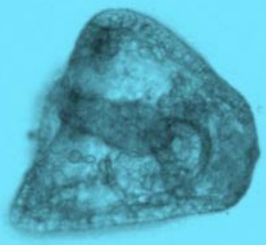
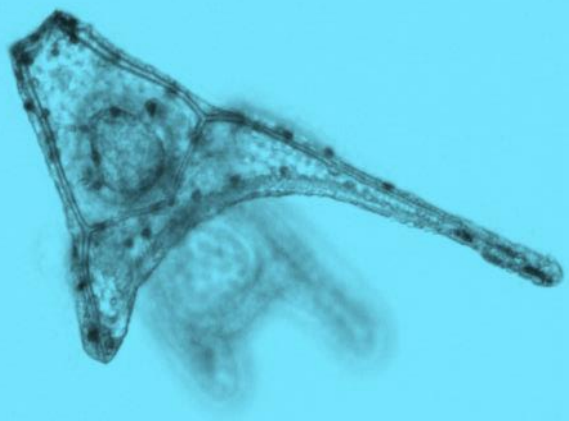
Final Report

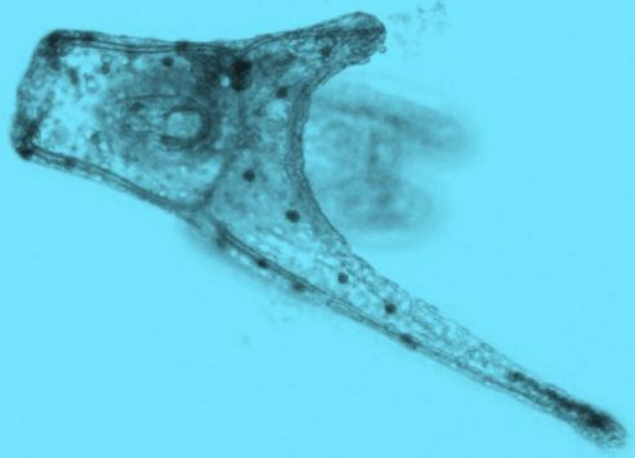
Phase I Porewater Toxicity Testing of Sediment from 25 Near-Shore Sites in St. Croix, USVI





Lisa A. May and Cheryl M. Woodley

NOS NCCOS CCEHBR & HML
Charleston, SC 29412



CRCP Project 1133
November 2016

INTRODUCTION

St. Croix (STX) is one of three major islands comprising the United States Virgin Islands (USVI) territory. The island encompasses a total land area of 84 square miles, with approximately 70 miles of coastal shoreline. Protection of critical reef habitats is the responsibility of the USVI Department of Planning and Natural Resources, U. S. National Park Service and NOAA, however threats remain to coral reef health from a variety of sources including pollution (LBSP), global climate change and overfishing. Impacts to several populations of *Acropora palmata* coral were found at Buck Island Reef National Monument (BUIS) and Salt River Bay National Historical Park and Ecological Preserve (SARI) in a 2013 survey of reproductive condition using histological analysis for the presence of gametes, prompting further investigation.

As the first step of an environmental investigation into the potential causes of reproductive failure in *Acropora palmata* corals in the waters of St. Croix, a survey of 25 near shore sites (including MPAs) was conducted during June 2015 to evaluate their toxicity potential. Toxicity of these sites was assessed using sediment porewater in the sea urchin (*Lytechinus variegatus*) embryo development bioassay. A subsequent effort was also initiated to characterize the observed toxicity in the original samples (preliminary phase I Toxicity Identification Evaluation (TIE)). In addition, sediment interstitial water (porewater) was evaluated for salinity, pH, dissolved oxygen, total ammonia nitrogen (TAN), nitrite, inorganic phosphate and total phosphorus (TP) prior to the bioassay.

METHODS

Collection Permits

Collections for this project were conducted under the following permits:
NPS Buck Island Permit #BUIS-2015-SCI-005
NPS Salt River Bay Permit #SARI-2015-SCI-003
DPNR Indigenous Species Research and Export Permit# DFW15057T
DPNR Indigenous Species Research and Export Permit# DFW15036X

Site Selection

The 25 sites were selected for sediment collections for bio-effects toxicity analysis in 2015 based on consultations with Dr. Thomas Dolan, Chief of the USVI Department of Planning & Natural Resources (DPNR) Bureau of Fisheries, who recommended sites along the north and south coasts of St. Croix. Mr. John Farchette, DPNR St. Croix East End Marine Park (STXEEMP) Ranger and Mr. Marlon Hibbert of the NOAA CRCP recommended collection points in the St. Croix East End Marine Park, a CRCP priority site. Dr. Zandy Hillis-Starr of the NPS assisted in determining

collection points in BUIS and SARI based on historical site information and helped to identify sites from the 2013 study of *Acropora palmata* reproductive condition. The 2013 study showed that *Acropora palmata* reproductive effort was poor (20 % or less colonies with gametes) at four sites within SARI and one site in BUIS. The collection sites are presented in Figures 1-3 and include SARI (7 sites, 4 identified with poor reproductive effort in 2013), BUIS (6 sites, 1 identified with poor reproductive effort) and 12 sites around the main island. A reference water column sample was taken near BUIS Site 3 for comparison.

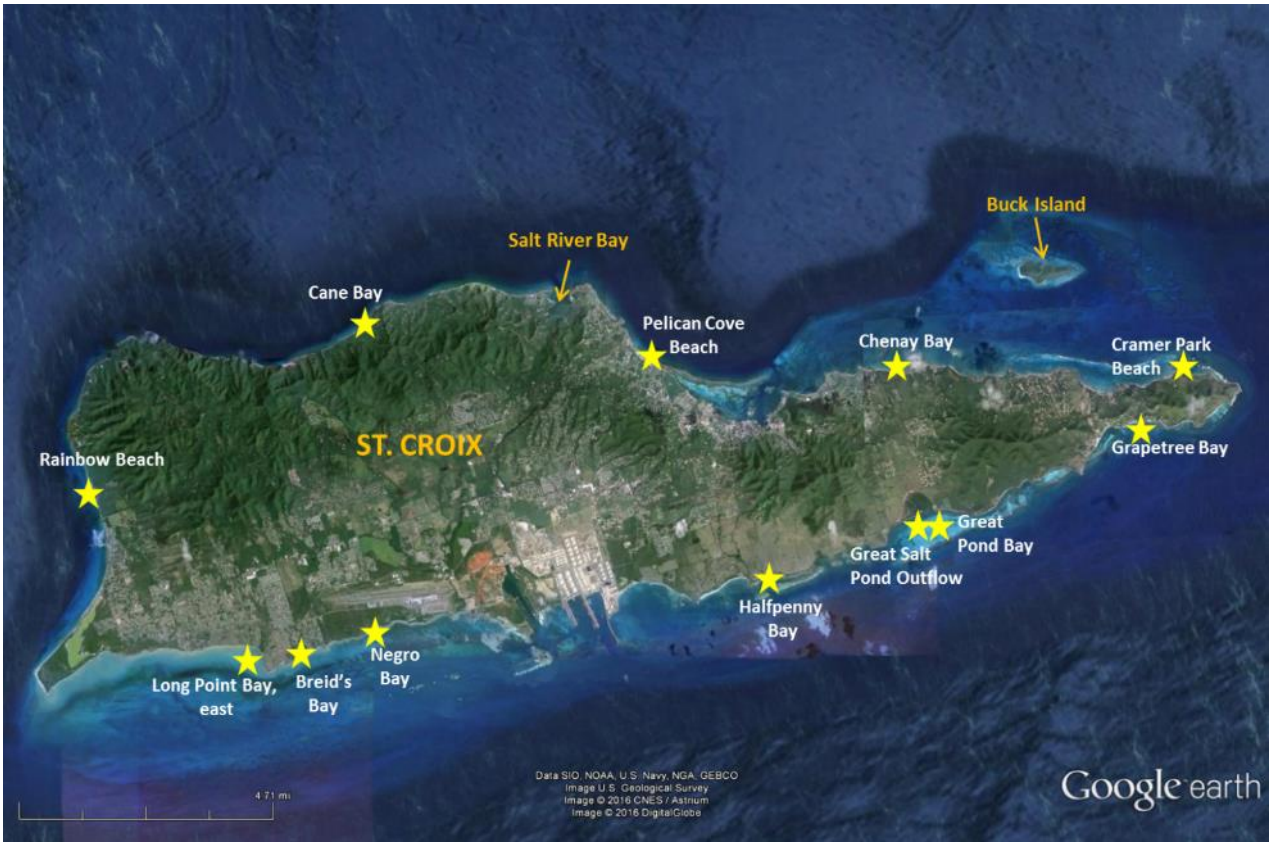


Figure 1. Sediment sampling sites around the island of St. Croix, USVI.

Figure 2. Detail of sediment sampling sites within the Salt River Bay National Historic Park. Sites with poor *Acropora palmata* reproductive effort in 2013 are indicated with red stars.

Figure 3. Detail of sediment sampling sites within Buck Island Reef National Monument (indicated with stars). *Acropora palmata* at Underwater Trail site (red star) showed poor reproductive effort in 2013.

Equipment Preparation

All glassware, Teflon sample vials, Teflon centrifuge tubes, and syringes were thoroughly cleaned (Chapman, *et al*. 1995). Briefly, glass and Teflon vessels were washed with an anionic, phosphate-free laboratory-grade detergent (e.g., Liquinox), rinsed five times with deionized water, and soaked in a 10% solution of hydrochloric acid in a Teflon tub for 30 min. Following the acid bath, vessels were rinsed three times with Type 1 water, rinsed three times with pesticide-free acetone and allowed to air dry upright for a few minutes on acetone-rinsed foil sheets in a chemical hood. Vessels were covered with a section of acetone-rinsed foil or sealed with a lid until use. New polypropylene syringes without rubber or silicon tipped plungers (30 mL, #4830001000, Henke Sass Wolf, Tuttlingen, Germany) were removed from packaging and the end was cut off. The barrel was separated from the plunger and the separate parts were rinsed once with Type 1 water, then placed in a clean glass vessel to soak in Type 1 water overnight. Syringe barrels and plungers were rinsed with pesticide-free acetone, dried on a clean sheet of acetone-rinsed foil, reassembled, and wrapped in clean foil until use. Teflon jars (90 mL) were washed in laboratory detergent, rinsed five times with Type 1 water, then rinsed three times with pesticide-free acetone and dried before replacing lid.

Sample Collection

Sediment samplers, free of personal care products, donned gloves prior to entering the water. Sediment samples were collected by snorkelers from near-shore waters using the modified syringe method. Briefly a 30-mL syringe with the end removed was inserted into the sediment. The plunger was slowly retracted as the syringe was pushed into the sediment, filling the barrel. A gloved hand was placed over the barrel as it was removed from the sediment to keep the sample from falling out (video of method: https://cdhc.noaa.gov/education/field_health.aspx). Upon return to the surface, syringes were used to fill two Teflon bags with sediment at each site (4-5 syringes). A water column sample collected on the eastern end of Buck Island was used as a reference (unimpacted) sample. All samples were placed in a cooler (on ice) until extraction, within 4 h.

Porewater Extraction

Sample interstitial waters were aspirated from sediment samples using a 10-mL glass pipet and placed in a clean 30 mL Teflon centrifuge tube. Samples were centrifuged at 1200 x g for 20 min. Clarified supernatant was transferred to a clean 90 mL Teflon vial and frozen. Samples were placed in a liquid nitrogen vapor dry shipper for transport to the laboratory. Upon arrival in Charleston, SC, samples were archived at -80 °C until assay initiation.

Water Quality Analysis

Frozen sediment porewater samples were thawed at 4 °C (24 h). Once completely thawed, samples were brought to room temperature and salinity was measured. Sample salinity was adjusted (only for Great Pond outflow and Salt River Bioluminescence Pond samples) using Type 1 water to a target of 35.0 ± 0.5 ppt. Following salinity adjustment, a 5-mL aliquot was removed to a clean 20 mL glass vial and dissolved oxygen and pH were measured using probes connected to a Thermo Orion 5-Star multimeter. Total ammonia nitrogen (TAN) was determined from 400 µL of sample using a colorimetric microplate assay based on a commercial (Red Sea, Houston, TX) kit. Ammonia standards for the assay were generated using 100 mg/L ammonia standard (Hach, Catalog #2406549) in a two-fold dilution series (0.13-8.0 mg/L) in 35 ppt artificial seawater (ASW, Pro-Reef Sea Salt, Tropic Marin, Wartenberg, Germany). Nitrite nitrogen concentration in each sample was verified with the USEPA diazotization method (#8507) using a microplate format for the Hach nitrite nitrogen assay kit (#2107169). Total inorganic phosphate concentrations were determined by the ascorbic acid method using a Hanna Checker kit (#HI 713-25) adapted to microplate format. Unionized ammonia (UAN) values were calculated using a standard method (Bower and Bidwell 1978). Total phosphorus was calculated as a percentage of total inorganic phosphate (32.62%). Following water quality analysis, sample porewaters (5 mL, 4 replicates) were placed in pre-cleaned, conditioned (5 mL Tropic Marin ASW, 35 ppt), 20-mL glass vials and held at 23.0 ± 0.5 °C.

Sea Urchin Embryo Development Toxicity Assay

Sediment porewater toxicity was determined according to the methods of Carr and Chapman (1992) and Carr et al. (1996). Gravid sea urchins (*Lytechinus variegatus*) were acquired from the

Florida Keys (Reeftopia, Key West, FL), and held for three months at 25 °C in a shallow glass-Teflon aquarium system containing artificial seawater (Instant Ocean Sea Salts, Blacksburg, VA, 35 ppt). Lighting was provided by six, 54W T-5 high-output fluorescent bulbs (3- Aquasun by UV Lighting Co.; 3-ATI blue plus bulbs (88 µmol/m^2/s at depth, 200 µmol/m^2/s at the surface) on a 12h:12h light:dark cycle. Urchins were fed a rotating diet of organic carrots, organic spinach, and seaweed (Julian Sprung's Sea Veggies®) three times per week.

Urchin spawning was initiated using 1-3 mL potassium chloride (0.5 M) injections into the coelom by inserting the needle through the peristomal membrane surrounding the mouth. Eggs were collected by inverting the female urchin over a beaker filled to the brim with artificial seawater (35 ppt, 25°C). The urchin aboral side was slightly submerged, so that the eggs were extruded directly into the seawater. After spawning was complete, the eggs were washed three times with an equal volume of fresh artificial seawater (Tropic Marin, 35 ppt) and enumerated on a Sedgewick-Rafter counting chamber. Sperm was collected dry by aspiration with a micropipet tip and placed in a sterile 0.5 mL polypropylene Eppendorf tube. Sperm was kept chilled (not directly on ice) until used. Sperm was diluted 1:250 in ASW to activate and cell concentration was determined and motility was verified from a 1:2000 dilution in ASW.

Prior to beginning the assay, optimal fertilization rates were determined using four dilutions of sperm in a fertilization pre-test. Embryos (~200 in 50 µL volume) were placed in pre-cleaned and conditioned 20 mL glass vials (Environmental Express, Charleston, SC) containing 5 mL of sample porewater (n=4/sample). Artificial seawater (35 ppt) and 4 mg/L sodium dodecyl sulfate in ASW were included as assay controls. Embryos were incubated for 48 h at 23 ± 0.5 °C under ambient fluorescent lighting on a 12h:12h light:dark cycle. Following incubation, an equal volume of 2X zinc-formalin fixative (Anatech, Poughkeepsie, NY) in ASW was added to each vial, and embryo developmental stage and developmental aberrations were scored, with a target of 100 embryos evaluated per sample replicate.

Phase I Toxicity Identification and Evaluation (TIE)

Eight sediment porewater samples were selected for toxicity reduction analysis based on results of the sea urchin development assay. Three BUIS sites (Underwater Trail, Scuba Mooring# 2 and West Beach), three SARI sites (Judith's Fancy, Site 3 and Sugar Bay), Great Pond Bay and Pelican Cove Beach were included. Since sites could be impacted by various sources of anthropogenic pollutants, a HyperSep™ C18 column (Thermo Fisher Scientific, Waltham, MA) was used for sample fractionation (binding nonpolar to moderately polar organic compounds from aquatic matrices). Columns (1 mL bed volume) were charged with 1 mL pesticide-free methanol and rinsed with 1 mL Type 1 water as per the manufacturer's instructions. One milliliter of the porewater sample (previously adjusted for appropriate salinity) was used to rinse the column before applying the remaining sample for collection using a vacuum manifold (~1 drop/s). Salinity and pH for filtered samples were verified prior to beginning the sea urchin development assay. Sea urchin embryos (*L. variegatus*) were treated with the column eluates (3 mL volume) as detailed above. Artificial seawater and SDS were used as assay controls.

Statistical analyses

Data (expressed as percent normal development for each replicate) were subjected to an arcsine square root transformation (Zar 1999) prior to other analyses. After transformation, the data met the assumptions that the model residuals followed the normal distribution and that the residual variances were homogeneous. A two sample t-test was used to compare the response of the artificial seawater (TM ASW) negative control to the positive control. A single factor ANOVA (PROC GLM) was performed on urchin development from the St. Croix field collected samples using the TM ASW negative control as the experimental control. A Dunnett's test for multiple comparisons versus controls was performed post-hoc to determine significant differences between treatment groups and control. A repeated measures ANOVA (PROC MIXED method=ML) was used to analyze a subset of the aforementioned samples before and after filtration through C18 SPE media. Sample SITE was the Between-Subject factor and FILTER (pre- or post-C18) was the Within-Subject factor. Post-hoc Contrasts where performed to compare pre-filtered samples versus post-filtered samples by SITE. Alpha was set to 0.05 for all statistical tests. Power for all tests was >0.9. All analyses were performed using SAS v9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS & DISCUSSION

This report presents the results of water quality measurements and sediment porewater toxicity testing of samples obtained during June 2015. It should be noted that these results represent one sampling time point during the dry season (low tourist visitation). Samples taken during a wet season and/or high tourist visitation will likely provide different results and further insights into the causes of impacts to St. Croix USVI reefs.

Water Quality Analysis

Water quality measurements were conducted on sediment porewater from 25 sites in the near shore waters of St. Croix, USVI (Table 1). Reference water collected at BUIS Site 3 had expected salinity, pH, dissolved oxygen and contained no detectable ammonia or phosphate. The pH and dissolved oxygen for each sample were measured to ensure these parameters were in normal range for sea urchin toxicity tests. Salinity adjustments with Type 1 water (Milli-Q) was required for only two samples, Great Salt Pond Outflow (98 %) and Salt River Bioluminescent Pond (95 %).

Nitrogen and phosphorus are essential elements for most living organisms; however, high levels of these nutrients are often indicators of land-based sources of pollution. Sources of these pollutants can include agricultural or urban run-off, sewage, industrial discharges, combustion of fuels and sedimentation (Carpenter et al. 1998). These nutrient increases lead to processes referred to as eutrophication that are detrimental to the structure and function of ecosystems (Paytan & McLaughlin 2007). Tests for levels of various forms of nitrogen (TAN, UAN, nitrite)

(Table 1) showed only one porewater sample from the Great Salt Pond Outflow that contained abnormally high levels (246.1 µg/L) of unionized ammonia (the most toxic fraction). This level exceeds the EC_{50} (150 µg/L; our unpublished data) for *L. variegatus* embryos during development. In addition, this level of unionized ammonia also exceeds the EC_{50} (73.58 µg/L) for developing *Acropora palmata* larvae (our unpublished data). Together these data indicate a heightened threat posed to developing sea urchin and coral embryos at this location due to ammonia toxicity.

Inorganic phosphate (as sodium phosphate) treatment at concentrations above 0.8 mg/L has been shown to cause arrested and abnormal embryonic development in *L. variegatus* embryos (Bottger and McClintock 2001). For coral, low-level inorganic phosphate exposure (0.09-0.50 mg/L) has been linked to weaker skeleton structure in acroporid corals (Dunn 2011). Recently it was demonstrated that sunscreens are a source of phosphates and ammonia in coastal waters (Tovar-Sanchez 2013). Twelve of the 25 samples contained levels of total phosphorus that exceeded the recommended limit for Class A, B, or C waters (USVI Integrated Water Quality Report 2010) (Table 1). Our findings indicate a significant threat potential from elevated phosphate levels in multiple locations of suitable sea urchin and coral habitat and should be considered a potential contributor to reproductive impairment in marine life.

Table 1. Nutrient load in reference, control and porewater samples. ND= not detected

Sample	Ammonia-nitrogen (mg/L)	Unionized ammonia[1] (µg/L)	Nitrite-nitrogen (mg/L)	Inorganic phosphate (mg/L)	Total phosphorus[2] (µg/L)
Buck Is. Scuba Mooring #2	0.815	26.1	0.004	1.125	**367.0**
Buck Is. Site 3	ND	0.0	0.007	ND	0.0
Buck Is. South Forereef	0.285	9.8	0.005	0.333	**108.6**
Buck Is. South Lagoon	0.110	3.9	0.008	0.031	10.1
Buck Is. Underwater Trail	0.404	16.2	0.004	0.572	**186.6**
Buck Is. West Beach	0.052	1.9	0.012	0.069	22.5
Breid's Bay	0.164	5.7	0.004	0.106	34.6
Buck Is. Reference Water	ND	0.0	0.012	ND	0.0
Cane Bay	0.216	7.1	0.003	0.044	14.4
Chenay Bay	0.551	17.3	0.005	0.119	38.8

Cramer Beach Park	0.151	5.7	0.008	1.716	**559.8**
Grapetree Bay	0.008	0.4	0.003	0.044	14.4
Great Pond Bay	0.170	7.3	0.004	0.232	**75.7**
Great Salt Pond Outflow	4.521	**246.1**	0.006	0.308	**100.5**
Halfpenny Bay	0.184	7.2	0.007	0.169	**55.1**
Long Point Bay, east	0.137	5.2	0.006	ND	0.0
Negro Bay	0.436	17.8	0.006	0.069	22.5
Pelican Cove Beach	ND	0.0	0.009	ND	0.0
Rainbow Beach	ND	0.0	0.003	ND	0.0
Salt River @ Gentle Winds	ND	0.0	0.005	ND	0.0
Salt River Bioluminescent Pond	0.241	9.2	0.003	0.157	**51.2**
Salt River Marina	0.656	28.7	0.013	0.371	**121.0**
Salt River Judith's Fancy	0.056	2.6	0.010	0.031	10.1
Salt River Site 2	0.081	4.2	0.008	0.157	**51.2**
Salt River Site 3	ND	0.0	0.007	0.245	**79.9**
Salt River Sugar Bay	0.261	11.7	0.005	0.308	**100.5**
Artificial Seawater (ASW)	ND	0.0	0.004	ND	0.0
4 mg/L SDS in ASW	ND	0.0	0.003	ND	0.0

[1] Unionized ammonia EC_{50} for L. variegatus = 174 µg/L. Toxic level is in bolded red numerals.
[2] Recommended total phosphorus for Class A, B and C waters is <50 µg/L. Samples with values above maximum are in bolded red numerals.

Sea Urchin Embryo Development Toxicity Assay

Green sea urchins (*Lytechinus variegatus*) are common from North Carolina throughout the Caribbean Sea to Brazil and are found in near-shore sea grass beds, rocky reef areas, and sandy or hard bottoms, as such, they provide a good model species for bioassays of samples from tropical and sub-tropical locations. The sea urchin embryo development assay was used to evaluate the effects of the sediment porewater samples and control waters. Seven of the 25 samples showed impacts to normal embryo development that were significantly different from

the negative control ($p < 0.05$ (#), $p < 0.0005$ (+) and $p < 0.0001$ (*); Figure 4); all other sample did not show toxicity in this bioassay. The artificial seawater control exposure (Figure 5A) resulted in less than 3% of embryos with retarded growth and less than 4% with malformations. Increased malformations such as missing appendages (Figure 5B-D) were observed at Pelican Cove Beach (average = 35% of total) (Figure 6) and three Salt River sites (Judith's Fancy, 27%; Site 3, 31%; Sugar Bay, 22%) (Figure 7). High percentages of embryos at retarded developmental stages were observed at the Great Salt Pond Outflow (96%) (Figures 5E & 6) and Buck Island Underwater Trail (69%) (Figures 5F & 8). The overall retarded development observed in the bioassay using sediment porewaters from the Great Salt Pond Outflow (Figures 5E & 6) is likely due to high total ammonia nitrogen in the sample water. This type of developmental pathology is consistent with that observed in the sea urchin, *Heliocidaris tuberculate*, when exposed to ammonium chloride (Byrne et al. 2008) resulting in an EC50 of 1.3 mg/L for this species. Although the origin of the toxicity observed in these assays has not been elucidated for these sites, scoring the different developmental anomalies has the potential of providing insight into the mechanisms of toxicity or the developmental programs being affected.

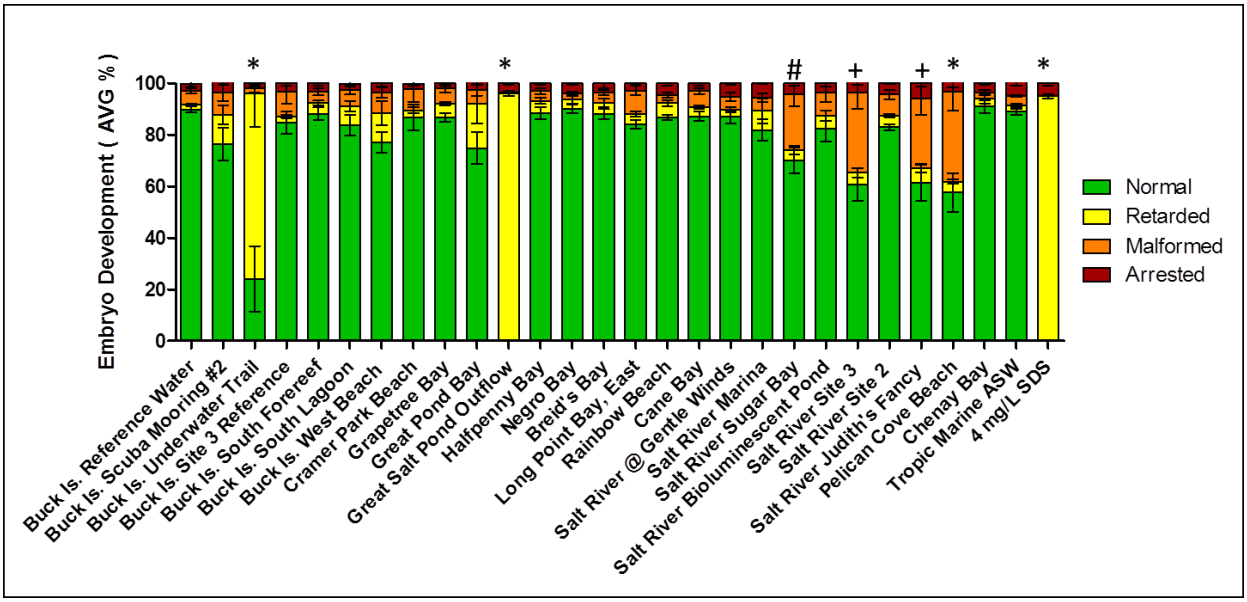


Figure 4. Percent normal (green), retarded (yellow), malformed (orange) and arrested (red) development for *L. variegatus* embryos in sample sediment porewaters and reference water from St. Croix, USVI (n=4). TM ASW = Tropic Marin artificial sea water (negative control), SDS = 4 mg/L sodium dodecyl sulfate in TM ASW (positive control). Treatments with significant differences in normal embryos as compared to the artificial seawater control are designated: $p < 0.05$ (#), $p < 0.0005$ (+) and $p < 0.0001$ (*).

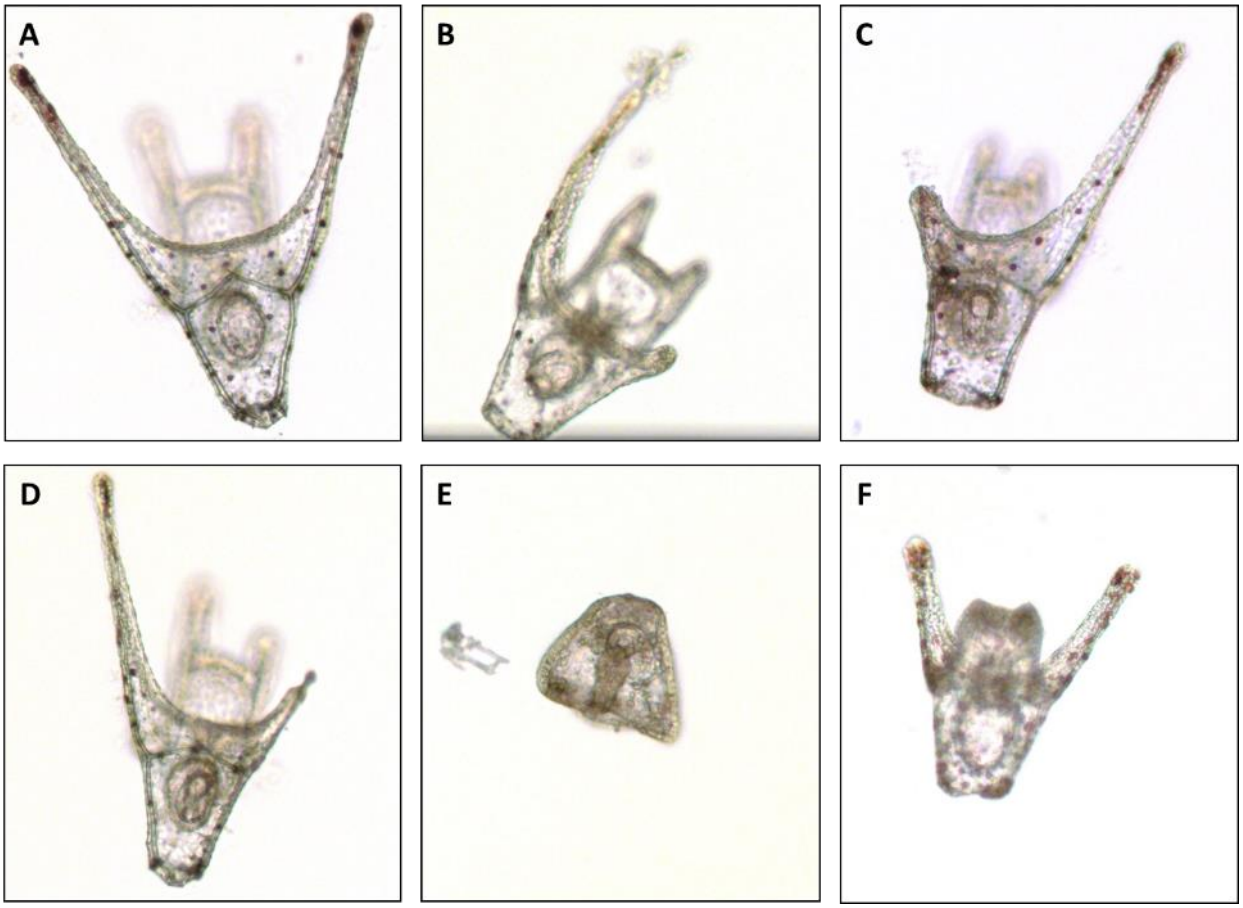


Figure 4. Example Example of *Lytechinus variegatus* 48 h embryo development following St. Croix, USVI sediment porewater treatment. Panel A: artificial seawater control (normal development), Panel B: Pelican Cove Beach (malformed arm), Panel C: Salt River Judith's Fancy (malformed arm), Panel D: Salt River Site 3 (malformed arm), Panel E: Great Salt Pond Outflow (retarded development, prism stage), Panel F: Buck Island Underwater Trail (retarded development, early pluteus stage). Increased malformations such as missing appendages were observed at Pelican Cove Beach and both Salt River sites. Increased numbers of embryos at retarded developmental stages were observed at the Great Salt Pond Outflow and at Buck Island Underwater Trail. All other treatments were not significantly different from the negative control. Magnification = 100X.

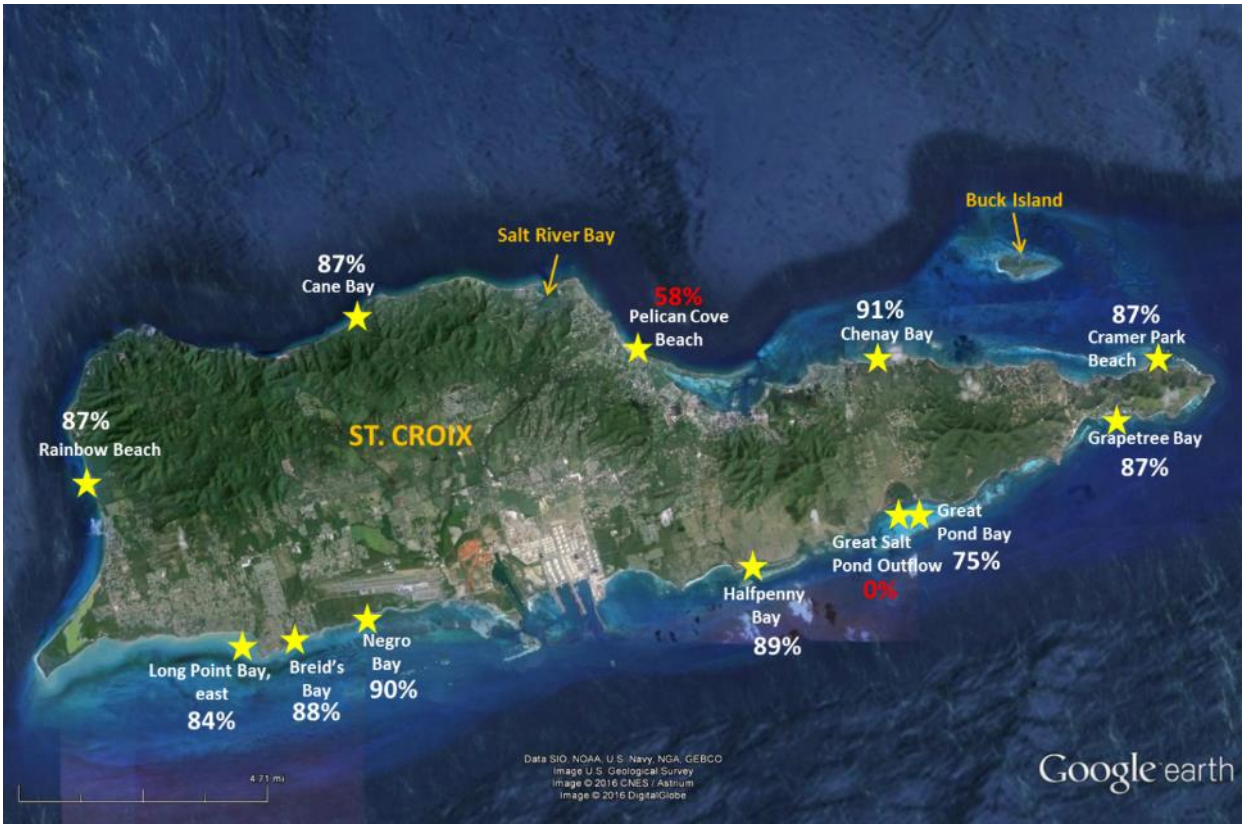


Figure 5. Map of St. Croix, USVI with results of sea urchin embryo development tests of sediment porewaters. Sampling sites (yellow stars) with percent normal embryos observed are listed for each location. Values in red indicate sites with percent normal embryos significantly different from the artificial seawater control.

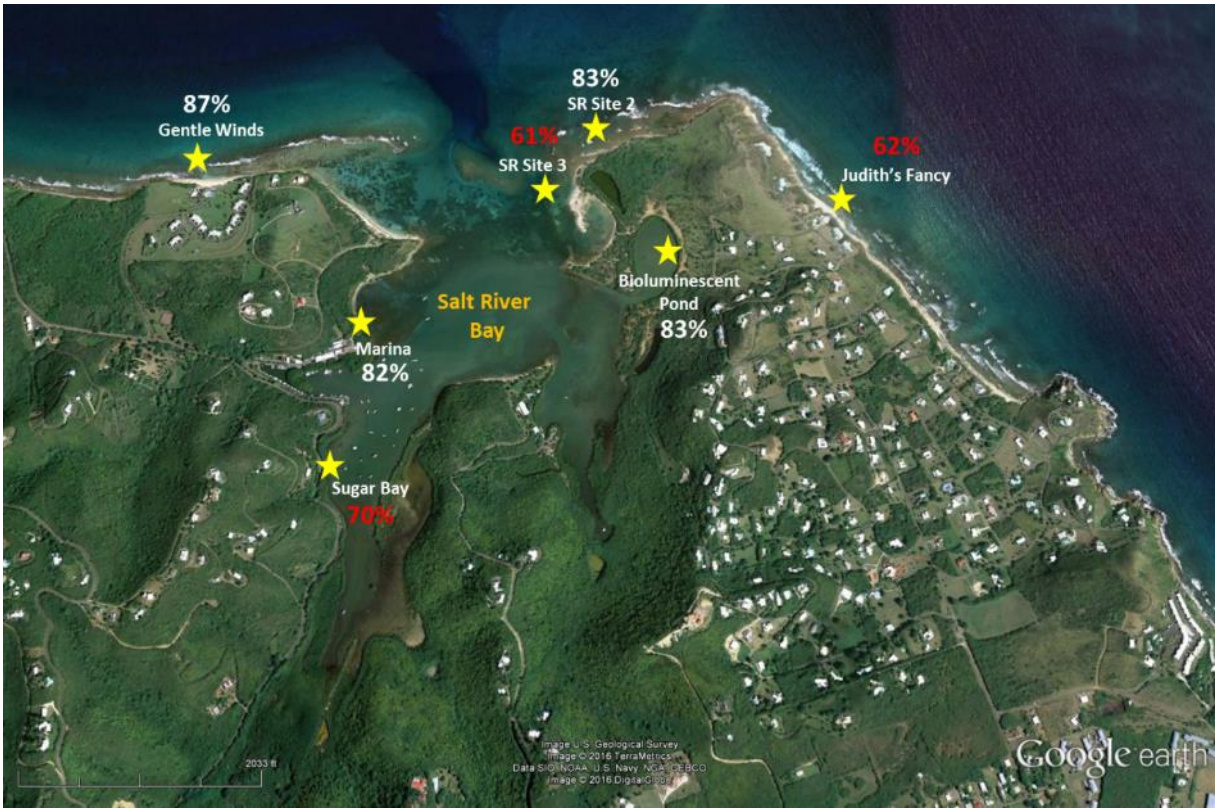


Figure 7. Map of Salt River Bay, St. Croix, USVI with results of sea urchin embryo development tests of sediment porewaters. Sampling sites (stars) with percent normal embryos observed are listed for each location. Values in red indicate sites with percent normal embryos significantly different from the artificial seawater control. Red stars indicate sites with low *Acropora palmata* reproductive effort in 2013.

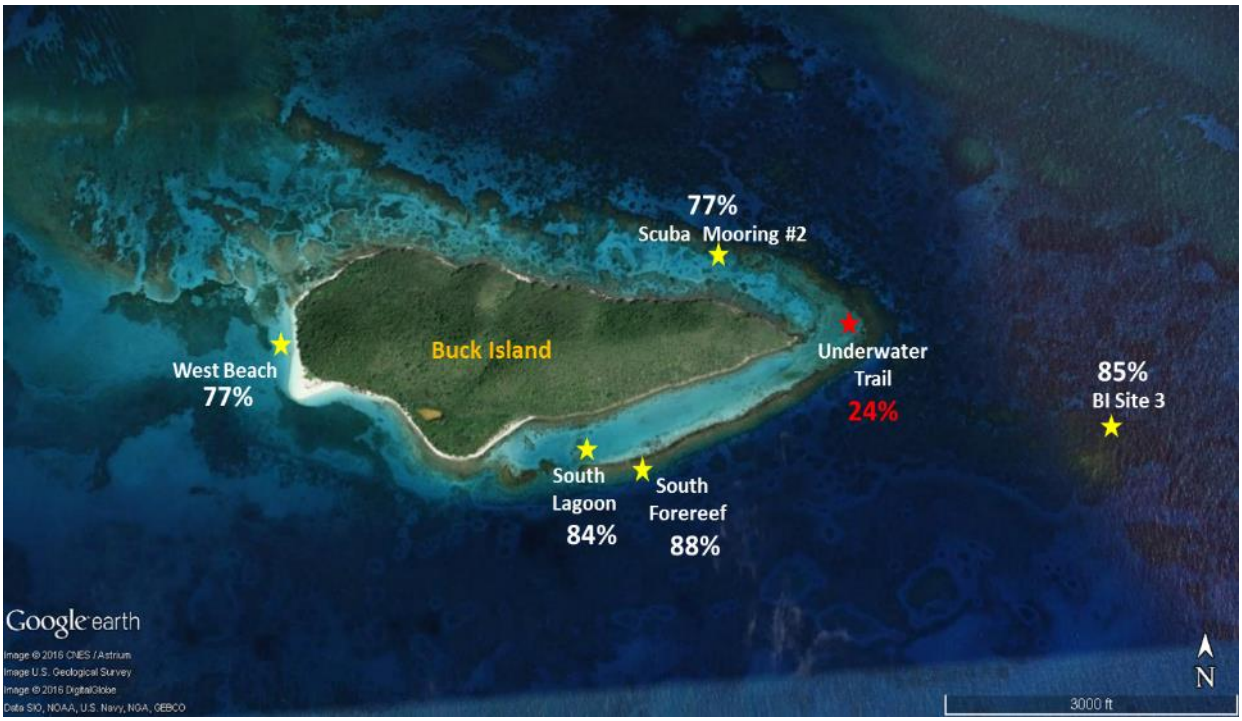


Figure 6. Map of Buck Island National Monument, St. Croix, USVI with results of sea urchin embryo development tests of sediment porewaters. Sampling sites (stars) with percent normal embryos observed are listed for each location. Value in red indicates site with percent normal embryos significantly different from the artificial seawater control. Red star indicates site with low *Acropora palmata* reproductive effort in 2013.

Phase I Toxicity Identification Evaluation with C18 Column

Phase I toxicity identification evaluations were conducted on eight of the samples showing toxicity ranging from 0% normal development to 76.4% in the original toxicity bioassay (Figures 4, 6-9). The toxicity reduction test treated the sediment porewater by filtering it over a C18 SPE column. Column treatment of sample porewater improved sea urchin embryo development outcome for all treatments, however three samples were significantly different from the TMASW control (BUIS Underwater Trail, Judith's Fancy and Pelican Cove Beach) (Table 2). C-18 SPE columns bind nonpolar or moderately polar compounds which likely contribute to sea urchin embryo toxicity at Buck Island Underwater Trail, Great Pond Bay, Pelican Cove Beach, and the three Salt River sites. At the BUIS Underwater Trail, these chemicals constitute the majority of the contaminants and may include hydrocarbons (i.e., boat motor operations), antifoulants and/or personal care products (e.g., certain sunscreens) from recreational swimmers and boaters.

Table 2. Results of Phase I TIE - Toxicity Reduction		
SITE	**% normal pre-treatment**	**% normal post-treatment**
Buck Is. Scuba #2	76.4	78.3
Buck Is Underwater Trail	**26.1**	**68.5***
Buck Is West Beach	77.0	76.8
Great Pond Bay	74.9	84.0
Pelican Cover Beach	**57.7**	**70.9***
Salt River Judith's Fancy	61.9	79.0
Salt River Site 3	**61.1**	**87.5***
Salt River Sugar Bay	70.4	82.0

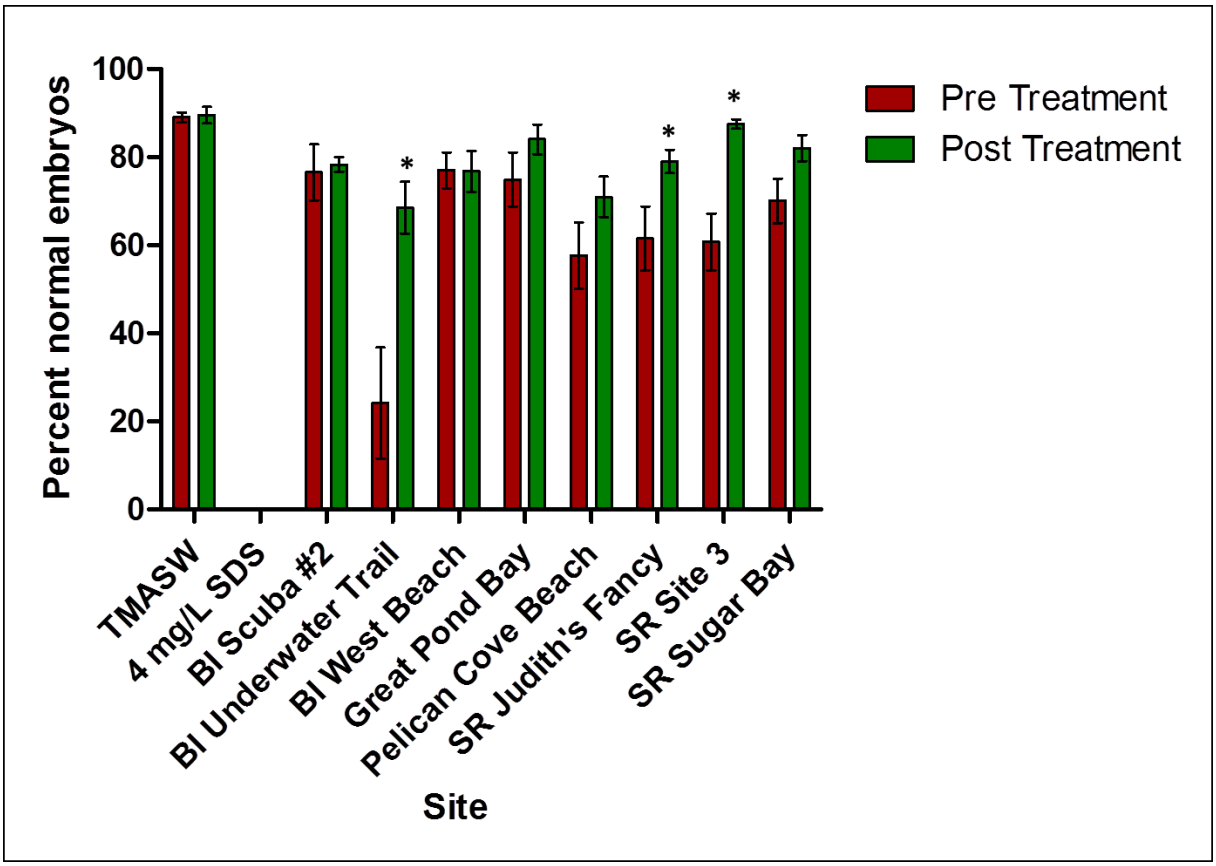


Figure 9. Results of Phase I TIE using a C18 column. Pretreatment percent normal embryos (red bars) and post-treatment percent normal embryos (green bars) indicate toxicity reduction at each site with column purification. Post treatment samples indicated with an asterisk (*) were significantly improved over the pretreatment. BI Underwater Trail $p<0.00001$; SR Judith's Fancy, $p<0.05$; SR Site #3, $p<0.001$.

CONCLUSIONS

Water quality

1. Sediment porewater from the Great Salt Pond Outflow site contained a level of unionized ammonia that is toxic to *L. variegatus* and coral (*Acropora palmate* larvae).
2. Sediment porewater from 12 sampling sites had levels of total phosphorus above the recommended limit for Class A, B and C marine waters in the USVI. We do not know if water column samples reflect the same phosphorus loads, however. Further study on the sources of phosphorus and the effects of phosphorus on marine organisms is needed.

Sea Urchin Toxicity

1. Six sites showed sea urchin embryo toxicity in the sediment porewater analysis (Great Salt Pond Outflow, Pelican Cove Beach, BUIS Underwater Trail, SARI Site 3, SARI Sugar Bay and SARI Judith's Fancy). As mentioned previously, the toxicity associated with the Great Salt Pond Outflow can be attributed to the toxic level of unionized ammonia.
2. The results of the Phase I toxicity identification evaluation indicate that most of the toxicity at the BUIS Underwater Trail, SARI Judith's Fancy and SARI Site 3 is due to non-polar or moderately polar compounds. This may include hydrocarbons, anti-foulants, detergents, and personal care products. The toxicity may be a cause of the reproductive failure in *Acropora palmata*, noted in our 2013 study.
3. Toxicity in sediment porewaters from Pelican Cove Beach and SARI Sugar Bay is partially due to non-polar and moderately polar compounds, however further investigation is needed to determine what other toxicants may be contributing to degraded water quality.

Acknowledgments

We very much appreciate the funding support for this work (CRCP Project 1133) provided by the Coral Reef Conservation Program to CMW.

We especially thank those assisting with field collections, Benjamin Wilson of Tobago Cays Marine Park, St. Vincent and the Grenadines; Zandy Hillis-Starr and Clayton Pollock of the National Park Service, St. Croix.

We appreciate the work of Carl Miller and Zachary Moffitt for their aquaculture expertise and maintaining sea urchins at the Coral Culture Facility, NCCOS, Charleston, SC.

We give a special thanks to Dr. Paul Pennington (NCCOS, Charleston SC) for conducting statistical analyses.

REFERENCES

Bottger SA and JB McClintock. 2001. The effects of organic and inorganic phosphates on fertilization and early development in the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). *Comp. Biochem. Phys. Part C.* 129:307-315.

Bower CE and JP Bidwell. 1978. Ionization of ammonia in seawater—Effects of temperature, pH, and salinity. *J. Fish. Res. Board Can.* 35:1012-1016.

Byrne M, DJ Oakes, JK Pollak, and E Laginestra. 2008. Toxicity of landfill leachate to sea urchin development with focus on ammonia. *Cell Biol. Toxicol.* 24:503-512.

Carpenter SR, NF Caraco, DL Correll, RW Howarth, AN Sharpley, and VH Smith. 1998. Nonpoint Pollution of Surface Waters with Phosphorus and Nitrogen. *Ecological Applications.* 8:559-568.

Carr SR and DC Chapman. 1992. Comparison of whole sediment and pore-water toxicity tests for assessing the quality of estuarine sediments. *Chem. Ecol*. 7:19-30.

Carr SR, DC Chapman DC, ER Long, HL Windom, G Thursby, GM Sloane, *et al.* 1996. Sediment quality assessment studies of Tampa Bay, Florida. *Environ. Toxicol. Chem.* 15:1218-1231.

Chapman G, D Denton, and J Lazorchak. 1995. *Short-term methods for estimating the chronic toxicity of effluents and receiving water to West Coast Maine and estuarine organisms,* U.S. Environmental Protection Agency, Washington, DC, EPA/600/R-95/136 (NTIS PB96261665).

Dunn JG, PW Sammarco and G LaFleur, Jr. 2011. Effects of phosphate on growth and skeletal density in the scleractinian coral *Acropora muricata*: A controlled experimental approach. *J. Exp. Mar. Biol. Ecol.* 411:34-44.

Patan, A and K McLaughlin 2007. The Oceanic Phosphorus Cycle. *Chem. Rev.* 107:563-576.

Tovar-Sanchez A, D Sanchez-Quiles, G Basterretzea, JL Benede, A Chisvert, A Salvador, I Moreno-Garrido and J Blasco. 2013. Sunscreen products as emerging pollutants to coastal waters. *PLOS One* 8(6): e65451.

Zar, JH. 1999. *Biostatistical Analysis*, 4th edition. Prentice Hall, Upper Saddle River, NJ, USA. p.278-280.