Grantees: Dr. Xaymara Serrano (PI)
Dr. Andrew Baker (co-PI)
Dr. Margaret Miller (collaborator)

Project Title: Effects of sedimentation stress in adult and early life stages of corals, including two ESA-listed species

Deliverables:
We planned to complete larval experiments aimed at assessing the effects of sedimentation on settlement success and respiration rates on the species *Orbicella faveolata*. In addition, we planned to complete sedimentation experiments with adult corals from the species *Porites astreoides* and *Siderastraea siderea*, and perform microbial analyses of sediment samples collected at relevant sites to detect the presence of fecal-indicator and/or potentially harmful bacteria. Finally, we conducted data analyses and began preparing a manuscript for submission to a peer-reviewed journal.

PROGRESS TO DATE

I. Experiments and preliminary analyses

All experiments were carried out at the Marine Technology and Life Science Seawater (MTLSS) complex at the University of Miami's Rosenstiel School (RSMAS). Two different sets of experiments were conducted, as follows:

Effects of sedimentation on larval performance

O. faveolata larval experiments were completed in the fall of 2017 as described in the ***POR 2015-15 Final Report*** (note that there were insufficient larvae from *Acropora palmata* for proposed experiments due to poor fertilization rates). Overall, findings suggest that the success of early life stages of *O. faveolata* may be compromised by the presence of PortMiami sediments during the pelagic phase. In addition, findings suggest both short- and long-term effects of sedimentation, as effects were still observed after corals were allowed to recover for 1 week (***see POR 2015-15 Final Report***). Together, these findings provide empirical data to local managers that might support the implementation of environmental policies aimed at minimizing the impacts of dredging operations, especially during expected coral reproductive seasons.

Effects of sedimentation on adult corals

Experiments were completed in the fall of 2017 and data is currently being analyzed (***see POR 2016-16 Interim Report***). Because of Hurricane Irma many of the *Acropora cervicornis* genotypes we intended to use in our experiments were no longer available, so experiments were conducted using *S. siderea* and *P. astreoides*. Preliminary analyses suggest species-specific effects of fine-grained sedimentation on adult coral respiration. Significant decreases in respiration were observed for *S. siderea* exposed to sediments collected near PortMiami (compared to those exposed to reef sediments), but no effects were observed for *P. astreoides* (***see POR 2016-16 Interim Report***). These findings are consistent with other work which suggests that Florida's "weedy" species may be more resistant to anthropogenic stressors compared to broadcast spawning species (e.g., Lirman et al. 2003). In addition, a comparison of the data obtained from the adult and larval experiments suggests that early life stages of corals may more susceptible to

the effects of sedimentation stress, consistent with previous literature (e.g., Fabricius, 2005; Jones et al. 2015).

II. Sediment sample collections and microbial analyses

Sediment samples were collected in 2016-2017 with permits from the FKNMS and Florida DEP at three different sites in SE Florida relevant to our experimental treatments: (1) PortMiami sediments, collected from a site 200 m north of the dredged channel ("Linear Reef" reported by Miller et al. 2016 as having detrimental effects on corals monitored and highest sediment depth measurements); (2) Emerald reef in Miami (from where adult experimental corals from the species *S. siderea* and *P. astreoides* were collected); and (3) Horseshoe reef in Key Largo (from where *O. faveolata* gametes were collected). Sediment samples were collected from the first 10 cm of the sediment layer in triplicate and ~1 g of each was preserved in RNAlater for Microbial Source Tracking (MST) assays and 16S amplicon sequencing. Note that MST markers look at rare members of the microbial community (using quantitative PCR) compared to 16S amplicon sequencing (which looks at the most abundant types of bacteria).

Microbial Source Tracking (MST) analysis

Sediment samples were extracted for purified metagenomic DNA representing all organisms within the sample at the time of extraction. These sediment samples were extracted with the commercial product "FAST-DNA Spin Kit for Soil" (MPBiomedicals/Life Technologies) per manufacturer recommendations. The purified metagenomic community DNA from these samples was separated into replicate aliquots, with some used for immediate analysis, and some archived for long-term storage. DNA samples were then analyzed by quantitative PCR for the presence of fecal-host markers by human sewage/septic, agricultural, and urbanized surface run-off using host-source MST assays previously described (e.g., Sinigalliano et al. 2010; Campbell et al. 2015).

A summary of the MST assay results for all sediment samples is given in Table 1. The highest levels of fecal-indicator bacteria were found for samples collected near PortMiami in 2016. One of these samples ("Port 4", see Table 1) showed high levels which would be considered a health concern, indicating potential exposure of settled sediments to septic/sewage waste and/or terrestrial run-off, and potentially hazardous conditions as result of high levels of human-fecal markers. In contrast, none of the reef sediments collected from Emerald or Horseshoe reefs showed bacterial fecal-indicator levels that would be considered of concern (although one of the Emerald samples collected near Miami in 2016 did show presence of fecal-indicator bacteria at low levels, see Table 1). Finally, none of the samples collected in 2017 indicated the presence of fecal-indicator bacteria, suggesting potential "flushing" of the system, perhaps due to sediment transport and hydrodynamic processes in the area.

16S microbial amplicon sequencing

DNA samples underwent standard protocols detailed in the Earth Microbiome Project (EMP, http://www.earthmicrobiome.org/emp-standard-protocols/16s/). Briefly, samples were PCR-amplified with the 16S rRNA 515F/806R primers that target the variable-4 (V4) region of the small subunit ribosomal DNA. Library preparations and sequencing on the MiSeq platform took place at Nova Southeastern University (Dania Beach, FL). The data was analyzed with the software package qiime-2.2018.2 using the dada2 pipeline to identify amplicon sequence variants (ASVs). To annotate the ASVs taxonomically, the qiime SILVA 128 99% clustered database was

used. The SILVA database was trained with the qiime-2 feature classifier option using “fit-classifier-naive-bayes” and the "extract reads" option was utilized to train the SILVA 128 database on the 16S rRNA V4 region. To evaluate potential important bacteria genera found at the Port of Miami and coral reef sediment samples, the qiime-2 random forest classifier was used with 1,000 trees. This is a supervised machine learning algorithm and can be used to classify bacteria taxa into categories (i.e., PortMiami sediments vs. reef sediments). Finally, to evaluate significantly differentially abundant bacteria present between PortMiami and reef sediments the software package EdgeR was used. The microbiota was evaluated for significant abundance with an exact test and considered significant if Bonferroni corrected p-values were < 0.05.

Overall, we found strong differences in bacterial communities observed in reef sediments (collected from Horseshoe and Emerald reefs), compared to sediments collected near PortMiami, regardless of year (2016 or 2017, see Figure 1). Although one of the samples collected from Emerald reef appears to harbor microbial communities more similar to PortMiami sediments than to other reef sediments (Figure 1), this may not be surprising given the proximity of this reef to Miami’s populated areas. These findings are consistent with recent literature which suggest that bacterial communities associated with different marine habitats (inlets vs. reef sites) tend to be different (Campbell et al. 2015). However, additional work could be aimed at also characterizing microbial communities present in water and/or coral samples in these habitats (in addition to sediment samples), as recent literature has shown that bacterial communities differ between compartments of the coral holobiont and/or surrounding water (Sweet et al. 2011).

Strong differences were also observed in the relative abundance of bacterial types present in reef sediment samples vs. those collected near the PortMiami (Figure 2). An important bacterial type (i.e., predictor of sites) from the deltaproteobacteria family Desulfobacteraceae was found in sediment samples collected near PortMiami at ~6 times higher abundance compared to reef sediments (Figure 2), and appear to be more abundant in all PortMiami samples collected in 2017 compared to 2016 (Figure 3), perhaps indicating the successful proliferation (i.e., growth) in this Port-type fine-grained sediments over the course of a one-year period. This is of concern, as recent work has correlated the presence of bacteria in this family with coral black band disease (Klaus et al. 2011). Desulfobacteraceae may be also responsible for the anaerobic production of hydrogen sulfide in these sediments (the black layer), as a result of sulfate reduction to sulphides to make energy (Klaus et al. 2011). This might be a consequence of the fine sediments at the Port (which favor anoxia) compared to the coarse-grained sediment on the reef. Taken together, these findings may support recent literature which suggests that dredging-associated sedimentation and turbidity may be a mechanism for the transmission of coral disease (Pollock et al. 2014).

Anticipated deliverables

The results from this project were presented at MOTE in April 2019 at the annual project presentations meeting and a manuscript is planned for completion in 2020-2021.

Description of purchases

Funds were used to finish analyses of all samples collected and hire a Master’s student to help with data analyses and manuscript preparation.

References cited

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Table 1. Summary of Microbial Source Tracking (MST) assay for all sediment samples collected in 2016-2017 (all direct DNA extractions). GE/g = Genome equivalents per gram, TSC/g = target sequence copies per gram, ND = not detected, DNQ = detected but not quantifiable (below quantification range). Values of health concern (≥1000 GE/g for Enterococci or ≥100 GE/g or TSC/g for human and/or dog bacteroides) are highlighted in red.

Sample (site-replicate-year)	**Marker and units**				
	Total Enterococci (Entero1A)	**Human Bacteroides (HF-183)**	**Human Bacteroides (HumM2)**	**Human Bacteroides (BacHum-UCD)**	**Dog Bacteroides (AOML)**
	GE/g sediment	**GE/g sediment**	**TSC/g sediment**	**GE/g sediment**	**TSC/g sediment**
PORT1-2016	56.0	12.6	ND	13.9	27.3
PORT2-2016	ND	ND	ND	ND	12.1
PORT3-2016	71.3	ND	ND	ND	**218.0**
PORT4-2016	**1819.0**	**496.8**	**760.1**	**512.3**	**2690.2**
EMERALD1-2016	180.8	60.6	43.5	15.3	ND
EMERALD2-2016	ND	ND	ND	ND	ND
EMERALD3-2016	ND	ND	ND	ND	ND
EMERALD4-2016	ND	ND	ND	ND	ND
PORT1-2017	ND	ND	ND	ND	ND
PORT2-2017	ND	ND	ND	ND	ND
PORT3-2017	ND	ND	ND	ND	ND
HORSESHOE 1A-2017	ND	ND	ND	ND	ND
HORSESHOE 1B-2017	ND	ND	ND	ND	ND
HORSESHOE 1C-2017	ND	ND	ND	ND	ND
HORSESHOE 2A-2017	ND	ND	ND	ND	ND
HORSESHOE 2B-2017	ND	ND	ND	ND	ND
HORSESHOE 2C-2017	ND	DNQ	ND	ND	ND

Figure 1. Differential abundance analysis. The Y-axis represent bacterial families that were significantly different ($p < 0.05$) between reef sediments (Emerald and Horseshoe reefs) and sediments collected near PortMiami.

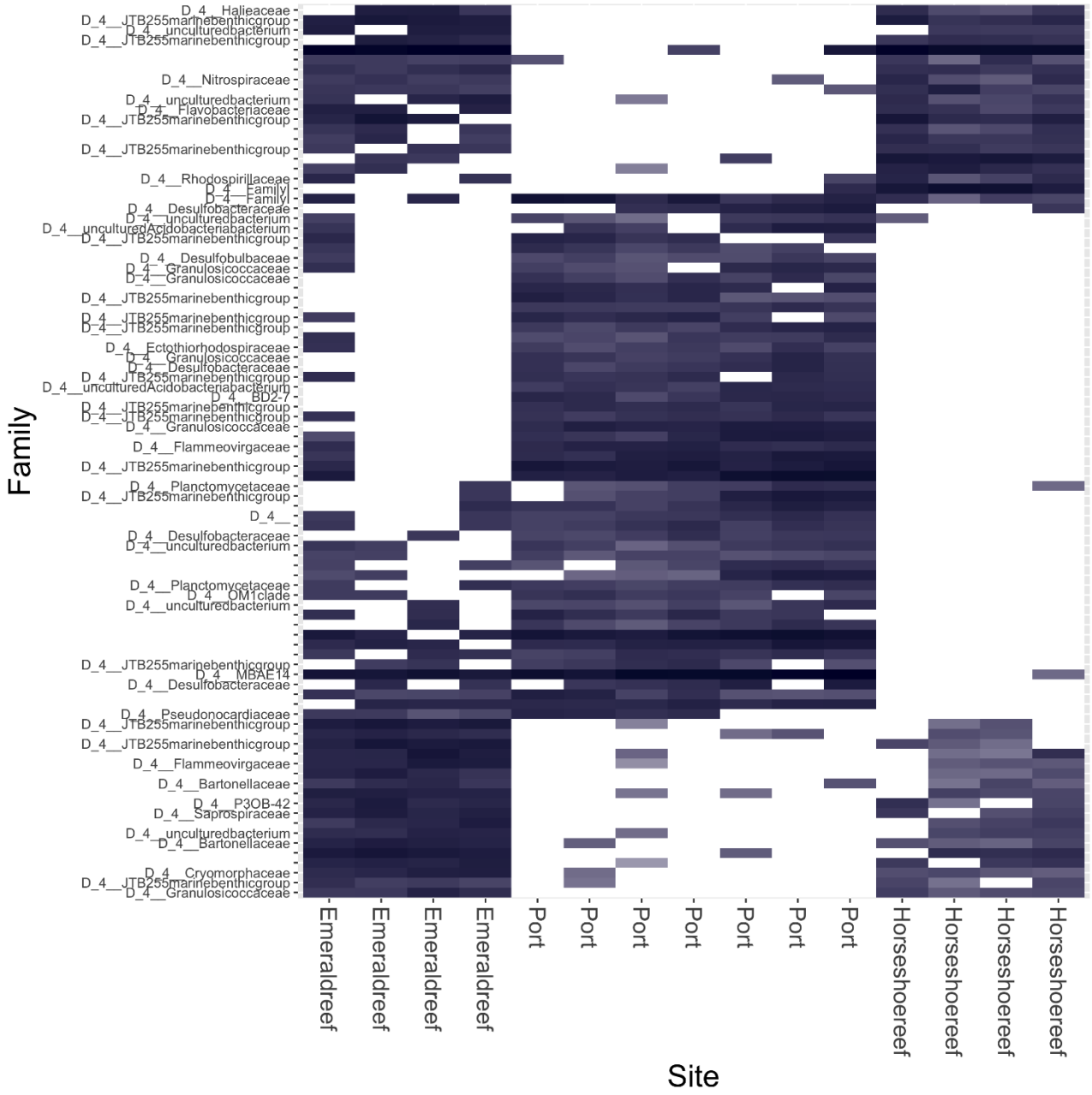


Figure 2. Relative abundance of the most abundant bacterial families (> 0.01 %) found in the sediment at each site.

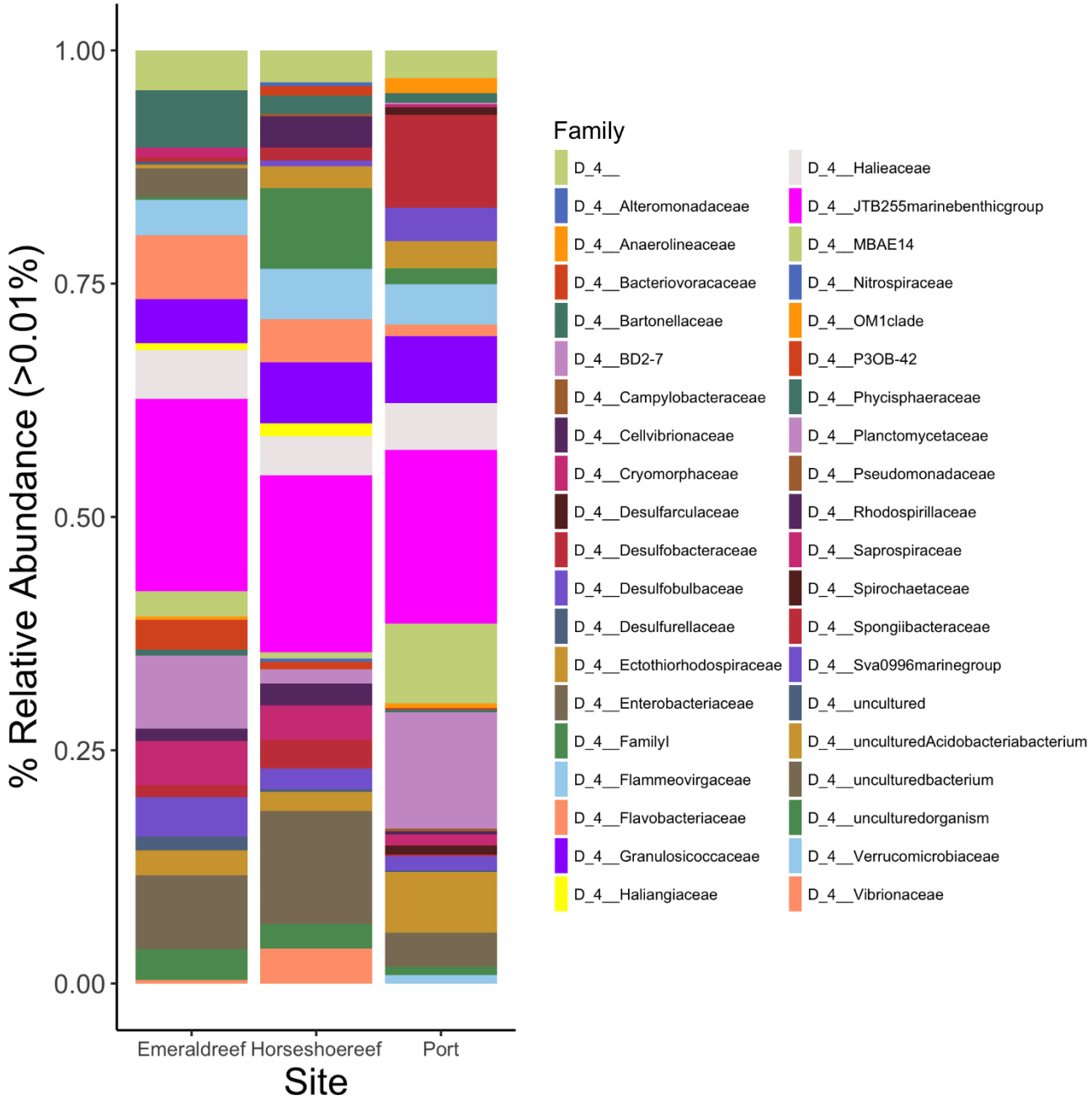
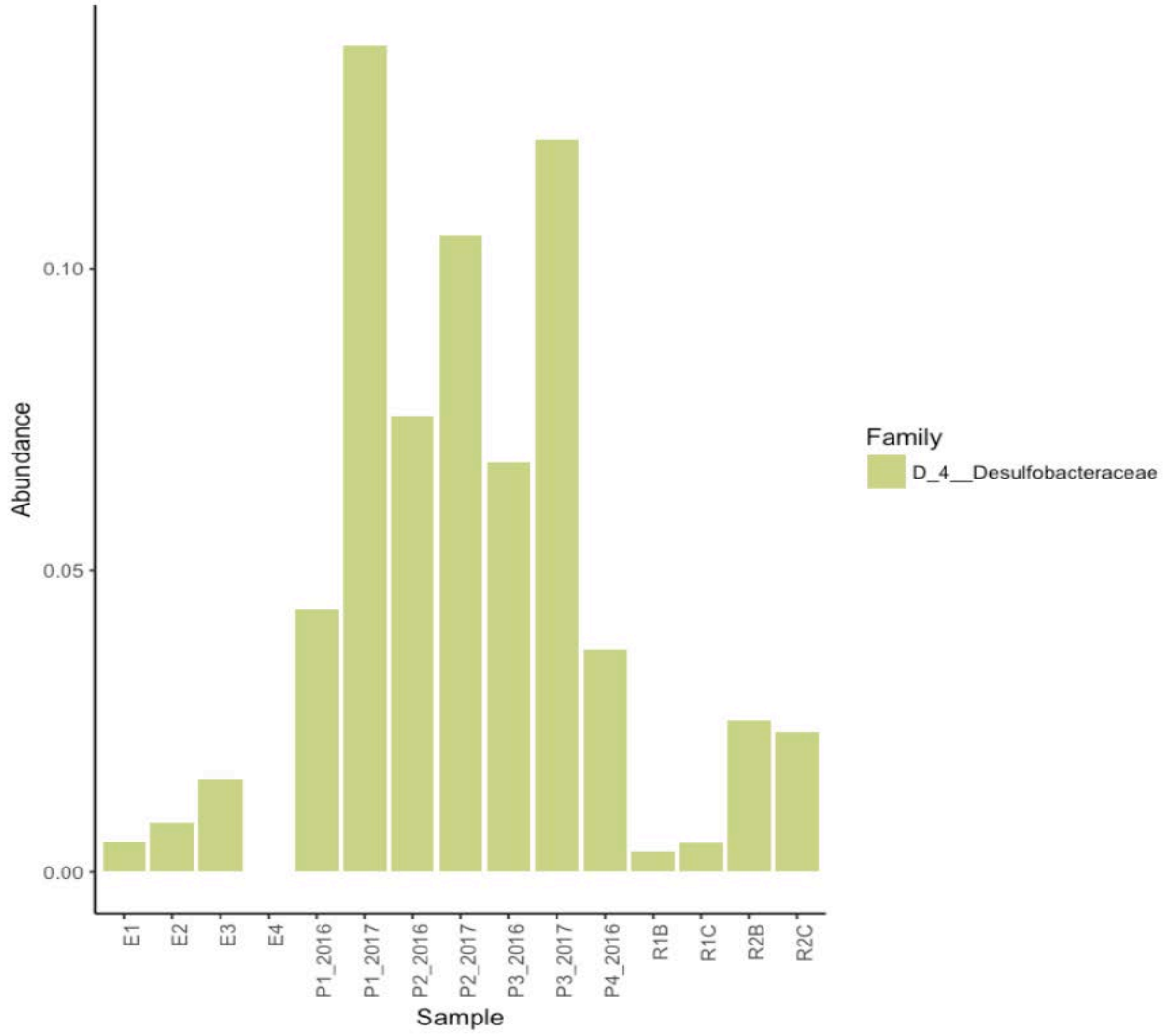


Figure 3. Relative abundance of the Proteobacteria family Desulfobacteraceae found in each sediment sample. Samples from Emerald reef were collected in 2016, whereas samples from Horseshoe reef were collected in 2017. P= Port, E=Emerald reef, R= Horseshoe reef



ATTACHMENT A: POR-2015-15-FINAL REPORT

Grantees: Dr. Andrew Baker (PI), Dr. Xaymara Serrano (co-PI), and Dr. Margaret Miller (collaborator)

Project Title: Effects of sedimentation stress in adult and early life stages of corals, including two ESA listed species

Report Period: November 2, 2016-August 31, 2017

Deliverables:
During Year 1 we planned on collecting *O. faveolata* and *A. palmata* larvae during the expected spawning season, as well as conducting experiments with these larvae. In addition, we planned to conduct proposed experiments with adult corals, which could be done any time in the year.

PROGRESS TO DATE

Field collection of gametes
Orbicella faveolata and *Acropora palmata* gametes were collected during the 2017 spawning season and larvae cultured as described in Szmant and Miller (2006). We obtained ~3,000 *O. faveolata* larvae to use in the experiments described below (but note that there were insufficient larvae from the species *A. palmata* due to poor fertilization rates).

Experiments and preliminary analyses
All experiments were carried out at the new Marine Technology and Life Science Seawater (MTLSS) building at the University of Miami's Rosenstiel School (RSMAS). Two different sets of experiments were conducted, as follows:

I. Effects of sedimentation on larval performance
Sediments were collected at two different sites: Horseshoe reef in Key Largo (our "Reef" sediment type, where parent colonies also originated from) and at a site near PortMiami (our "Port" sediment type). Sediments were collected with permits from the FKNMS and Florida DEP. Fifty larvae were added to individual glass vials containing 45 mL filtered seawater. Vials were allocated randomly to one of five different treatments: (1) a control (no sediment), (2) a low "Reef" sediment treatment (~0.2 grams of sediment from Horseshoe reef added), (3) a low "Port" sediment treatment (~0.2 grams of sediment from the Port site added), (4) a high "Reef" sediment treatment (~1 gram of sediment from Horseshoe reef added), and (5) a high "Port" sediment treatment (~1 gram of sediment from the Port site added). Nine to ten replicates were used per treatment. Vials were then attached to a titer plate shaker and left for 24 h (initial trials were conducted to assess "best" exposure times that caused minimal mortality in control larvae). After 24 h of exposure, swimming larvae remaining in each vial were counted and examined with a microscope to determine the proportion that survived (Figure 1). A subset of larvae (N = 6 per vial) was also used to assess changes in respiration rates (data not shown). Larvae were then allowed to recover for 1-week (without sediments) before being transported back to Key Largo to conduct the settlement assays (see below).

The settlement assays were conducted by pooling all the larvae remaining in the vials per treatment and adding fifteen larvae to individual petri dishes containing filtered seawater and a piece of rubble collected from Horseshoe reef to act as settlement cue. Nine replicates were usedper treatment. After 24 h, the total number of larvae still swimming, or that had settled on the rubble, were scored to determine the proportion that had settled (Figure 2). Overall, preliminary analyses suggest significant effects of the "Port" type of sediment in the survival of *O. faveolata* larvae after 24 h of exposure (Figure 1), and on settlement after a 1-week recovery period (even at the low dose treatment, see Figure 2). Together, these findings suggest that the success of early life stages of *O. faveolata* may be compromised by the presence of PortMiami sediments during the pelagic phase. In addition, these findings suggest both short- and long-term effects of sedimentation, as effects are still observed after corals are allowed to recover for 1 week. Together, these findings provide empirical data to local managers that might support the implementation of environmental policies aimed at minimizing the impacts of dredging operations, especially during expected coral reproductive seasons.

I. Effects of sedimentation on adult corals

Sediments were collected at two different sites: Emerald reef near Miami (our "Reef" sediment type and where coral colonies were also collected from) and at the same site near PortMiami, as described above. Coral colonies were collected with permits from the Florida Fish and Wildlife Commission. Replicate "mini" cores were taken from four different coral colonies from each of two species (*Siderastrea siderea* and *Porites astreoides*, after preliminary trials showed that mini-cores of these species had the highest survival over time), and randomly allocated to experimental aquaria. Two independent aquaria were used per treatment and there were 7 different treatments: (1) a control (no sediment), (2) a low "Reef" sediment treatment (20 mg cm^{-2} d^{-1} of sediment from Emerald reef added directly on top of each core), (3) a low "Port" sediment treatment (20 mg cm^{-2} d^{-1} of sediment from the Port site added directly on top of each core), (4) a high "Reef" sediment treatment (80 mg cm^{-2} d^{-1} of sediment from Emerald reef), (5) a high "Port" sediment treatment (80 mg cm^{-2} d^{-1} of sediment from the Port site), (6) a high "Reef" sediment treatment with shading, and a (7) a high "Port" sediment treatment with shading. The last two treatments were used to mimic the loss in PAR as a result of increased turbidity.

Overall, physiological changes were assessed for each core at (1) the initial time point (prior to exposure), (2) the end of a 96 h exposure to experimental treatments, and (3) after a 2-week recovery period. At each of these time points, we measured respiration rates (using a new 1,700 uL microplate reader developed by Loligo Systems®), partial/total tissue loss (via photographs) and took I-PAM measurements to assess bleaching susceptibility of treatment corals. Experiments were recently completed and data is being analyzed.

Microbial Source Tracking Analysis of sediment samples

Sediment samples were collected from the two sites described above for initial analyses. These samples are currently stored in NOAA/AOML to be extracted and analyzed.

Plan of action for the next 9 months

The majority of proposed experiments were completed at the end of August 2017. In Year 2, we

plan to work with MOTE Marine Lab, the University of Miami's *Rescue A Reef* Program and/or the Coral Reef Conservation Foundation (CRF) to determine the availability of *A. cervicornis* genotypes we can use to complete our experiments with adult corals. In addition, the remains of Year 2 will be dedicated to analyzing all the data from experiments and preparing manuscripts for publication.

Anticipated deliverables

We hope to submit 1-2 manuscripts from the work conducted in Years 1-2 in 2017-2018. All data and results from this project will be presented in a final technical report anticipated to be available by April 2018.

Description of purchases

Funds have been used to set up and conduct proposed experiments. Remaining funds will be used to complete the experimental work and conduct analyses of all samples collected.

Figure 1. Percentage of larval survival in *Orbicella faveolata* after a 24 h exposure to experimental treatments. Error bars represent SEM. Asterisks indicate significant differences with respect to the control treatment ($p < 0.05$, one-way ANOVA and Dunnet's post hoc comparison test). Data was arcsine square root transformed prior to statistical analysis in order to meet assumptions of normality.

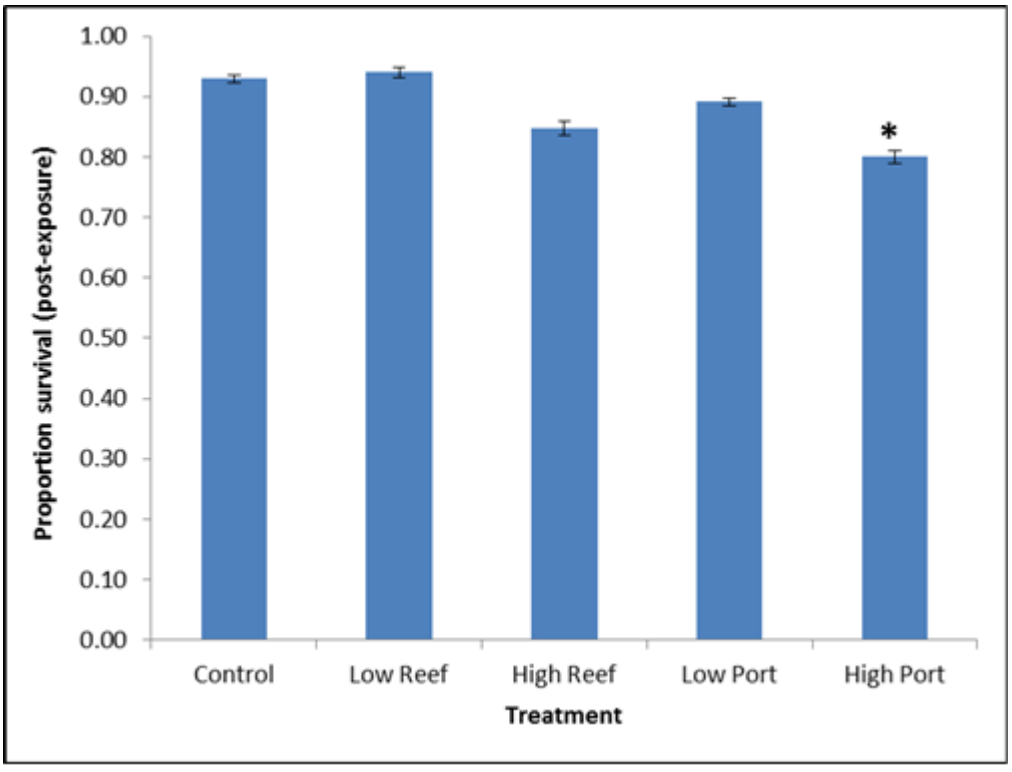
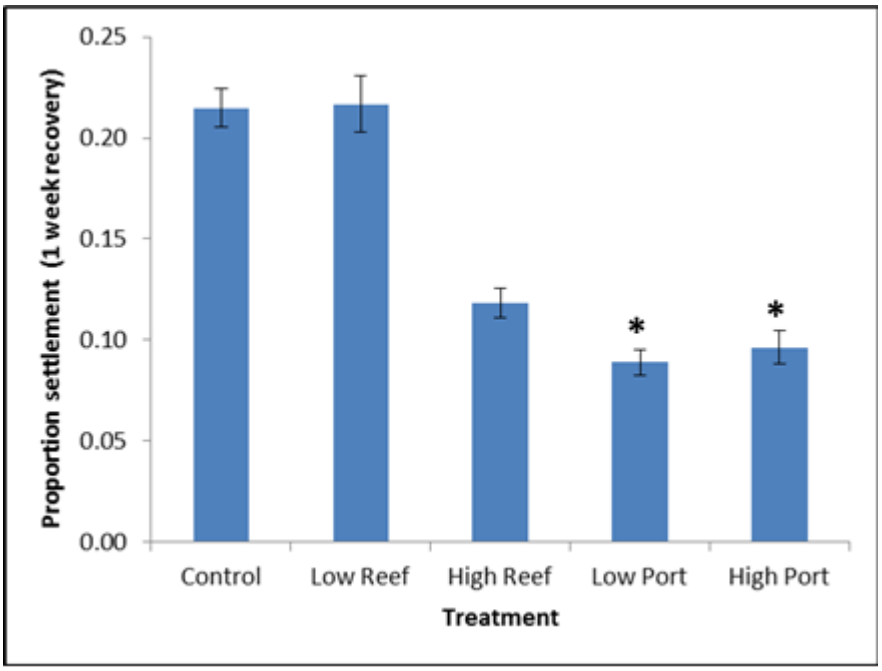


Figure 2. Percentage of *Orbicella faveolata* larvae settled in assays conducted after a 1-week recovery period. Error bars represent SEM. Asterisks indicate significant differences with respect to the control treatment ($p < 0.05$, one-way ANOVA and Dunnet's post hoc comparison test). Data was arcsine square root transformed prior to statistical analysis in order to meet assumptions of normality.



ATTACHMENT B: POR-2016-16 INTERIM REPORT

Grantees: Dr. Xaymara Serrano (PI), Dr. Andrew Baker (co-PI), and Dr. Margaret Miller (collaborator)

Project Title: Effects of sedimentation stress in adult and early life stages of corals, including two ESA listed species

Report Period: September 1, 2017-December 31, 2017

Deliverables:
In Year 2 we planned to conduct the remaining experiments with *Orbicella faveolata* and *Acropora palmata* larvae (if sufficient larvae were available) looking at the effects of sedimentation on settlement success and respiration rates. In addition, we planned to complete the experiments with adult corals and perform analyses of samples collected for all species. Finally, we planned to dedicate Year 2 to complete the data analyses and prepare manuscripts to be published.

PROGRESS TO DATE

Experiments and preliminary analyses
All experiments were carried out at the new Marine Technology and Life Science Seawater (MTLSS) building at the University of Miami's Rosenstiel School (RSMAS). Two different sets of experiments were conducted, as follows:

I. Effects of sedimentation on larval performance

Orbicella faveolata gametes were collected during the 2017 spawning season and experiments were completed in August as described in the ***final report of POR 2015-15*** (note that there were insufficient larvae from the species *A. palmata* for proposed experiments due to poor fertilization rates). Overall, findings suggest that the success of early life stages of *O. faveolata* may be compromised by the presence of PortMiami sediments during the pelagic phase. In addition, findings suggest both short- and long-term effects of sedimentation, as effects were still observed after corals were allowed to recover for 1 week (***see final report of POR 2015-15*****)**. Together, these findings provide empirical data to local managers that might support the implementation of environmental policies aimed at minimizing the impacts of dredging operations, especially during expected coral reproductive seasons.

II. Effects of sedimentation on adult corals

Sediments were collected at two different sites: Emerald reef near Miami (our "Reef" sediment type and where coral colonies were also collected from) and at a site near PortMiami (our "Port" sediment type), with permits from the FKNMS and Florida DEP. Coral colonies were collected with permits from the Florida Fish and Wildlife Commission. Replicate "mini" cores were taken from four different coral colonies from each of two species (*Siderastrea siderea* and *Porites astreoides*, after preliminary trials showed that mini-cores of these species had the highest survival over time), and randomly allocated to experimental aquaria. Two independent aquaria were used per treatment and there were 7 different treatments: (1) a control (no sediment), (2) a low "Reef" sediment treatment (20 mg cm^{-2} d^{-1} of sediment from Emerald reef added directly on

top of each core), (3) a low "Port" sediment treatment (20 mg cm^{-2} d^{-1} of sediment from the Port site added directly on top of each core), (4) a high "Reef" sediment treatment (80 mg cm^{-2} d^{-1} of sediment from Emerald reef), (5) a high "Port" sediment treatment (80 mg cm^{-2} d^{-1} of sediment from the Port site), (6) a high "Reef" sediment treatment with shading, and a (7) a high "Port" sediment treatment with shading. The last two treatments were used to mimic the loss in PAR as a result of increased turbidity. Then, physiological changes were assessed for each core at (1) the initial time point (prior to exposure), (2) the end of a 96 h exposure to experimental treatments, and (3) after a 2-week recovery period. At each of these time points, we measured respiration rates (using a new 1,700 uL microplate reader developed by Loligo Systems®), partial/total tissue loss (via photographs) and took I-PAM measurements to assess bleaching susceptibility of treatment corals (only for species *S. siderea*). Experiments were completed and data is currently being analyzed.

Preliminary analyses suggest species-specific effects of sedimentation stress on adult coral respiration. Significant effects on respiration were observed for the species *S. siderea* (Figure 1, right panel) but not for *P. astreoides* (Figure 1, left panel). For *S. siderea*, high doses of the PortMiami sediments appear to decrease respiration rates with respect to corals exposed to reef sediments, suggesting detrimental effects on metabolism. However, no significant effects were observed in photochemical efficiency (Fv/Fm) for *S. siderea* at any time point (Figure 2, no data available for *P. astreoides*), suggesting no effects on bleaching susceptibility (at least for the short tested period). Further analyses will reveal if there are any latent effects after a 2-week recovery period and whether corals displayed partial/total tissue loss.

Overall, data from both set of experiments preliminary suggests that early life stages of corals are more susceptible to the effects of sedimentation stress from PortMiami than adult corals. However, effects on adult corals appear to be species-specific, and suggest that *P. astreoides* may be more resistant to sedimentation stress compared to *S. siderea*. This is not entirely surprising, as this "weedy" species appears to be the only scleractinian coral that is becoming a more prominent component of coral reef communities throughout the Caribbean (Green et al. 2008). Further work could be aimed at assessing whether detrimental effects may be observed for *P. astreoides* after a longer exposure period.

Microbial Source Tracking Analysis of sediment samples

Sediment samples were collected from the two sites described above for initial analyses. These samples are currently stored in NOAA/AOML to be analyzed.

Unanticipated obstacles

In Year 2, we planned to work with the University of Miami's *Rescue A Reef* Program to determine the availability of *A. cervicornis* genotypes we could use to complete our experiments with adult corals. However, as a result of Hurricane Irma many of the *A. cervicornis* genotypes we intended to use were no longer available for use in the experiments. In addition, preliminary trials revealed poor survival of *A. cervicornis* "mini-cores" prior to experiments suggesting this species was not a good candidate for proposed experiments.

Plan of action for the next 6 months and anticipated deliverables

Proposed experiments with adult corals (2 species) and larvae (1 species) were completed in August 2017. The remains of Year 2 will be dedicated to finish the analyses of experimental data

and prepare manuscripts for publication. We hope to submit at least one manuscript from the work conducted in Years 1-2 in 2018. Finally, all data and results from this project will be presented in a final technical report anticipated to be available by April 2018.

Description of purchases

Remaining funds will be used to finish analyses of all samples collected and hire a Master's student to help with data analyses and manuscript preparation.

Figure 1. Average respiration rates (± SE) measured in *Porites astreoides* (left panel) and *Siderastrea siderea* (right panel) adult "mini-cores" after 96-h exposure to a no sediment control (NS), low "Reef" sediment treatment (RL), high "Reef" sediment treatment (RH), low "Port" sediment treatment (PL), or high "Port" sediment treatment (PH). A total of N = 7-15 replicate wells were used per treatment. Note difference in scale of y-axis for species. Treatments not connected by the same letter are significantly different ($p < 0.05$; one-way ANOVA and Student's t posthoc comparison test).

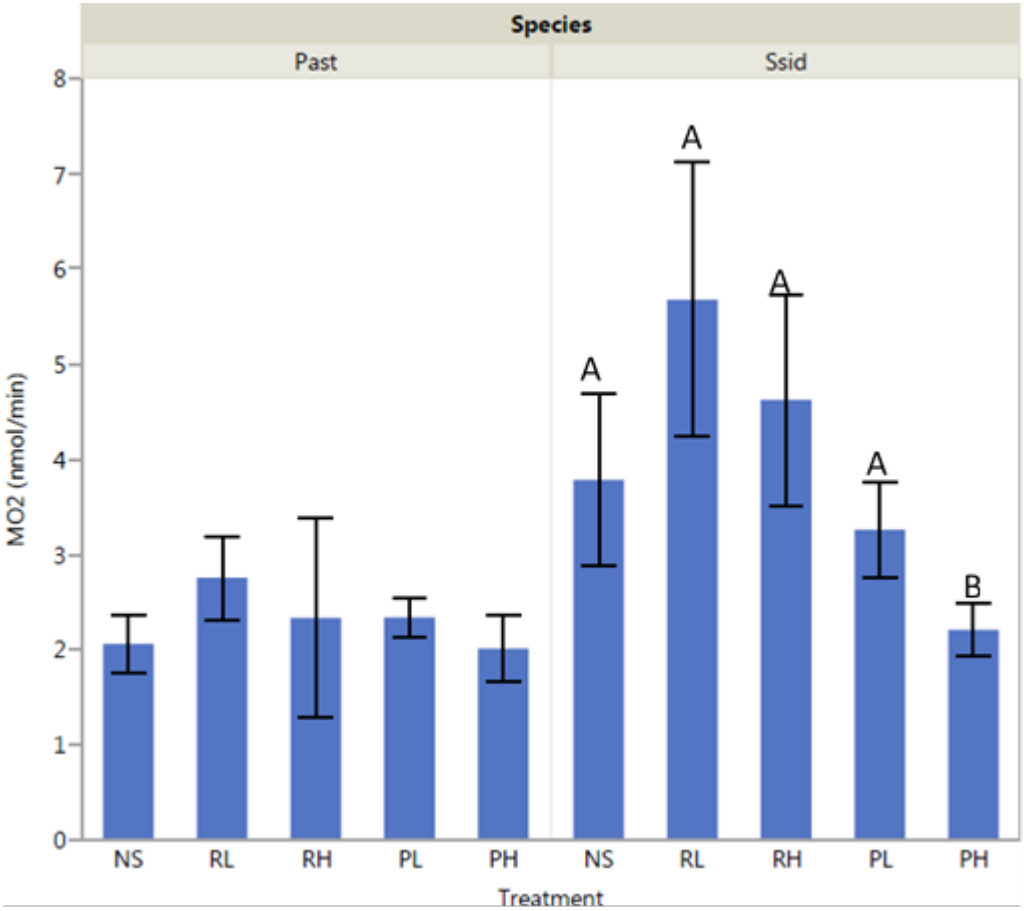


Figure 2. Maximum quantum yield of photosystem II (Fv/Fm) of dark-adapted *Symbiodinium* in experimental corals. Average Fv/Fm values (± SE) measured in *Siderastrea siderea* adult "mini-cores" after 96-h exposure to a no sediment control (NS), low "Reef" sediment treatment (RL), high "Reef" sediment treatment (RH), low "Port" sediment treatment (PL), or high "Port" sediment treatment (PH). No significant differences were found among treatments at any time point.