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NAME OF RECIPIENT ORGANIZATION:	Nova Southeastern University
PRINCIPAL INVESTIGATOR:	Amy C. Hirons (PI) Cameron Baxley
PROJECT TITLE:	Impacts of land-based sources of pollution on two Caribbean coral species
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PROJECT SUMMARY

Elevated levels of two common land-based pollutants, phosphate found in fertilizer and endosulfan found in pesticide, have been detected in canals, inlets, and oceanic waters of South Florida. Although both have been shown to have biologically deleterious effects, few studies have tested environmentally realistic levels on Caribbean corals and none have examined the threshold of effects. The levels of orthophosphate and endosulfan were measure in the nearshore water column and sediments, and in the tissues of two Caribbean coral species, *Montastraea cavernosa* and *Porites astreoides*. Laboratory dosing experiments using a range of concentrations of the two pollutants were performed to determine the effects on coral health and function. No detectable levels of orthophosphate were found in any coral dosing tanks for the five treatment concentrations. At the same time, an unprecedented algae bloom occurred that obscured the sides of the tanks and covered both coral species, likely utilizing the available limiting nutrient. Endosulfan (I, II, and sulfate) was not detected in ocean water or sediment. Endosulfan II was not detected in the tank water or either coral species at any of the treatment levels and endosulfan sulfate was only discernible at extremely low levels, if at all, in all treatments. Endosulfan I was continuously removed from each

treatment tanks' water from the beginning to end of the five day exposure, reducing the amount ultimately found in the water by 41-88%. Tissue incorportation of endosulfan concentrations was on the order of one-one thousandth. P. asteroides struggled to survive throughout the entire experiment. No noticeable trends were observed in zooxanthellae condition or density for either experiment. Increases in endosulfan concentration resulted in increases in number and size of the mucous secretory cells. Phosphate appeared to be a greater stressor as compared to endosulfan. Increased swelling of mucous secretory cells and widespread atrophy in epithelia cells coincided with the highest exposure of phosphate.

INTRODUCTION

The effects of land-based sources of pollution, including increased nutrients, chemicals and pesticides, on coral reef ecosystems, are of particular concern off of the heavily developed southeast coast of Florida. Coral reefs generally occur in waters with low levels of inorganic nutrients (0.2 to 0.5 μM ammonium, 0.1 to 0.5 μM nitrate, and <0.3 μM phosphate, Furnas 1991). Recent water quality monitoring by the National Oceanic and Atmospheric Administration (NOAA) Florida Area Coastal Environment (FACE) program has identified levels of phosphate approaching 2 μM in the outflow from the Port Everglades Inlet, Ft. Lauderdale, Florida (Featherstone pers. comm.). Additionally, pesticide residues have been documented in the south Florida canal system and Biscayne Bay (Miles and Pfeuffer 1997, Pfeuffer and Rand 2004, Harman-Fetcho et al. 2005). Also, flame retardants have been found in tissues of marine organisms off southeast Florida's coast (Fair et al. 2010). Increasing coastal population density and storm event frequency associated with climate change are expected to result in increased coastal runoff (Landsea 2000a, 2000b, Goldenberg et al. 2001, Houghton et al. 2002, Karl and Trenberth 2003). As such, identifying the specific effects of land-based sources of pollution on coral reef ecosystems is of utmost importance.

Significant increases in nutrient concentrations (>1 μM inorganic nitrogen, >0.3 μM phosphorus) have been shown to have negative effects on coral growth rates (Hallock and Schlager 1986, Stambler et al. 1991, Ferrier-Pagès et al. 2000, Renegar and Riegl 2005). Multiple studies have found that the effects of elevated phosphate are concentration dependent and compared to nitrate, may have greater effects at lower concentrations (Kinsey and Davies 1979, Walker and Ormond 1982, Tomascik and Sander 1985, Ferrier-Pagès et al. 2000, Renegar and Riegl 2005, Renegar et al. 2008). Phosphate may act as a crystal poison (Simkiss 1964) and, as a limiting nutrient, may affect the coral zooxanthellae symbiosis (Renegar et al. 2008). However, the majority of these studies have utilized phosphate concentrations that are orders of magnitude higher than those tested on even polluted reefs (Szmant 2002). To date, no investigation of the threshold of effects at sub-lethal phosphate concentrations has been performed. Such research is of critical importance to definitively link coral reef degradation in southeast Florida to pollutant exposure.

Several studies have demonstrated the deleterious effects of toxicants such as pesticides and herbicides on non-target species. Endosulfans, a group of neurotoxic

insecticides, are one of the compounds found in the highest concentrations in the south Florida canal system (Miles and Pfeuffer 1997, Scott et al. 2002, Pfeuffer and Matson 2002, 2003, Key et al. 2003, Pfeuffer and Rand 2004, Harman-Fetcho et al. 2005). Estimated regional usage of endosulfan from 1992-1995 was 36 tons per year, largely on commercial agricultural lands (Miles and Pfeuffer 1997). Harman-Fetcho et al. (2005) report total endosulfan mean and maximum concentrations of 11 and 98 ng/L, respectively, in south Florida canals. Previous studies focusing on non-cnidarian invertebrates in the C-111 canal system have suggested that endosulfan may already be causing chronic toxic effects in bivalves, including clams and oysters (Scott et al. 2002). Endosulfan acts as a neurotoxin, xenoestrogen, and an endocrine disruptor. Previous research has shown long term ontogenetic consequences to short term, low concentration endosulfan exposure in the form of decreased growth rate, delayed maturity and decreased fecundity in Japanese killifish (*Oryzias latipes*) (0.01-0.1µg/L; Gromley and Teather 2003), altered estrogen dependent gene expression in rats (0.006mg/kg/day; Varayoud et al. 2008), and decreased settlement and metamorphosis of coral larvae (*Acropora millepora*) (0.1-0.3µg/L; Markey et al. 2007). Harman-Fetcho et al. (2005) also reported an off shore presence of endosulfan in Biscayne Bay. As a result, the potential lethal and sub-lethal effects of endosulfan on corals are of concern to the northern portions of the Florida reef tract.

The pesticide endosulfan is composed of two chemical isomers, endosulfan I and endosulfan II; they are present in a 7:3 ratio. Endosulfan II converts to the more stable endosulfan I at high temperatures. Endosulfan sulfate is a reaction product of endosulfan and is found in the environment due to oxidation by biotransformation (Cotham and Bidleman 1989).

MATERIAL AND METHODS

Multiple laboratory dose response experiments were undertaken using phosphate and endosulfan to assess examining the physiological effects of two types of pollutants on two species of coral, *Montastraea cavernosa* and *Porites astreoides*. Methods to evaluate the effects of these stressors included chemical analysis of pollutant levels in the water, sediments and coral tissue, histological analysis of tissue structure, and identification of genetic stress markers.

Field

Twelve whole coral colonies of each species, *Montastraea cavernosa* and *Porites astreoides*, were collected from the middle reef of the South East Florida Reef Tract, 2 km north of Port Everglades (26° 08’ N, 80° 05’ W). Colonies were removed with hammer and chisel and transported in 80 gal. marine coolers containing local seawater. The colonies were places in land-based coral nursery at Nova Southeastern University’s Oceanographic Center. Seawater was sampled at the site of coral collection in 1 L amber glass bottles at 0.5 m and 8 m depths. These samples were designated surface and bottom water samples, respectively. Several centimeters of bottom sediment were collected in a

250 ml clear glass bottle. All samples were kept cool and dark and transported to Florida Spectrum laboratories for chemical analyses.

Water and tissue chemistry

Phosphate concentrations in seawater were determined as the concentration of orthophosphate ion as phosphorus using EPA Method 365.1. The method detection limit was 3.0×10^{-3} mg/L and the practical quantification limit was 9.0×10^{-3} mg/L for orthophosphate. Detection of endosulfan isomers endosulfan I, endosulfan II, and endosulfan sulfate required use of EPA Method 8081A for organochlorine pesticides. The method detection limit was 4.5×10^{-3} µg/L and the practical quantification limit was 1.35×10^{-2} µg/L for endosulfan I. The method detection limit was 5.8×10^{-3} µg/L and the practical quantification limit was 1.74×10^{-2} µg/L for endosulfan II. The method detection limit was 2.6×10^{-3} µg/L and the practical quantification limit was 7.8×10^{-3} µg/L for endosulfan sulfate (http://water.epa.gov). The method detection limit is the minimum level of target analyte that can be determined with 99% confidence. The practical quantification limit is the lowest level that can be reliably achieved within specified limits of precision and accuracy during routine laboratory operating conditions.

Florida Spectrum Environmental Services Inc. analyzed the sediment and water samples collected at the time of coral collection and no discernible levels of endosulfan isomers were detected in the sediment or water. The planned dosing levels were below instrumentation detection limits and an increase in the dosing factor from ng/L to µg/L or mg/L range was recommended. Endosulfan experimental concentrations were increased by a factor of 100 to assure detection.

Dosing experiment

Fifteen, forty gallon SECOR dosing tanks, each with two, 20 gallon input/output tanks, were cleaned and placed in a water table for temperature control. Three replicate tanks for five treatment concentrations for each of the two pollutants were used. The tanks were filled with seawater and covered with additional UV screening and polyethylene vinyl acetate (PEVA) shower curtains to prevent freshwater input from precipitation and to limit evaporation. Daily temperature and salinity measurements were recorded with a YSI meter during the fourteen-day phosphate experiment and five-day endosulfan experiment. A thermometer in each water bath was connected to a chiller which would turn on once the water temperature exceeded a temperature maximum (25° C). Due to seasonal temperature fluctuations, the temperature probe was moved from the water bath to the 40 gallon tanks.

Each dosing tank used one circulation pump situated halfway down the side of the tank to pass seawater over, but not directly on, the corals. Air lines utilized polypropylene tubing connected to submerged glass pipettes. Lines were checked and any salt residue was removed every 48-72 hours. The pump for the seawater well which provided water to the tanks failed after the first five days of the phosphate experiment so seawater was replaced with Instant Ocean mixed with reverse osmosis (RO) water in 55 gallon drums and pumped into the 20 gallon tanks for water changes. Instant Ocean was also used during the endosulfan experiment. Peristaltic pumps were used to perform daily water

changes by removing 10 gallons of seawater from the 20 gallon tanks and moving it to the 40 gallon tanks via rigid polypropylene tubing. Rubber stoppers held the tubing at the 10 gallon mark in the 20 gallon tank to ensure only 10 gallons were exchanged. The rate of water exchange was one gallon per hour. Only 10 gallons of seawater per day were exchanged so no more than 25% of new water was introduced daily. Sources familiar with maintaining healthy corals indicated that water changes of 50% or more at one time can be stressful for a number of coral species (O'Neil pers. comm.). A large number of peristaltic pumps had diminished water flow after 48-72 hours and they were replaced with new pumps. The pumps appeared to have failed due to leaks in the tubing, most likely where the inner peristaltic tubing joined the polypropylene tubing. After day five, Instant Ocean was pumped into the 40 gallon dosing tanks within a 10 minute period versus the 12 hour interval the peristaltic pumps would have.

Colonies of both coral species were segmented into 3 x 3 cm fragments and placed in a 1000 gallon fiberglass holding tank. Water temperature was maintained at 25° C with continuous water flow. The corals were not fed immediately prior to or during the experiment in order to control extraneous phosphate concentration and bindable lipids for endosulfan. These colonies/fragments were allowed to acclimate for the same amount of time before the commencement of the phosphate and endosulfan experiments prior to placement in the SECOR dosing tanks. Each 40 gallon tank within a treatment contained four fragments of each species from distinct colonies. The daily dose of each concentration of phosphate and endosulfan was added directly to the 40 gallon tanks containing the coral fragments after the daily water change. The chemical was added over the circulation pump to aid in mixing throughout the tank. Phosphate and endosulfan waste water was collected in 55 gallon drums and disposed through the University's Environmental Health and Safety officer in Facilities Management.

The ambient seawater in the seawater system consistently had phosphate levels approximately 0.25 µM above our expected starting value of 0 µM. We adjusted our phosphate concentrations to account for the naturally occurring phosphate in the seawater which changed the treatment levels to control (0.25 µM), 0.5 µM, 0.75 µM, 1.25 µM, and 2.25 µM $P\text{-}PO_4^{3-}$ during the first week of the phosphate experiment. When the pump for the seawater well ceased functioning after five days, Instant Ocean replaced water from the seawater well and the experimental phosphate levels were returned to the original dosing concentrations. Seawater samples from each of the fifteen dosing tanks were collected daily during the fourteen day experiment to test for orthophosphate levels.

Endosulfan was dissolved in a 99% acetone carrier totaling 1 ml and three concentrations were administered to the dosing tanks, 500 ng/L, 5000 ng/L, and 10,000 ng/L. Additionally, one treatment consisted of Instant Ocean in RO water and another treatment consisted of 1 ml of acetone only. A 20 gallon water change for each of the fifteen tanks was performed on day three of the five day experiment. The endosulfan dose was added directly to the 40 gallon tank after the water change. Seawater samples from each of the fifteen dosing tanks were collected daily during the five day experiment.

At the completion of both chemical exposure experiments, coral fragments of each species and replicate were cut and separated for chemical, histological, and genetic analysis. Tissue samples for chemical analysis were frozen in a standard -20° C freezer. The tissue was removed from the calcium carbonate exoskeleton with a Dremel cutting tool, ground, and sent for chemical analysis. Histological samples were preserved in commercial Z-fix. *M. cavernosa* tissues were preserved in Trizol and P. asteroides tissues were preserved in qPCR fixative for genetic analysis.

Histology

Coral samples of both species were decalcified over a period of three to five days using acidic EDTA. After decalcification the tissues were trimmed with a new razor blade and placed in in perforated plastic processing cassettes. The cassettes were placed in 70% ethanol and moved through an automated tissue processor using increasing concentrations of ethanol, xylene, and paraffin for embedding. Blocks were split to tissues could be sectioned for slides in the sagittal and cross-section orientation. Microtome sections were cut at 5 μm, floated on a water bath and placed on new glass slides. Slides were allowed to dry for 2-4 hours before tissues on the slides were stained with hematoxylin and eosin. Slides were examined with an Olympus compound microscope at 40 x magnification to observe the size of mucocytes of gland cells, zooxanthellae density, and thickness and condition of the epidermis, gastrodermis, and calicodermis, and photomicrographs were taken.

RESULTS

Phosphate

Daily water samples were collected and analyzed by Florida Spectrum. No detectable phosphate was found in any of the seawater samples for any phosphate concentration. However, a highly visible algal bloom occurred in each of the experimental tanks throughout the phosphate experiment, qualitatively increasing with higher phosphate concentrations. The algae were on the corals as well as on the perimeter of the tanks and throughout the water column. The algal blooms could have further stressed the corals as they were distributed throughout the tanks by their circulation pumps. Similar results (high algae, no phosphate) were obtained by other studies utilizing various phosphate concentrations (McCorquodale pers. comm.). At the culmination of the dosing experiment, no mortality was observed.

Endosulfan

Due to maintenance difficulties with the SECOR dosing tanks, the endosulfan exposure experiment had to be completed in two parts. One-third of the experiment, one replicate of the five treatments, was conducted three weeks before the remaining two replicates of the five treatments.

No detectable levels of endosulfan I, II, or endosulfan sulfate were found in either the surface and bottom waters and the sediment samples when the coral colonies were collected. Daily water samples from the fifteen experimental tanks were collected and

analyzed for the three isomers. None of the endosulfan isomers was detected in any of the control or acetone treated tanks. Endosulfan II was not detected in any of the treatment tanks either at the beginning or the end of the five day experiment. The levels of endosulfan sulfate remained relatively unchanged from the beginning to the end of the experiment at all treatment levels, ranging from not detected at the 500 ng/L concentration to 0-74 ng/L at the 5000 ng/L concentration to 0-131ng/L at the 10,000 ng/L concentration.

Endosulfan I consistently showed a decrease at all three endosulfan treatment concentrations from the beginning to the end of the exposure experiment. Replicate one, which was conducted three weeks before the remaining two replicates, showed a 43% decrease at the 500 ng/L concentration, a 41% decrease at the 5000 ng/L concentration, and a 42% decrease at the 10,000 ng/L concentration. Replicates two and three each showed a 77% decrease at the 500 ng/L concentration, a 77% and 83% decrease at the 5000 ng/L concentration, and a 88% and 79% decrease at the 10,000 ng/L concentration.

P. astertoides exhibited high mortality throughout the endosulfan dosing experiment. Only one-third of total fragments had any living tissue remaining at the end of the exposure. *M. cavernosa* survived all exposures.

Increases in endosulfan I found in *M. cavernosa* tissues appeared to be in portion to the amount of endosulfan exposure (10 times increase between 500 and 5000 ng/L and 2 times increase between 5000 and 10,000 ng/L). After exposure to 500 ng/L, the average concentration of endosulfan I was 1.6×10^{-8} ng/kg wet weight. After exposure to 5000 ng/L, the average concentration was 8.9×10^{-8} ng/kg wet weight, and after exposure to 10,000 ng/L, the average concentration was 20.4×10^{-8} ng/kg wet weight. These increases of endosulfan I nearly matched the increase in concentrations between treatment exposures.

Only one-third of the *P. asteroides* fragments survived in one replicate and did not survive treatment exposures in the two remaining replicates. After exposure to 500 ng/L, the average concentration of endosulfan I was 0.4×10^{-8} ng/kg wet weight. After exposure to 5000 ng/L, the average concentration was 2.3×10^{-8} ng/kg wet weight, and after exposure to 10,000 ng/L, the average concentration was 2.5×10^{-8} ng/kg wet weight.

Histopathological condition for corals

General observations were recorded concerning tissue quality and any changes between treatments for each exposure experiment. The size of mucocytes of gland cells, zooxanthellae density, and the thickness of the epidermis and gastrodermis were targeted as indicators of coral tissue health. Photomicrographs of the coral tissue were taken at 40x magnification and reviewed and assigned a rating of 0-4 in order to provide semi-quantitative data. Normal tissue quality with good integrity was assigned a value of 0; mildly stressed tissue that exhibited slight swelling was assigned a value of 1; widespread and marked swelling was assigned a value of 2; severe swelling and decrease in the number of epidermis mucous secretory cells (MSC) was assigned a value of 3; widespread loss of MCS and cell or tissue atrophy was assigned a value of 4.

Further details of rating conditions are summarized in Table 1. Table 2 shows the resulting rating for the exposure treatments.

Phosphate-treated corals showed a marked decrease in tissue quality with increased exposure. An increase in the thickness of the gastrodermis occurred in the treated corals, along with an increase in the number and size of mucous secretory cells in the gastrodermis. The epidermis of the control coral contained numerous mucous secretory cells, whereas the treated corals showed a limited number of mucous secretory cells. The epidermis of the highest phosphate-dosed coral was completely absent of mucous secretory cells (Fig 1C). The epithelial columnar cells of the control and lowest dosed corals showed no noticeable differences (Fig 1A&B). However, the epithelial columnar cells of the highest dosed coral exhibited severe atrophy and granularity. Furthermore, an increase in the endolithic community occurred with an increase in phosphate dose (Fig 1D). All other cells appeared to remain normal and intact throughout the exposure to stress. Zooxanthellae densities seemed to remain similar throughout all corals even though corals appeared to be lighter in color at the end of the experiment.

Overall, the quality of the endosulfan-treated corals remained very similar among the coral treatments (Fig 2). The zooxanthellae seemed to remain healthy and showed no change between coral treatments. There were no signs of mucous secretory cells or any major trends of tissue detachment. The gastrodermis of all the corals were similar in thickness and number and size of mucous secretory cells. Both the gastrodermis and epidermis contained a few cells within about 200 µm span that contained darkly stained granules. The epidermis did not have any extended cilia for any of the treatments. However, the noticeable change that increased with endosulfan exposure was the increase in epidermis thickness and size of mucous secretory cells.

Gene expression

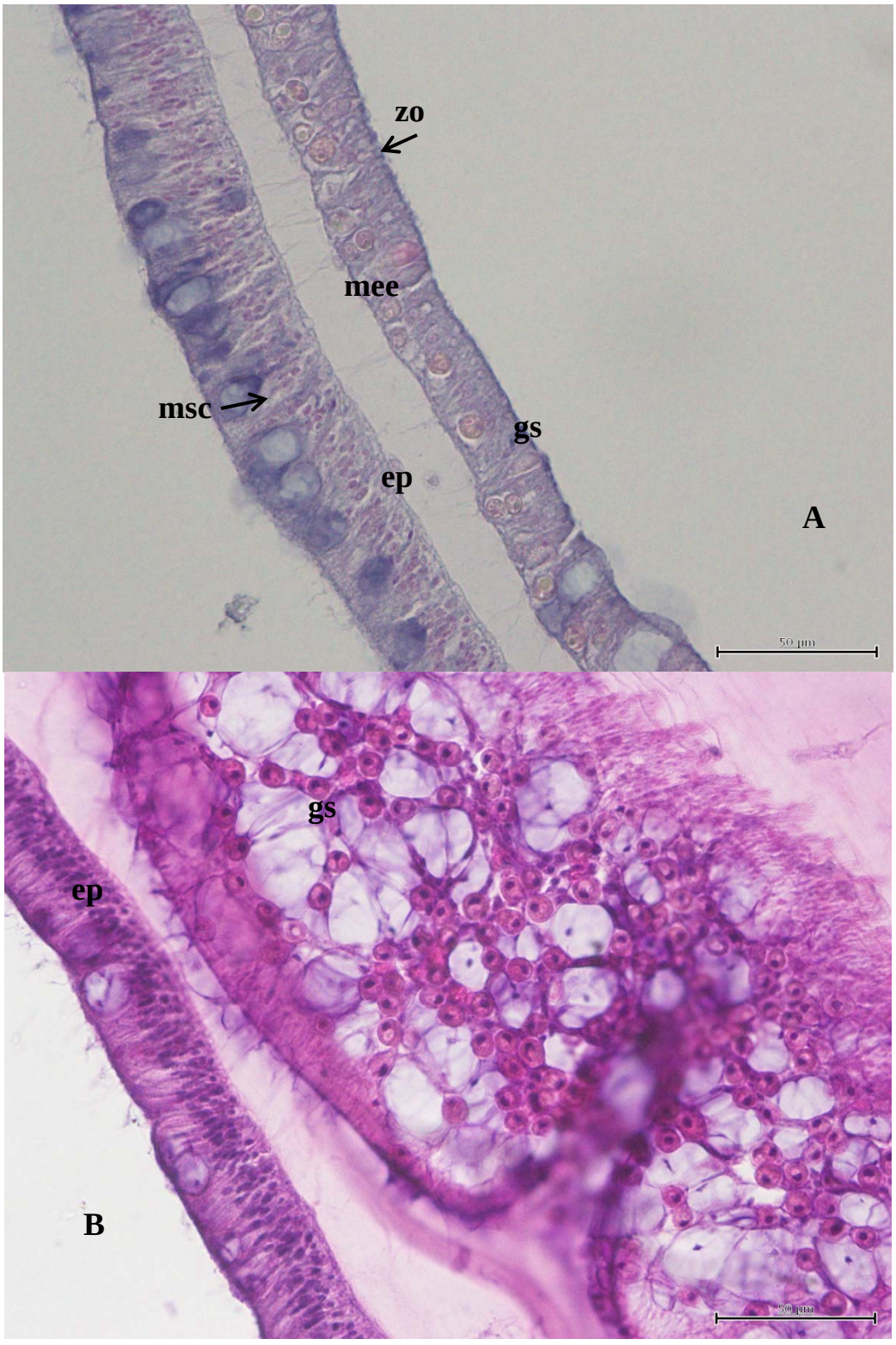
RNA has been extracted from all surviving fragments of both coral species. Due to packaging damage during shipment, a number of *Montastrea* samples were lost. The test for genetic markers in both coral species from the phosphate exposure experiment will be limited. Genetic assays were concentrated on the remaining *Montastrea* and *Porites* samples for the phosphate and endosulfan experiments. Obtaining genetic stress markers from *Porites spp.* has proven difficult in the genetic community (Edge and Shearer, pers. comms.). As of this date, no final results have been received from Dr. Shearer at Amegen Consulting, Inc. Unbeknownst to the PI, Amegen may be consulting with S. Edge in the Czech Republic for additional interpretation of the results.

Table 1. Rating scale for histological changes in *Montastraea cavernosa* samples based on tissue and cellular changes and/or trends.

Range	Coenosarc
0	Healthy tissue and cellular integrity. Distinct nuclei and membranes. No swelling of tissue and mucous secretory cells.
1	Above conditions + minor swelling of epidermis and/or mucous secretory cells
2	All mucous cells and epidermis layer exhibit swelling.
3	Presence of cell debris and localized cell atrophy. Mucous secretory cells decrease in number/ exhaust mucous.
4	Above conditions + large amount of cellular and membrane atrophy Tissue degradation.

Table 2. Results for *Montastraea cavernosa* tissue quality from exposure to endosulfan or phosphate. Slides stained with Harris's hematoxylin and eosin and numbers are the median of histological ratings for each treatment.

Treatment	Rating
Phosphate Control	1
Phosphate .25	1
Phosphate 2.0	3
Endosulfan Control	0
Endosulfan Acetone	0
Endosulfan 500 ng/L	1
Endolsulfan 10,000 ng/L	1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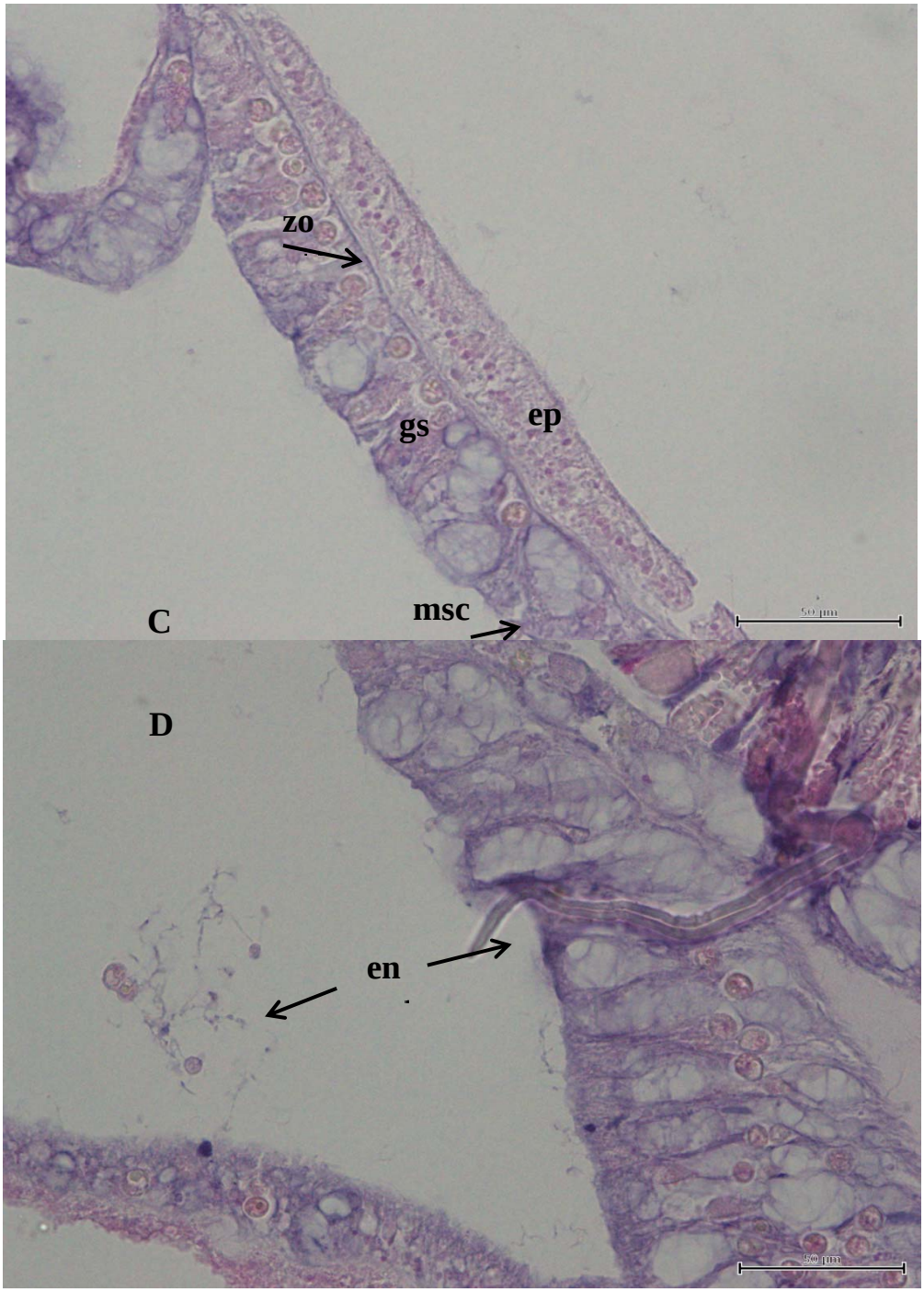
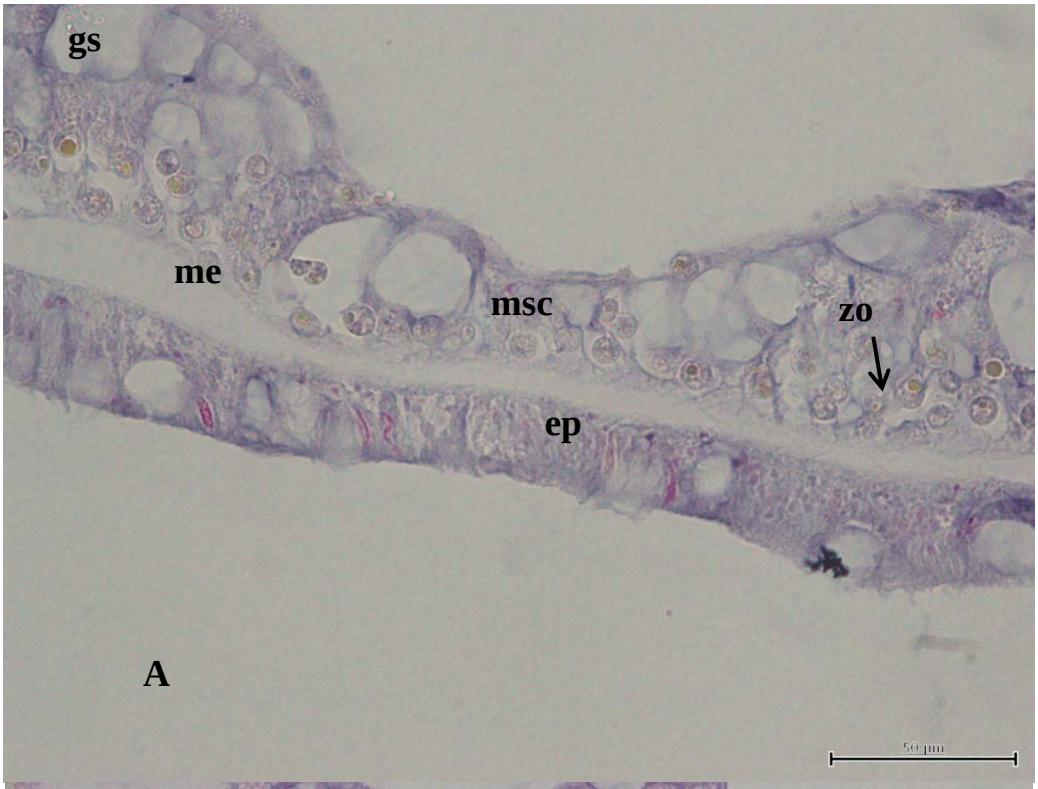


FIGURE 1 Photomicrographs of *Montastraea cavernosa* tissues stained with hematoxylin and eosin, showing changes in tissue due to different levels of phosphate. (**A**) Longitudinal section through the coenosarc of the control coral. (**B**) Longitudinal section through the coenosarc of the lowest phosphate-dosed coral, (**C &D**) Longitudinal section through the coenosarc of the highest phosphate-dosed coral. All scale bars 50 µm. (Abbrevaitions: en, endolithic community; ep, epidermis; gd, gastrodermis, me, mesoglea; msc, mucous secretory cells; zo, zooxanthellae)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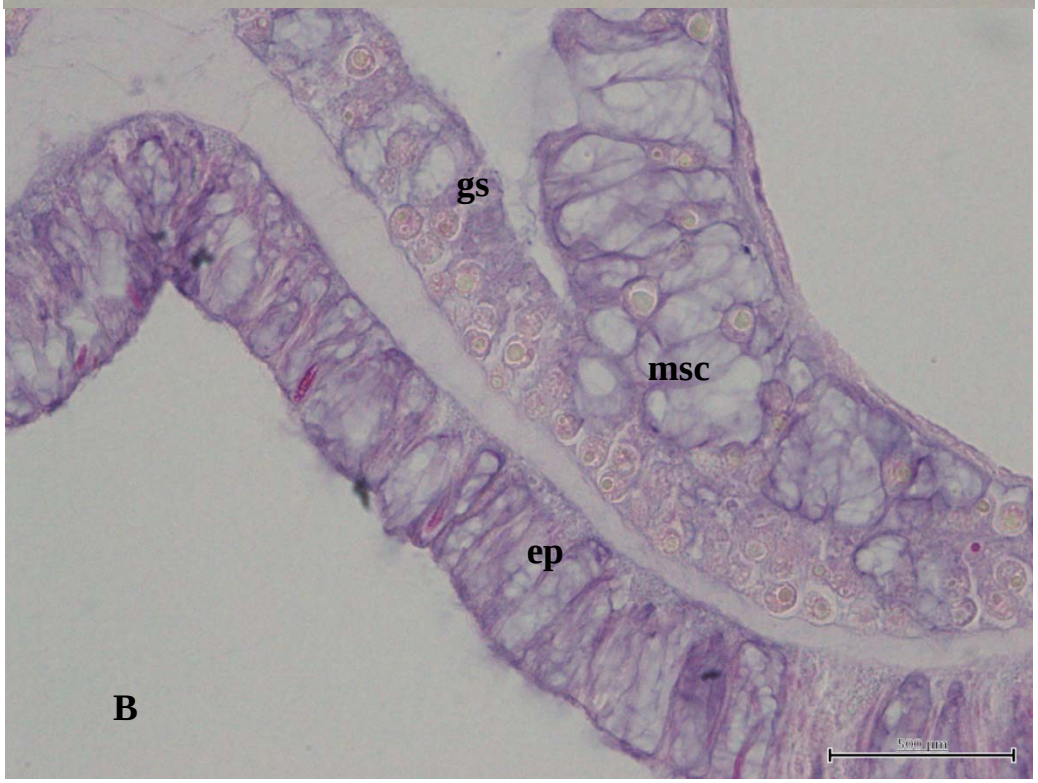
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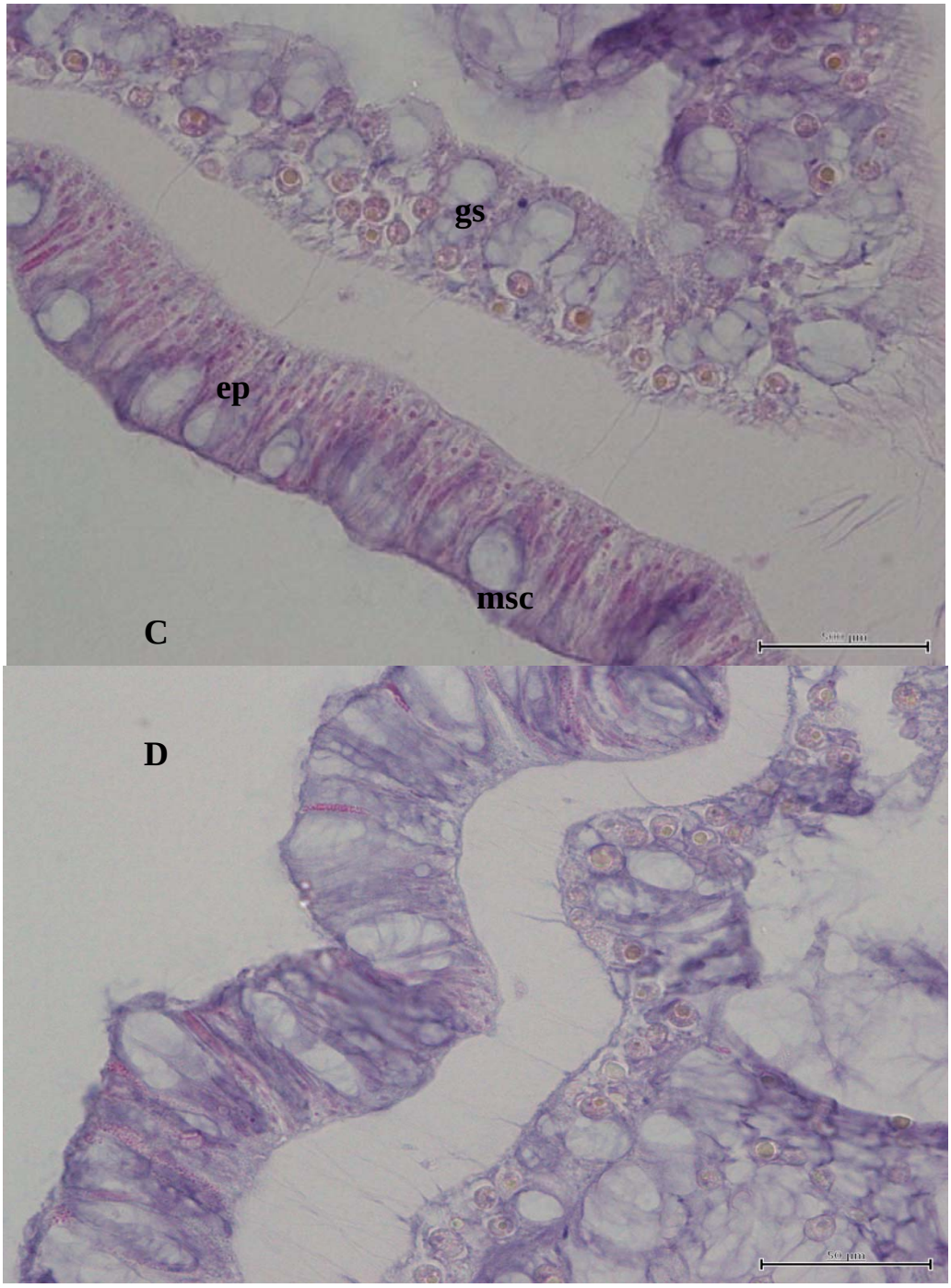

FIGURE 2 Photomicrographs of *Montastraea cavernosa* tissues stained with hematoxylin and eosin, showing changes in tissue due to different levels of phosphate. (**A**) Longitudinal section through the coenosarc of the control coral. (**B**) Longitudinal section through the coenosarc of the solvent-control coral, (**C**) Longitudinal section through the coenosarc of the lowest endosulfan-dosed coral, (**D**) Longitudinal section through the coenosarc of the highest endosulfan-dosed coral. All scale bars 50 µm. (Abbrevaitions: en, endolithic community; ep, epidermis; gd, gastrodermis, me, mesoglea; msc, mucous secretory cells; zo, zooxanthellae)

DISCUSSION

Chemicals

Endosulfan isomers bind to lipid so either lipid concentration in the seawater was too low for the endosulfan to bind to the organic material or concentration of endosulfan was too low to be detected in the nearshore seawater samples. Previous biochemical analyses of the study area's sediment concluded lipid was in evidence. Therefore, endosulfan concentrations, if present, were in low enough concentrations to not be detected.

The lack of endosulfan II was to be expected given that it occurs in lower concentrations in pesticides and also its ready conversion to endosulfan I. The very low concentrations of endosulfan sulfate may have resulted from the short duration of the experiment. The five day period may not have been long enough for biotransformation of endosulfan I to endosulfan sulfate. The large decreases observed in endosulfan I were consistent across all three endosulfan concentrations. The first replicate had 41-43% removal from the water while the two remaining replicates had 77-88% removal from the beginning dose to the last day of exposure. Ambient weather conditions remained the same and the temperature controls of the SECOR dosing tanks also were consistent during the three week period as well as consistent use of Instant Ocean in all tanks.

Histology

No noticeable trends were observed in zooxanthellae condition or density for either experiment. Endosulfan-treated corals revealed an increase in stress with endosulfan concentration by an increase in number and size of the mucous secretory cells. Phosphate seemed to present itself as a greater stressor as compared to endosulfan. Not only was there an increase in swelling of mucous secretory cells, but the epithelia cells exhibited widespread atrophy with the highest dose of phosphate.

Sources of stress

The algal bloom in the 1000 gallon holding tank lasted ten days and largely covered all coral colonies of both species. After siphoning the algae from the corals, ***M. cavernosa*** did not appear to exhibit any deleterious effects. However, ***P. asteroides*** suffered severe tissue loss and mortality. While there was no mortality of either species during the phosphate experiment, and subsequent algal bloom, ***P. asteroides*** appeared to have greater tissue loss than ***M. cavernosa***.

Midway through the phosphate dosing experiment, the pump delivering seawater from the saltwater well at our coastal facility ceased functioning. Natural seawater in the dosing tanks was replaced with Instant Ocean. Phosphate concentrations in the water were adjusted to maintain consistent levels for the remainder of the experiment. The transition between the two different water sources may have contributed additional stress to the corals.

Porites asteroides survival was clearly challenged throughout the experiment, from time of collection through exposure to phosphate and then endosulfan. Whether ***P. asteroides*** was more susceptible to endosulfan can only be answered by a repeat of the

experiment and conducting the endosulfan exposure prior to the phosphate. Endosulfan I was incorporated into their tissues at lower concentrations than that of *M. cavernosa*. While the concentration increase in *P. asteroides* tissues appeared proportional between 500 and 5000 ng/L, that relationship did not increase two-fold as expected between 5000 and 10,000 ng/L. Whether this was a result of lethal exposure to endosulfan can only be determined with further study.

PROJECT DIFFICULTIES

While the hypotheses associated with this project appeared straightforward, the complexity of maintaining healthy coral colonies and plugs in both a new land-based coral nursery and multi-tank dosing system, proved challenging. The associated coral colony plug deaths and equipment failures led to two, six-month no-cost extensions.

Coral colonies, approximately 25 x 25 cm, and fragments of *Montastrea cavernosa* and *Porites astreoides* were collected in October 2012. A series of cold snaps throughout the winter, where water temperature was lower than 70 ° F in the outdoor seawater tanks, occurred as recently as early April 2013. Warm water changes were employed each time to help raise the temperature but each time the coral appeared lighter and stressed. Alkalinity spiked to higher than normally recognized amounts on at least three occasions which further stressed the corals. A significant algal bloom occurred in the nursery holding tank. The combination of these two events led to tissue necrosis in both species, but predominantly *P. asteroides*. As a result additional coral colonies were collected after an extension to our collection permit was granted. Two unexplained water quality events occurred, each in the span of less than seventy-two hours (over a weekend) which resulted in bleaching and subsequent death of approximately 25% of *Porites astreoides* colonies. *Montastrea cavernosa*, while stressed, survived better than *Porites astreoides*. Additionally, a four-fold increase in the number of coral plugs was estimated as necessary to get adequate RNA for each species. All coral colonies for the experiment were then collected from one site to eliminate response variables which could not be identified or controlled.

The SECOR dosing system had integrated chillers which were not designed for outdoor use, resulting in some failure during both experiments. While the tanks were monitored at least once daily, chiller failure at other times of the day resulted in water baths reflecting ambient air temperature until chillers were replaced (< 24 hours). Replacement involved drainage of the suspect water bath so water in the 40 gallon tanks was unable to be chilled and warmed up to ambient air temperature. These temperature fluctuations likely contributed additional stress to both coral species.

Because the SECOR system was located outdoors, a variety of insects and small reptiles would utilize the water baths as a freshwater source. When any of these organisms died while in the water, their remains were transported through the water bath output line to the chillers which reduced water flow. This reduced the efficiency of the chillers.

Some research has shown that endosulfan binds to certain grades of plastic compounds (Downs pers. comm). Plastics were limited in the 40 gallon tanks to circulation pumps. Upon investigation with the pump manufacture, plastic composition could not be identified. Glass pipettes were utilized in place of air stones to limit plastic introduction.

Peristaltic pumps repeatedly failed during the phosphate experiment and had to be replaced throughout the first week. During the second week of the experiment, Instant Ocean was pumped into the 40 gallon dosing tanks within a 10 minute period versus the 12 hour interval the peristaltic pumps would have. No filtration exists in the SECOR system so water changes were the only mechanism to remove dissolved and particulate matter. The pump failures prevented synchronous water changes which may have led to the corals being exposed to waste products for longer periods of time than previously scheduled.

After the fifth day of the fourteen-day phosphate dosing experiment, the pump delivering seawater from the saltwater well at our coastal facility ceased functioning. Natural seawater in the dosing tanks was replaced with Instant Ocean for the remainder of the phosphate experiment and the endosulfan experiment.

The package of preserved coral tissues from the phosphate exposure experiment was damaged in shipment from Nova Southeastern University, Florida to Amegen Consulting, Inc., Georgia. Several of the tubes leaked which damaged the preserved corals and also marred the codes on the exterior of the vials for identification. The remaining coral specimens were pooled by species and known phosphate exposure. A double layer of packing material and boxes were used during shipment of the coral tissues following the endosulfan experiment. A different labeling system was also used (ink and internal vial labels). We were informed that those samples arrived safely.

PRESENTATIONS/OUTREACH/PUBLICATIONS

Informative tours of the land-based coral nursery and dosing tank experiments were provided a minimum of twice per month through the course of project. These lecture-based tours were provided to visiting researchers, potential University donors, and prospective graduate students. Informative presentations were provided to local user groups, ie. Hollywood Beach Community Center. The results of this study are directly contributing to a Masters of Science thesis (Cameron Baxley) and results are expected to be published in the scientific journal Coral Reef.

FUTURE WORK

The highest concentration of phosphate in this study appeared to cause greater stress than the experimental levels of endosulfan. The start of this study coincided with more frequent water sampling having been undertaken by Broward County and NOAA.

Until then, concentrations of the two pollutants in nearshore waters were relatively unknown. Frequent water chemistry analysis based on water flow patterns at specific locations, ie. freshwater output / seawater inlet, nearshore, reef tracts, and outfalls, would provide finer scale environmental pollutant concentrations. These data would then more accurately focus experimental exposure of coral specimens to pollutant levels.

Seasonality in South Florida, resulting predominantly in salinity but also temperature fluctuations damaging to coral species, has a known impact on corals. These variables should be studied in combination with pollutant concentrations in a strictly controlled environment.

LITERATURE CITED

Ainsworth TD, Hoegh-Guldberg O (2009) Bacterial communities closely associated with coral tissues vary under experimental and natural reef conditions and thermal stress. Aquat Biol, 4: 289-296.

Bhavan, P.S. and Geraldine, P. 2000. Histopathology of the hepatopancreas and gills of the prawn *Macrobrachium malcolmsonii* exposed to endosulfan. Aquatic Toxicology 50:331-339.

Brown, F. R., Winkler, J., Visita, P., Dhaliwal, J., Petreas, M. (2006) Levels of PBDEs, PCDDs, PCDFs, and coplanar PCBs in edible fish from California coastal waters. Chemosphere 64: 276–286.

Chou, C., Hsiao, Y., Ko, F., Cheng, J. (2010) Chronic exposure of 2,2_,4,4_-tetrabromodiphenyl ether (PBDE-47) alters locomotion behavior in juvenile zebrafish (Danio rerio). Aquatic Toxicology 98: 388–395.

Cotham, WE, Bidleman, TF. (1989) Degradation of malathion, endosulfan, and fenvalerate in seawater and seawater/sediment microcosms. J Agric Food Chem 37:824-828.

Darnerud PO. (2003) Toxic effects of brominated flame retardants in man and in wildlife. Environ Int 29:841–53.

Edge, S.E.,Morgan, M.B., Snell, T.W. 2008. Temporal analysis of gene expression in a field population of the Scleractinian coral *Montastraea faveolata*. Journal of Experimental Marine Biology and Ecology. 355:114–124.

Edge, S.E. 2007. Using microarrays to quantify stress responses in natural populations of coral. PhD dissertation. Doctor of Philosophy in Biology, Georgia Institute of Technology, School of Biology. Atlanta, Georgia. 164 pp.
Edge, S.E.,Morgan, M.B, Gleason, D.F., Snell, T.W. 2005. Development of a coral cDNA array to examine gene expression profiles in *Montastraea faveolata* exposed to environmental stress. Marine Pollution Bulletin. 51:507–523

Ferrier-Pages C, Gattuso J-P, Dallot S, Jaubert J (2000) Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral *Stylophora pistillata*. *Coral Reefs*, 19:103-113.

Furnas MJ (1991) Nutrient status and trends in waters of the Great Barrier Reef Marine Park. In: Yellowlees D (ed) Land uses, patterns and nutrient loadings of the Great Barrier Reef Region. James Cook Univ. Townsville, 162-179.

Goldenberg SB, Landsea CW, Mestaz-Nuñez AM, Gray WM (2001) The recent increase in hurricane activity: causes and implications. Science 293:474–479

Gromley KL and Teather KL (2003) Developmental, behavioral, and reproductive effects experienced by Japanese medaka (*Oryzias latipes*) in response to short-term exposure to endosulfan. *Ecotox. and Environ. Safety.* 54: 330-338.
Hallock P, Schlager W (1986) Nutrient excess and the demise of coral reefs and carbonate platforms. Palaios 7:389–398

Harman –Fetcho JA, Hapeman CJ, McConnell LL, Potter TL, Rice CP, Sadeghi AM, Smith RD, Bailek K, Sefton KA, Schaeffer BA, Curry R (2005) Pesticide occurrence in selected south Florida canals and Biscayne Bay during high agricultural activity. *J. Agric. Food Chem.* 53: 6040-6048.

Houghton JT, Ding Y, Griggs DJ, Noguer M, van der Linden PJ, Dai X, Maskell K, Johnson CA (2002) Climate change 2001: the scientific basis. Cambridge University Press, Cambridge

Karl TR, Trenberth KE (2003) Modern global climate change. Science 302:17191723
Key P, Fulton M, Harman-Fetcho J, McConnell L (2003) Acetylcholinesterase Activity in Grass Shrimp and Aqueous Pesticide Levels from South Florida Drainage Canals. Arch. *EnViron. Contam. Toxicol.* 45: 371-377.

Kinsey DW, Davies PJ (1979) Effect of elevated nitrogen and phosphorus on coral reef growth. Limnol. Oceanogr., 24:935-940.

Landsea CW (2000a) Climate variability of tropical cyclones: past, present and future. In: Pielke RA Sr, Pielke RA Jr (eds) Storms. Routledge, New York, p 220–242

Landsea CW (2000b) El-Nino Southern Oscillation and the seasonal predictability of tropical cyclones. In: Diaz HF, Markgraf V (eds) El Niño: multiscale variability and global regional impacts. Cambridge University Press, Cambridge, p 149–182
Markey KL, Baird AH, Humphrey C, Negri AP (2007) Insecticides and a fungicide affect multiple coral life stages. *Marine Ecol. Prog. Ser.* 330: 127-137.

Miles CJ and Pfeuffer RJ (1997) Pesticides in canals of South Florida. *Arch. Environ. Contam. Toxicol.* 32: 337-345.

Morgan, MB, Edge, SE, Snell, TW (2005) Profiling differential gene expression of corals along a transect of waters adjacent to the Bermuda municipal dump. Marine Pollution Bulletin. 51:524–533

Otludil, B, Cengiz, EI, Yildirim, MZ, Ünver, O, Ünlü, E (2004) The effects of endosulfan on the great ramshorn snail *Planorbarius corneus* (Gastropoda, Pulmonata): a histopathological study. Chemosphere 56: 707-716.

Pfeuffer R, Matson F (2002) Pesticide Surface Water and Sediment Quality Report: November 2002 Sampling Event; The South Florida Water Management District: West Palm Beach, FL.

Pfeuffer R, Matson F (2003) Pesticide Surface Water and Sediment Quality Report: March 2003 Sampling Event; The South Florida Water Management District: West Palm Beach, FL.

Pfeuffer R, Rand G (2004) South Florida Ambient Pesticide Monitoring Program. Ecotoxicology, 13: 195-205.

Renegar DA, Blackwelder PL, Moulding AM (2008) Coral ultrastructural response to elevated pCO2 and nutrients during tissue repair and regeneration. Proc of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008

Renegar DA, Riegl B (2005) Effect of nutrient enrichment and elevated CO2 partial pressure on growth rate of the Atlantic zooxanthellate coral *Acropora cervicornis*. Mar Ecol Prog Ser 293:69-76

Scott G, Fulton M, Wirth E, Chandler G, Key P, Daugomah, J, Bearden D, Chung K, Strozier, E, DeLorenzo M, Silvertsen S, Dias A, Sanders M, Macauley J, Goodman L, LaCroix G, Thayer G, Kucklick J (2002) Toxicological Studies in Tropical Ecosystems: an Ecotoxicological Risk Assessment of Pesticide Runoff in South Florida Estuarine Ecosystems. J. Agric. Food Chem. 50: 4400-4408.

Simkiss K (1964) Phosphates as crystal poisons of calcification. Biol. Rev., 39: 487-505.
Snell, T.W., Brogdon, S.E., Morgan, M.B. 2003. Gene expression profiling in ecotoxicology. Ecotoxicology. 12(6):475-483

Stambler N, Popper N, Dubinsky Z, Stimson J (1991) Effects of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. Pac. Sci., 45:299-307.
Tomascik T, Sander F (1985) Effects of eutrophication on reef building corals: I: Effects of eutrophication on the reef building coral *Montastrea annularis*. Marine Biology, 87:143-155.

Velasco-Santamaria, YM, Handy, RD, Sloman, KA (2011) Endosulfan affects health variables in adult zebrafish (*Danio rerio*) and induces alterations in larvae development. Comparative Biochemistry and Physiology C. doi:10.1016/j.cbpc.2011.01.001.

Varayoud J, Monje L, Bernhardt T, Munoz-de-Toro M, Luque EH, Ramos JG (2008) Endosulfan modulates estrogen-dependent genes like a non-uterotrophic dose of 17β-estradiol. Reproductive Toxicology. 26: 138-145.

Vargas-Angel B, Riegl B, Gilliam D, Dodge RE (2006) An experimental histopathological rating scale of sedimentation stress in the Caribbean coral *Montastrea*

cavernosa. *In: Proceedings of the 10th International Coral Reef Symposium* (Okinawa, Japan), pp. 1168–1173.

Walker DI, Ormond RFG (1982) Coral death and phosphate pollution in Aquaba, Red Sea. Mar. Poll. Bull., 13:21-25.