtOH

- All Ssid R1 and almost all R2

Corals moved inside aquaria, not nutrients added yet

Oct 25, 2017

EtOH wash -> DNA/0.1X TE

- Ssid R1 (except the first set of 48) and almost all R2, by BG and CD

Organic Extraction last Ssid R2 and Acer R1

- CTAB CD, Chl PR, 1st ETOH CD
- Last 19 samples of Ssid R2

Oct 26, 2017

EtOH wash -> DNA/0.1X TE

- First Ssid R1 (48 samples) by AP

2nd EtOH last 19 samples in Ssid R2

- Used the NaOH made yesterday and added a - control to the extraction

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Nov 06, 2017

IPAM 3 for Ssid

- Values look better!
- min=0.47 (22-24), mean and median ~0.55, max 0.59 (28-23)

Nov 10, 2017

Buoyant weight before sampling for Ssid R1 (Control and N)

- Data taken by CD and BG

Nov 15, 2017

Buoyant weight before sampling for Ssid R1 (NP) and R2

- R1 NP by BG and PR
- R2 Control by BG and PR
- R2 N by PR
- R2 NP by CD

Tissue samples T=4

- Ssid samples taken by CD
- Ofav samples taken by PR

Pictures T=4 for Ssid

Nov 16, 2017

IPAM T=4 for Ssid and Ofav

- Ssid values declined, the increased in T=3 is reverted and values look similar to T=2
  - It was maybe sampling/handeling?

NUTRIENT ADDITION

Nov 17, 2017

STARTED NUTRIENT ADDITION!!!

- To start concentrations:
  - N: 1mL of NH4Cl [Stock 1 = 300mM] in 30L filtered sea water
  - NP:1mL of NH4Cl [Stock 1 = 300mM] + 1mL NaH2PO4 [Stock 1 = 30mM] in 30L filtered sea water
- To keep concentrations
  - N: 24mL of NH4Cl / day [Stock 2 = 6.25uM]
  - NP: 24mL of NH4Cl + NaH2PO4 / day [Stock 2 = 6.25uM + 1.25uM]

Split Ofav cores

- Cores were split to balance YII values in each treatment
- Some were not labeled, so ramdomly allocated

Nov 19, 2017

Fed Ssid and ofav

- Outside the aquariums with the larvae food + reef chilli

Insecticide dip for Ofav and Revive dip for Ssid

Nov 20, 2017

Ssid ProK incubation

- All Ssid samples by CD

Ofav moved inside the aquariums

- Check labels and re-label some
- Pictures with color scale

Nov 22, 2017

Fed Ssid and Ofav

- Inside the aquariums with reef chilli

Nov 23, 2017

IPAM time = 5

- AP and CD
- Values in Ofav colony 34 declined
- Values in Ssid increased, more in R2

Water change and horizontal rotation

Nov 25, 2017

Initial buoyant weight Ofav T= 5

- After initial samples and already in Nutrients

Nov 27, 2017

ProK incubation o all Ofav samples (initial time point)

- 100uL aliquots + 6uL of ProK by RG and AP

Nov 29, 2017

IPAM time = 6

- Values in Ofav increased (AP and BG)
- Values in Ssid increased back to time 4! (AP and PR)

Water change and horizontal rotation

Water sample for nutrient analysis

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* ~ 50 mL from each aquarium
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Nov 30, 2017

Measured light in all aquaria

Water samples day 2 for nutrient analysis

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* ~ 50 mL from each aquarium
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Dec 06, 2017

IPAM time = 7

- PR and CD
- ~ Constant values

Water change and vertical rotation

Dec 8, 2017

CTAB first batch of Ssid sampled in 11-15-2017

- 1st block Ssid 20-1 to 28-3
- 2nd block Ssid 28-6 to 24-23

- No negative control, started by BG

Dec 10, 2017

1st ETOH first batch of Ssid sampled in 11-15-2017

- AP

Dec 11, 2017

2nd ETOH first batch of Ssid sampled in 11-15-2017

- AP

Dec 12, 2017

ETOH wash first batch of Ssid sampled in 11-15-2017

- AP

Dec 13, 2017

1st ETOH

- second batch of Ssid (65 samples) + first batch of Ofav sampled in 11-15-2017 (Colony 34 from 34-1 to 34-30s)
- CTAB by CD
- We ran these O.fav samples and they have late coral CTs, we do not know why!!!!

IPAM time = 8

- CD and AP
- Ssid values increased a bit, specially for N, Control looks lower
- Ofav values increased a bit, Control looks lower

Water change and horizontal rotation

Dec 14, 2017

2nd ETOH:

second batch of Ssid (65 samples) + first batch of Ofav sampled in 11-15-2017 (Colony 34 from 34-1 to 34-30) by CD

Organic Extraction and 1st ETOH

- second batch of Ofav sampled in 11-15-2017 (Colony 34 from 34-1 to 34-49, Nutrients and CO2) + Ofav CO2
- by CD

Ofav BW before samples

- Data taken by CD and my mom

Ssid tissue samples + Picture with color scale

- Taken by CD

Dec 15, 2017

ETOH wash

- second batch of Ssid (65 samples) + first batch of Ofav sampled in 11-15-2017 (Colony 34 from 34-1 to 34-30)
- by AP

2ndt ETOH

- second batch of Ofav sampled in 11-15-2017 (Colony 34 from 34-30 to 34-49, Nutrients and CO2) + Ofav CO2
- by AP

Ofav tissue samples (T=8) + Picture with color scale

- Taken by PR

Sid BW after samples

- Data taken by CD and my mom

ProK for all Ofav and all Ssid R1 up to 30-14

Dec 16, 2017

ETOH wash

- second batch of Ofav sampled in 11-15-2017 (Colony 34 from 34-30 to 34-49, Nutrients and CO2) + mess of Ofav in CO2 and Nutrients
- by AP

Feeding and cleaning

- All spp fed with green food.

Dec 17, 2017

Water change and vertical rotation

- AP

New Stock 2 of nutrients

- AP

Dec 19, 2017

ProK incubation Ssid R2 from Dec 14

- from 20-26 to 20-30 (55 samples) with last Acer samples

Dec 20, 2017

Organic extraction Ssid R2 from Dec 14

- from 20-26 to 20-30 (55 samples) with last Acer samples
- CTAB AP- 1st EtOH PR

ProK incubation last of Ssid R2 from Dec 14

- from 20-36 to 30-28 (26 samples)
- PR

Dec 21, 2017

Second EtOH of Ssid R2 from Dec 14

- from 20-26 to 20-30 (55 samples) with last Acer samples
- AP

Cleaned tanks

- AP, corals left without nutrients

Dec 22, 2017

EtOH wash of Ssid R2 from Dec 14

- from 20-26 to 20-30 (55 samples) with last Acer samples
- AP

Cleaned corals + horizontal rotation

- AP

Dec 23, 2017

Organic extraction of all Ofav samples from Dec 14 and some R2 Ssid

- All Ofav samples
- Ssid from 20-36 to 28-23 (21 samples)

Dec 27, 2017

Second EtOH Ofav samples from Dec 14 and some R2 Ssid

- All Ofav samples
- Ssid from 20-36 to 28-23 (21 samples)
- Worked with S.Diaz de Villegas

Fed corals + vertical rotation

Dec 28, 2017

Organic Extraction and 1st EtOH R1 Ssid and 4 last samples R1 (from Dec 14)

- AP and NC

Dec 29, 2017

2nd EtOH R1 Ssid and 4 last samples R1 (from Dec 14)

- AP and NC

Jan 03, 2018

Fed corals + horizontal rotation

- With S.D

IPAM time = 9

- SD and AP
- All values declined for Ofav, except N R2
- All values declined for Ssid.

Jan 04, 2018

EtOH wash R1 Ssid and 4 last samples R1 (from Dec 14)

- Worked with S.Diaz de Villegas
- DNA in 0.1 TE

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

Jan 10, 2018

Fed corals and stayed in extra tank for cleaning next day

- CD and SD

Jan 11, 2018

Cleaned corals + vertical rotation

- CD and SD

Jan 16, 2018

Cleaned tanks + horizontal rotation

- CD and SD

Jan 17, 2018

qPCR to check A and B presence

- Plate 1 Ofav (StepOnePlus)
- Plate 1 (New machine) and 2 Ssid (StepOnePlus)
- Ran by CD with SYBR (expired in 2010) instead TaqMan
- All the plates show amplification of A and B, including in the NTC, I think the primers create primer dimer and the SYBR was not specific enough showing increasing florescence signal.
- Melting curved showed multiple and and lower temperature peaks than the positive controls.
- Some of the Ofav samples showed earlier curves for B, re-run these plate with TaqMan MM instead SYBR

Jan 18, 2018

qPCR to check for A and B using TaqMan MM

- Rerun of Plate 1 (Ofav) using TaqMan MM instead of SYBR
- Same setup
- Run by CD
- Samples from colony 31 and colony 6 have B symbionts. Other colonies are D dominated

Water change

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

Jan 19, 2018

IPAM T=10 + Pictures

- CD and AP
- Ssid declined, but simmilar to IPAM before the last one

Corals back to treatments

- Corals moved inside the tanks and nutrients added
- Vertical rotation

Jan 22, 2018

Buoyant weight for Ofav

- CD

Jan 23, 2018

Water change by CD

Jan 24, 2018

qPCR Ssid from Dec 14

- Plates 3, 4 and 5 ran by CD in StepOnePlus
- Ssid ran with SYBR and CD with TaqMan MM

- Old + control for plates3, made a new one from the rest of the plates.

Jan 25, 2018

qPCR Ssid from Dec 14

- Plates 6 and 7 ran by AP in StepOnePlus

IPAM T=11

- Ofav R2 declined, R1 stable

Pics T=11

- SD

Jan 26, 2018

qPCR Ssid from Dec 14

- Plates 7 and 8 ran by AP in StepOnePlus

Nutrient Stock Replenished/HOBOs checked

- Changed the bottles, but used stock made before.

Ofav Tissue samples (Time=12)

- PR

Jan 28, 2018

Water change + vertical rotation

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

Jan 29, 2018

ProK incubation Ofav samples from Jan 26

- By SD and CD

Jan 30, 2018

IPAM T=12

- CD and AP

qPCR Ofav samples with no B

- Plates 2 and 3, coral with SYBER, melt curve added.
- CD

Feb 01, 2018

Buoyant weight before samples for Ssid

- CD

Tissue samples for Ssid

- CD

RAMP UP

Feb 02, 2018

ProK incubation for Feb 1 Ssid samples

- CD

Ctab and organic extraction of all Ofav samples from Jan 26

- CD and PR

Thermal stress begins

- Temperatures increased 1 degree (26 –> 27)
- AP

Feb 03, 2018

Organic and 1st EtOH precipitation for Ssid samples taken on Feb 1

- SD
- 30-9, 30-13, 20-38, 20-45, and 23-54 were all messed up need to be reextracted

2nd EtOH precipitation, EtOH wash, and resuspension for Ofav samples taken on Jan 26

- RESUSPENDED IN 1xTE!!!!
- 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

2nd EtOH precipitation for Ssid samples minus the 6 that were messed up over the weekend

- SD

Feb 06, 2018

Temperature increase 29

Feb 07, 2018

IPAM time=13

- IPAM done by CD and AP

Water samples taken

- CD

Temperature increase 30

- Corals were at 29 degrees and temperature increased to 30 at the end of the day

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Feb 08, 2018

Water samples taken

- SD

Feb 09, 2018

Buoyant weight after samples Ssid

- NP R2; C, N and NP R2
- CD and SD

Feb 12, 2018

Begin tissue blasting for Ssid samples sacraficed on Feb 5

- 10 samples taken from the -80
- Took alliquots for:
  - Cell counts (1mL + 50uL lugols)
  - DNA (1mL + 10% SDS)
  - Chlorophyll A (~ 2-3mL filtered + 2mL of Methanol)
  - Lipids (~ 2-3mL filtered + moved back to -80)

Salinity and Tempereture

- 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

IPAM and Pictures time=14

- IPAM done by CD and AP

Water change

- Vertical and horizontal rotation

Feeding

- PR

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

Pictures with colorscale taken of Ofav

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

Feb 18, 2018

Added fresh water to aquaria

~ 100mL of RO water was added to each one

Feb 19, 2018

Salinity and Temperature

- 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 dones by CD and AP

Water changes and feeding

Got HOBOs data from Jan-Feb

Feb 23, 2018

Razor blade samples taken

- Ssid done by CD
- Ofave done by Prati

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

IPAM and pictures with color scale time=18

- done by CD, AP, and SD

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

CTAB incubation, organic extraction, and 1st EtOH precipitation

- Ofav and Ssid samples taken on Feb 23, 2018 (Done by CD and SD)

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

2nd EtOH precipitation for all Ofav and Ssid samples

- Done by AP and CD

Mar 01, 2018

Water changes and feeding

- Vertical and horizontal rotation

IPAM and Pics T=19

- Done by CD and AP

EtOH wash for samples taken on Feb 23, 2018

- Done by CD and SD

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)

ProK incubation dying corals

Mar 05, 2018

Blasted The rest of Ssid fragments

- done by CD, AP, SD and CL

Water changes and feeding

- Vertical and horizontal rotation

IPAM and Pics T=20

- Done by CD and AP

SDS and Prok incubation of blastate-samples

Mar 06, 2018

Chlorophyll for all Ssid fragments blasted on Mar 05, 2018

Tissue samples for Ofav and Ssid

- CD took Ssid and SD took Ofav

qPCR Ofav with BCD

- Plates # ??? samples from date???

Mar 07, 2018

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

qPCR run for Ofav colonies 6 and 31 from December sample date

- Run by CD

ProK incubation 3/6/18 samples

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

IPAM T=20?

- AP and CD

Took fragments to blast and put them in -80

- AP and CD

Mar 09, 2018

qPCR for Ssid samples

- 27-87 to 30-38 from 12/14/17 the rest of the samples are from 2/1/17

CTAB incubation, organic extraction, and 1st EtOH precipitation

- Ssid samples up to 30-21 (done by CD and PR)

Mar 10, 2018

4 qPCR plates for Ssid samples

- 2/1/18
- Ssid Pax-C sybr green, C/D TaqMan

- Step One machine
- Run by CD

Mar 12, 2018

2 qPcr plates for Ssid samples

- 2/1/18
- Ssid Pax-C sybr green, C/D TaqMan
- Step One machine
- Run by CD

Rerun of Ofav Plate 5 in qPCR

- original plate had amplification in NTC ** Primer dimer
- Step One machine
- 0.02 threshold with sybr
- Run by CD

Mar 13, 2018

CTAB incubation, Organic extraction, and 1st EtOH for Ofav and second part of Ssid samples taken on 3/6/18

- Done by CD

Mar 14, 2018

Tissue blasting for all Ofav samples sacraficed on Feb 5

- Samples taken from the -80
- Took alliquots for:
  - Cell counts (1mL + 50uL lugols)
  - DNA (1mL + 500DNAB + 83uL of 10% SDS) SDS incubation for 1:30 at 65C
  - Chlorophyll A (~ 2-3mL filtered + 4mL of Methanol)
  - Lipids (~ 2-4mL filtered + moved back to -80)

2nd ETOH for Ofav and last Ssid samples taken on 3/6/18

- Done by SD and CD

Mar 15, 2018

Chl-a for all Ofav samples sacraficed on Feb 5 (before bleaching)

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 19, 2018

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

- Done by CD

Mar 20, 2018

Blasted all Ofav fragments collected on Mar 08 (bleaching)

- done by CD, AP, SD
- Samples taken from the -80
- Took alliquots 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), very few samples had enough blastate for lipids

SDS incubation of blastate samples

Mar 21, 2018

Chl-a for Ofav fragments collected on Mar 08

- done by CD, AP

PCR ITS-2 selected samples to sequence symbionts

- fragments per genotype
- Done by SD

Cell counts for before bleaching

- Ssid by CL

Mar 22, 2018

qPCR for Ssid in Nutrients Plate 17 and 18

Re-extract Ofav 20-29 (Pro-K) because no DNA sample was found

- ProK with blastate samples
- Done by SD

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

- Done by SD

PCR ITS-2 gels to sequence symbionts

- Done by SD

Mar 23, 2018

qPCR for Ssid in Nutrients Plates 19 and 20

- Done by CD

Mar 26, 2018

Blasted all the Ssid cores after bleaching

- 42 cores
- CL, CD and AP
- Samples taken from the -80
- Took alliquots 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 (~ 3-6mL filtered + 4mL of Methanol)
  - Lipids: only 2 samples had enough blastate for lipids

SDS incubation of blastate samples

Mar 27, 2018

Chl-a extraction for Ssid cores after bleaching

- 42 cores
- SD, CD and AP

ProK incubation Ssid blasted on Mar 26

- SD

IPAM Ofav and Ssid after 3 weeks of recovery

- AP and CD

Mar 28, 2018

Organic extraction for Ssid and Ofav blasted cores after bleaching

- SD

April 4, 2018

qPCR plate 22 for Ssid Nutrients

- Checking if amplifcation cycles are consistent from time point to time point
- Chose 1 sample per genotype and ran it from each time point to compare
- CD

April 5, 2018

qPCR plates 6-9 for Ofav Nutrients

- Ofav sybr green SC assay
- Taqman assay for C/D and B (plates 8 and 9)

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

2nd EtOH precipitation done for one set of Ssid

- CD

qPCR for Ssid Plate 24

- Vac fug broken, hand spin *CD

Apr 13, 2018 (Friday the 13th)

qPCR for Ssid Nutrients plates 25, 27-31

- Ssid syrb green assay
- Taqman for C/D
- Done by CD

2nd EtOH precipitation for the remaining Ofav and Ssid sets

- CD

Apr 20, 2018

IPAM (T23) Ofav and Ssid after ~6 weeks of recovery

- AP and CD

Apr 26, 2018

1:2 dilutions for 4 cell count samples to recount

- 34-38, 6-86, 20-21, and 6-118
- high SE and >50 cells per chamber
- 20uL of sample, 20uL DNAB
- done by CD

Apr 30, 3018

qPCR for Ssid Nutrients Plates 32-35

- Done by CD

May 07, 2018

qPCR for Ofav Nutrients Plates 10-16

- Done by CD

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 be CD and AP

May 17, 2018

qPCR for Ofav Nutrients from 03/06/18

- Check for A,B,C,D symbionts post bleachin and to rule out contamination
- Master mix: Actin primers an 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, OfacSC and AcerCaM primers respectively

- this will be used for copy #, fluormetry corrections, and specialized/species super positive qPCR sample
- Done by CD

qPCR for Ssid Nutrients from 03/06/18

- Check for A,B,C,D symbionts post bleachin and to rule out contamination
- Master mix: Actin primers an probes and EMM
- 1:10 super (+) used
- Done by CD
