Photochemical Release of Sediment Bound Brevetoxin (PbTx-2) From Resuspended Sediments

G. Brooks Avery Jr.^{a,*}, Wesley Mickler^a, Emily Probst^a, Ralph N. Mead^a, Stephen A. Skrabals^a,
Robert J. Kieber^a, and J. David Felix^b

a. Department of Chemistry and Biochemistry, University of North Carolina Wilmington, 601
South College Rd., Wilmington, N.C. 28403

averyg@uncw.edu

meadr@uncw.edu

skrabals@uncw.edu

kieberr@uncw.edu

b. Present address: Science & Engineering, Physical & Environmental Sciences
Texas A&M University-Corpus Christi Office: Natural Resources Center Texas A&M
University-Corpus Christi 6300 Ocean Dr. Corpus Christi, TX, 78412.
joseph.felix@tamucc.edu

Key words

Brevetoxins, photochemical release, resuspended sediments, PbTx-2

Abstract

Marine sediments from coastal Florida, USA impacted by *Karenia brevis* blooms were resuspended in seawater and irradiated in a solar simulator to determine if sedimentary bound PbTx-2 is photolytically released into the aqueous phase. All bulk and size-fractionated (<10-20 μm) sediments exhibited photorelease of PbTx-2 after a six-hour full spectrum irradiation. The magnitude of photorelease of a size-fractionated sediment ($250 \pm 20 \text{ pmol g}^{-1}$) was greater on a per gram basis compared to an analogous bulk sediment ($1.66 \pm 0.89 \text{ pmol g}^{-1}$). Experiments conducted with photosynthetically active radiation ($\lambda=400\text{-}700 \text{ nm}$) indicated that these less energetic wavelengths are also capable of releasing toxin from sediments. Two of three sediments autoclaved prior to irradiation with full spectrum sunlight exhibited a statistically significant photorelease of PbTx-2 into the aqueous phase suggesting abiotic processes are important in photoproduction of the toxin. The importance of PbTx-2 production from exposure of resuspended sediments to sunlight was estimated using photorelease data from the current study and previously reported total suspended solid concentrations for this region. The PbTx-2 produced from this process could account for 0.5 – 13% of extracellular brevetoxin concentration previously reported during a low level *Karenia brevis* bloom. Results of this study have significant implications for water quality management because they suggest a potentially important vector of toxin to overlying waters and ecosystems even in the absence of a *Karenia brevis* bloom.

1.0 Introduction

Harmful algae blooms of the dinoflagellate *Karenia brevis* occur annually, primarily along the Florida Gulf Coast, USA releasing a suite of lipophilic neurotoxins (brevetoxins) into coastal waters resulting in significant negative impacts on marine life, human health, fisheries and tourism (Baden, 1989; Baden et al., 2005; Brand et al., 2012; Maze et al., 2015). Human exposure to brevetoxins is generally through inhalation of aerosolized sea spray during a bloom or through consumption of contaminated shellfish resulting in neurotoxic shellfish poisoning. Shellfish harvesting areas are routinely monitored for *K. brevis* and regulatory agencies close shellfish harvesting when *K. brevis* cell counts exceed 5.0×10^3 cells L⁻¹ to prevent human exposure to brevetoxin laden shellfish (Watkins et al. 2008; Fleming et al., 2005).

There is a paucity of knowledge on the fate of brevetoxins in marine waters after a bloom has subsided and *K. brevis* cell counts return to baseline levels. Known sinks of brevetoxins in marine water columns are biological uptake and metabolism (Redshaw et al., 2010; Shetty et al., 2010; Bricelj et al., 2012), export via aerosols (Pierce et al., 2003) sorption to sediments (Pierce et al., 2004; Hitchcock et al., 2012; Mendoza et al., 2008), and photodegradation (Kieber et al., 2010). Mendoza et al. (2008) reported detectable levels of brevetoxins in surface sediments collected five months after a *K. brevis* bloom. These findings confirm that marine sediments act as a reservoir of brevetoxins when *K. brevis* is not in bloom, possibly storing toxins for future remobilization.

One possible mechanism for remobilization of brevetoxins sorbed onto sediments is by photochemical release following resuspension of sediments. Sediment resuspension occurs through natural or anthropogenic processes including, wind driven wave action, biotic disturbance, dredging, and boat propeller wash. Previous studies have reported that dissolved

organic carbon (DOC) and nutrients are released into the water column from resuspended river and estuarine sediments upon exposure to simulated sunlight (Kieber et al., 2006; Mayer et al., 2006; Southwell et al., 2010). Therefore, it is possible that brevetoxins are also capable of being released by this process.

Understanding photochemical release of brevetoxins from resuspended sediments is important for assessing the impacts of sediment resuspension events in areas affected by *K. brevis* blooms. The primary goal of the current research was to determine if sedimentary bound brevetoxin (PbTx-2) is released into the aqueous phase through resuspension and exposure to simulated sunlight. Sediments from various sites previously exposed to red tide events were resuspended in a seawater matrix and exposed to simulated sunlight to determine the magnitude of brevetoxin photochemically released into the aqueous phase. Controls on the magnitude of the photochemical release were also examined including the role of irradiation wavelength, particle size and biological processes.

2.2 Methods

2.1 Standards and Reagents

All solvents were HPLC grade or better and purchased from Fisher Scientific. Brevetoxin standards (> 95% purity by HPLC) were purchased in units of 100 µg from MARBIONC (Wilmington, NC) packaged under inert gas in ampules and stored at -20°C until needed. Stock solutions were prepared by dissolving the toxin in 0.5 mL of acetone, sonicating for 30 seconds and transferring to a 2 mL Teflon screw capped vial. An additional 0.5 mL aliquot of acetone was added to the ampoule and sonicated for 30 seconds to ensure quantitative transfer of brevetoxin into the vial. Prepared brevetoxin stock solutions were stored at 4°C at a final concentration of 100 µg mL⁻¹. A Milli-Q Plus water system (Millipore, Bedford MA) provided

deionized water used in sample preparation. Mobile phase solvents for instrumental analysis were LC-MS grade purchased from Fisher Scientific.

2.2 Sediment Collection Sites

All sampling sites are indicated in Figure 1. All offshore sediments were collected by Florida Fish and Wildlife Conservation Commission (FWC) aboard a small vessel using a Ponar sampler. North Florida sediments (NFn) were collected offshore in the Gulf of Mexico and were sandy and contained fine shell hash. Central Florida sediments (CFn) were collected close to shore or onshore with either a Ponar sampler or by hand with a small shovel. Sediments from these sites varied greatly in composition ranging from sandy beach sediments to organic-rich tidal creek sediments. Sample CF1 was an organic-rich sediment collected from a bay. Samples CF2 and CF3 were collected approximately 3 to 5 km offshore with a Ponar grab sampler and were sandy and contained fine shell hash. Sample CF4 was collected from a beach and was composed almost entirely of shell hash material. Sample CF5 was an organic-rich sediment collected from a tidal creek. South Florida (SFn) samples SF1 and SF2 were collected 3 km offshore using a Ponar grab sampler and contained predominately fine shell hash. For all collections, the top layer of each sediment grab (approximately 2 cm) was skimmed off and placed in a resealable plastic bag and stored in a cooler until laboratory processing. Only the top surface sediment layer was used in simulated solar irradiation experiments.

2.3 Bulk Sediment Extraction

All sediment samples were extracted with acetone to determine if detectable levels of PbTx-2 were present prior to simulated solar irradiation experiments following published procedures (Mendoza et al., 2008). Bulk wet sediments from each site were placed in a small Kapak® pouch and freeze dried for 24 h in a Labconco FreeZone 4.5 benchtop freeze drying

system (-47 °C, <0.05 torr). A known mass of dry sediment was weighed into a 40 mL screw capped vial for extraction. Twenty mL of acetone was added and each vial and sonicated for 10 minutes in a Branson 3510 ultrasonic cleaner. The extract was passed through a Whatman® glass microfiber filter to remove particles. The extraction procedure was repeated two additional times and the extracted solvent combined and concentrated under reduced pressure using a rotary evaporator. Once the solvent volume was reduced to approximately 0.5 mL it was transferred to an HPLC vial with 3 small volume rinses and blown to dryness under a gentle stream of N₂ gas. The dried residue was reconstituted in 20% (v:v) ACN/H₂O and brevetoxins quantified using LC-MS.

2.4 Bulk Sediment and Size Fractionated (<10-20 µm) Irradiation Experiments

Samples containing only coarse grained sediments (sand and shell hash) were prepared for simulated solar irradiation by weighing approximately 3 g of bulk wet sediment into a quartz tube containing 70 mL of filtered seawater collected from Wrightsville Beach, NC (WBSW). Samples containing fine-grained sediments were size fractionated (<10-20 µm) prior to experiments to simulate sediments that would likely remain in the water column following a resuspension event (Xia et al., 2004). In order to size fractionate sediments, approximately 50 g of wet sediment was added to a 2 L Nalgene FLPE fluorinated carboy with filtered WBSW. The carboy was vigorously shaken by hand for two minutes and placed on the laboratory bench and allowed to settle for 17.5 minutes to obtain the <10-20 µm size fraction (Jackson 1973). The top layer of suspension was siphoned off into a clean combusted beaker. The beaker containing the size fractionated suspension mixture was placed on a stir plate to maintain a homogenous sediment suspension while apportioning into quartz tubes for simulated solar irradiation experiments.

Quartz tubes for initial, light and dark samples were prepared in quadruplicate. Initial samples were dispensed into quartz tubes and immediately filtered and prepared for brevetoxin extraction. Light samples were placed into the solar simulator uncovered. Dark samples were wrapped in aluminum foil and placed into the solar simulator. All sediment suspensions were exposed to simulated solar irradiation for 6 h.

A Spectral Energy solar simulator LH lamp housing with a 1000 W Xe arc lamp was used for all solar irradiation experiments. The lamp was powered by a LPS 256 SM power supply equipped with AM1 filter to remove wavelengths < 290 nm. The light spectrum of the solar simulator was designed to mimic high noon mid-summer natural sunlight at 34°N latitude. For full spectrum simulated solar irradiation experiments only the AM1 filter was used on the solar simulator. For PAR simulated solar irradiation experiments an additional filter was used in tandem with the AM1 filter to remove wavelengths between 290-400 nm.

A gyrotory shaker table operating at approximately 100 rpm was used inside of the solar simulator to maintain sediment suspension throughout irradiation. The shaker table inside the solar simulator was rotated 90° clockwise 3 times throughout the duration of the experiment to ensure equal irradiance of each replicate sample.

2.5 Autoclaved Sediment Irradiation Experiments

Three sediments were autoclaved prior to simulated solar irradiation to investigate the role of biological vs. abiotic processes on sedimentary photochemical release of PbTx-2. A Sanyo MLS 3750 autoclave was used to sterilize sediment and seawater suspensions at 121°C for 60 min. Results from the autoclaved sediment simulated solar irradiation experiments were compared to analogous non-autoclaved sediment experiments.

2.6 Simulated Solar Irradiation (Full Spectrum and PAR)

Photosynthetically active radiation (PAR; 400-700 nm) penetrates much deeper into the water column than the higher energy portion of the solar spectrum (Wörmer et al., 2010).

Experiments were conducted using full spectrum solar irradiation and only the PAR portion of the solar spectrum to investigate the wavelengths of light responsible for the photorelease of PbTx-2.

2.7 Aqueous Phase Brevetoxin Extraction

After solar irradiation samples were promptly suction filtered using a combusted and preweighed Whatman glass microfiber filter (0.7 μm) to separate the aqueous and sediment phases. The flask containing the aqueous phase was loaded onto a conditioned 500 mg Agela C18 solid phase extraction cartridge and passed through with a flow rate of approximately 3 mL min^{-1} . The flask was rinsed with Milli-Q and added to the aqueous phase. Once the entire seawater volume was passed through the SPE cartridge it was rinsed with 6 mL of Milli-Q to remove sea salts and allowed to dry under a vacuum for 5 minutes. The cartridge was slowly eluted into a clean combusted 20 mL screw capped vial with 10 mL of acetone. The acetone eluent was blown down under a gentle stream of prepurified N_2 gas at a temp of 40 °C and transferred into an HPLC vial with 3 small volume rinses. Recovery of PbTx-2 using this method is >90% (Kieber et al., 2010). The HPLC vial containing acetone eluent was blown down to dryness and reconstituted in v:v 20% ACN/ H_2O to a final volume of 125 μL . The samples were analyzed by LC-MS immediately after preparation.

2.9 Instrumental Analysis and Quantification.

An Agilent 1290 Infinity LC equipped with a reversed-phase Kinetex C18 column (2.1 x 150 mm, 1.7 μm) was used for chromatographic separation of samples. The mobile phases were (A) 100% HPLC grade water with 0.1% formic acid and (B) 100% HPLC grade acetonitrile with

0.1% formic acid. The gradient started 80:20 A/B and slowly increased to 1:99 A/B over 79 minutes. The injection volume was 5 μL and the mobile phase flow rate was 0.3 mL min^{-1} . A Bruker Amazon SL ion trap mass spectrometer was used for the detection of PbTx-2. Ionization of the samples was done using an (+) APCI source (drying temp. 200 $^{\circ}\text{C}$, nebulizer 44.00 psi, drying gas 12.0 L min^{-1}). (+) APCI was selected as an ionization source for PbTx-2 analysis because of its low limits of detection (mass on column 7.7×10^{-4} pg) and increased analytical sensitivity over (+) ESI (Mead et al., 2014). Identification of PbTx-2 was by the protonated molecular ion (895.0 m/z) and subsequently used to quantify the concentration.

High resolution mass spectrometry was performed on an Agilent 1290 LC coupled to a Bruker MicroQTOF II with APCI (+) ionization. All chromatographic conditions were the same as the low resolution sample analysis. An internal mass axis calibration was performed during the analysis using Agilent tune mix.

PbTx-2 concentrations were quantified using a 4 point external standard calibration curve. Standards were prepared by serial dilution of a PbTx-2 stock solution. The stock was pipetted into preweighed HPLC vials and diluted with acetone. After appropriate dilutions were made HPLC vials were blown to dryness using a gentle stream of N_2 and low heat (40 $^{\circ}\text{C}$). Dried HPLC vials were reconstituted with 20% v:v ACN/ H_2O to a final volume of approximately 500 μL and the concentration of PbTx-2 in each standard vial calculated gravimetrically. PbTx-2 standard concentrations ranged from 0.5 nM to 50 nM.

3.Results and Discussion

3.1 Full Spectrum Bulk and Size Fractionated Sediment Suspension Irradiations

A total of nine bulk and five size fractionated (<10-20 µm) sediment suspensions were prepared and irradiated with full spectrum simulated sunlight to determine the photochemical release of PbTx-2. Mean aqueous phase PbTx-2 concentrations were greater in all nine bulk light-exposed sediment suspensions compared to dark controls (Figure 2) suggesting that release of toxin occurred in these samples upon exposure to light. The amount of photoreleased toxin should be viewed as a minimum estimate because of the concurrent photodegradation of aqueous phase PbTx-2 observed in earlier studies (Kieber et al. 2010.)

A second series of irradiation experiments were conducted with size-fractionated (<10-20 µm) sediments to determine how particle size influenced photoproduction results. This fraction is considered to remain suspended in the photic zone during relatively low energy resuspension events (Hill et al., 2000; Xia et al., 2004). All five of these photochemical experiments conducted with full spectrum simulated solar irradiations resulted in greater mean aqueous phase PbTx-2 concentrations in the light-exposed samples compared to dark controls similar to what was observed when bulk sediments were irradiated (Figure 3). All size fractionated sediments exhibited a much larger photorelease of PbTx-2 on a per gram basis compared to bulk sediment solar irradiations most likely because of an increased surface area per mass of suspended particles and higher percent organic carbon. Results of both bulk and size fractionated irradiation experiments are significant because they confirm for the first time that photochemical processes release sediment-bound PbTx 2 from resuspended sediments. This has important implications because in the absence of a *K. brevis* bloom this process may remobilize PbTx 2 into the water column resulting in the negative effects associated with this toxin if threshold toxicity levels are reached.

Sediment-bound PbTx-2 concentrations and water column *K. brevis* counts at time of sediment collection were examined to determine possible relationships between these parameters. Sediments were collected from areas affected by *K. brevis* blooms between December 2012 to September 2014 (Table 1). There was a medium to high concentration of *K. brevis* bloom (1.0×10^5 to $> 1.0 \times 10^6$ cells L⁻¹) at the time of sediment collection in the vicinity of North Florida sample sites (NFx), Central Florida (CFx), and South Florida 1 (SF1) (<http://myfwc.com/research/redtide/statewide/>). Sediments at South Florida 2 (SF2) were collected approximately a month after a short-lived medium concentration bloom. There does not appear to be any correlation between *K. brevis* counts in the water column and sediment concentrations of PbTx-2. For example, CF sites had order of magnitude higher concentrations of sedimentary PbTx-2 but were collected under similar *K. brevis* water column conditions as all sites with the exception of SF2. Also, SF2 had similar sediment PbTx-2 concentrations as the NFx sites and were collected when *K. brevis* had not been present in the water column for several months. The results of this study are consistent with recent research that suggests brevetoxin concentrations are not directly related to cell counts and are influenced by environmental factors (Hardison et al., 2012; Hardison et al., 2013a; Hardison et al., 2013b; Hardison et al., 2014).

Similar results were obtained by (Flewelling (2008) who found that sedimentary phase brevetoxin concentrations were not correlated with water column *K. brevis* cell counts. In that study, sediment concentrations of brevetoxins and *K. brevis* cell counts were obtained bimonthly at four separate sites for five months. Three of these sites exhibited the greatest concentrations of sedimentary phase brevetoxins when *K. brevis* cell counts were the lowest for the sampling period. One site did not contain *K. brevis* cells, but had measureable sedimentary phase

brevetoxin concentrations that decreased over the sampling period. Therefore, when considering the possible impacts of photoreleased PbTx 2 from resuspended sediments it cannot be assumed that the impact is insignificant during times when *K. brevis* is not in bloom. Nor can it be assumed that water column concentrations of PbTx-2 are directly related to *K. brevis* counts. This has important implications for monitoring programs based solely on *K. brevis* counts because brevetoxin impacts may exist during resuspension events alone long after a bloom has ceased.

3.2 Sediment Suspension Irradiation with PAR

The effect of irradiation wavelength on PbTx-2 photoproduction from resuspended sediments was also evaluated. This has important implications with respect to the depth at which photochemical release of the toxin from resuspended sediments occurs. Photosynthetically active radiation penetrates deeper than shorter wavelengths of light such as UVA that can be absorbed by chromophoric dissolved organic matter (CDOM). Three sediment suspensions were exposed to PAR only to determine if these wavelengths of light would engender the release of PbTx-2 (Figure 4).

In all three sediment suspensions there was a greater mean aqueous phase PbTx-2 concentration in the light-exposed samples compared to dark controls similar to what was observed during full spectrum irradiations. The magnitude of photorelease from bulk sediment site NF3 was similar for both PAR and full spectrum exposure (1.66 ± 0.89 and 1.51 ± 0.97 pmol g⁻¹ respectively) suggesting that for this sediment, PAR was the most important wavelength for engendering release of the toxin. Photorelease of PbTx-2 from the other two sites was significantly smaller during PAR exposure compared to full spectrum suggesting that high energy UV wavelengths were relatively more important in these suspensions. Results presented

in Figure 4 are significant because they suggest that wavelengths of light in the PAR portion of the spectrum (400-700 nm) can induce significant photochemical release of PbTx-2 from suspended sediment particles allowing for this process to occur at relatively deeper locations in the water column.

3.3 Autoclaved Bulk Sediment Suspension Irradiation

A series of three autoclaved sediment resuspension experiments were conducted with full spectrum simulated solar irradiation to evaluate the role of biological processes on the photorelease of PbTx-2. Two of the three autoclaved sediments had significantly higher concentrations of PbTx-2 in light-exposed samples relative to dark controls suggesting abiotic processes can drive photorelease of the toxin (Figure 5). Sediment site CF4 had a greater aqueous phase concentrations of PbTx-2 in the dark control relative to the light exposed sample; however, large standard deviations were associated with the analytical measurements in this experiment potentially masking the interpretation of results.

3.4 Aqueous Phase Photo-products from Resuspended Sediments

Preliminary structural elucidation of photoproducts, produced during resuspension experiments, were examined using a combination of low and high resolution mass spectrometry conducted on sediments from two stations (NF 3 and NF 5). Extracted ion chromatograms from NF 3 consisted of several new peaks that were not present in the dark control and initial samples (Figure 6 and S1). The letters used to identify peaks in the aqueous phase of sediment site North Florida 3 were the same as the letters used to identify the peaks in the aqueous phase of sediment site North Florida 5. All of the new photoproducts formed (A-D) have shorter retention times and a greater $M+1$ m/z ratio compared to PbTx-2 (peak E). The decrease in retention time is due to

the formation of oxidized functional groups on the molecule that leads to a decrease in retention time given the separation was performed on a reverse phase column. The identify of the photoproducts is not known but based upon high resolution mass spectrometry data, the molecular formulas are all oxidized when compare dto PbTx-2 (peak E). Further research is on going regarding the formation and structure elucidation of these photoproducts.

3.4 Implications

The environmental relevance of photorelease of PbTx-2 from resuspended sediments can be determined by comparing PbTx-2 concentrations observed during a *K. brevis* bloom to an estimated release based on our laboratory experiments. To calculate a range of potential impact of this process on water column PbTx-2 concentrations, a range of previously published TSS concentrations from field locations as well as a range of photoreleased PbTx-2 from our laboratory experiments were used. For TSS concentrations, we assumed a range of 25 - 140 mg L⁻¹ reported for the Cedar Creek basin (Leonard et al., 1995). This site is located approximately 120 km southeast of the NF sampling sites in the current study in an area along the Florida Gulf Coast that is periodically impacted by *K. brevis* blooms. TSS values of approximately 25 mg L⁻¹ were measured in the absence of a wind driven sediment resuspension event while maximum measured TSS values of 140 mg L⁻¹ were observed during passage of an extra tropical low pressure system during a spring tide (Leonard et al. 1995). This is similar to other TSS values in the region such as those reported in Old Tampa Bay after strong sustained winds during spring tides (Schoellhamer 1995). These TSS values are also in the range for experiments conducted in the current study (40-80 mg L⁻¹).

The concentration of PbTx-2 released during the two size-fractionated sediment experiments with the greatest release (52 and 247 pmol g⁻¹ respectively) were used to calculate net photoproduction after a six hour sunlight exposure. Concentrations of photoproduced PbTx-2 at these sites ranged from 1.3 pm L⁻¹ for the low TSS release rate to 35 pm L⁻¹ for the high TSS high release rate (Table 2). Pierce et al. (2007) reported total extracellular brevetoxin concentration (PbTx-1 + PbTx-2 + PbTx-3) during a low level bloom (1.4 x 10⁴ cells L⁻¹) of 0.24 µg L⁻¹ or 268 pm L⁻¹. The photochemical release of PbTx-2 from resuspended sediments, based on results presented in Table 2, produces between 0.5 – 13% of the total toxin observed during an actual bloom. This has important implications since the potential concentrations of PbTx-2 from this process are similar in magnitude to that observed during an active *K. brevis* bloom. As a result the negative environmental effects associated with this toxin may exist during resuspension events occurring at locations containing PbTx-2 from previous blooms.

This study is the first, to the best of our knowledge, illustrating the formation of new photoproducts during resuspension of authentic toxin laden sediments. These new compounds may exhibit different toxicities and trophic transfer potential compared to brevetoxins. In a related study it has been shown photochemical transformation of brevetoxins form biologically active products but this biological activity decreased with increase in light exposure (Khan et al., 2010).

Acknowledgements:

Financial support of this project was provided by the National Science Foundation grant OCE-1154850 and the Division of Chemistry (CHE-1039784). The authors would also like to thank Dr. Alina Corcoran at Florida Fish and Wildlife Conservation Commission.

References

- Abraham, A. et al., 2006. Characterization of polar brevetoxin derivatives isolated from *Karenia brevis* cultures and natural blooms. *Toxicon*, 48(1): 104-115.
- Baden, D.G., 1989. Brevetoxins: unique polyether dinoflagellate toxins. *The FASEB Journal*, 3(7): 1807-17.
- Baden, D.G., Bourdelais, A.J., Jacocks, H., Michelliza, S. and Naar, J., 2005. Natural and derivative brevetoxins: Historical background, multiplicity, and effects. *Environmental Health Perspectives*, 113(5): 621-625.
- Brand, L.E., Campbell, L. and Bresnan, E., 2012. *Karenia*: The biology and ecology of a toxic genus. *Harmful Algae*, 14: 156-178.
- Bricelj, V.M., Haubois, A.-G., Sengco, M.R., Pierce, R.H., Culter, J.K., and Anderson, D.M., 2012. Trophic transfer of brevetoxins to the benthic macrofaunal community during a bloom of the harmful dinoflagellate *Karenia brevis* in Sarasota Bay, Florida. *Harmful Algae* 16:27-34.
- Fleming, L.E. et al., 2005. Initial Evaluation of the Effects of Aerosolized Florida Red Tide Toxins (Brevetoxins) in Persons with Asthma. *Environmental Health Perspectives*, 113(5): 650-657.
- Flewelling, L. J. 2008. Vectors of brevetoxins to marine mammals. Univ. South Florida Diss.

FWC, Florida Fish and Wildlife Conservation Commission. Accessed May 20th, 2015

<http://www.myfwc.com/research/redtide>

Hardison, D.R., Sunda, W.G., Litaker, R.W., Shea, D. and Tester, P.A., 2012. Nitrogen limitation increases brevetoxin in *Karenia brevis* (Dinophyceae): Implications from bloom toxicity. *Journal of Phycology*, 48(4): 844-858.

Hardison, D.R., Sunda, W.G., Shea, D. and Litaker, R.W., 2013a. Increased Toxicity of *Karenia brevis* during Phosphate Limited Growth: Ecological and Evolutionary Implications. *Plos One*, 8(3).

Hardison, D.R., Sunda, W.G., Shea, D., Tester, P.A. and Litaker, R.W., 2013b. When hungry, *Karenia brevis* goes toxic. *Phycologia*, 52(4): 41-41.

Hardison, D.R., Sunda, W.G., Tester, P.A., Shea, D. and Litaker, R.W., 2014. Increased cellular brevetoxins in the red tide dinoflagellate *Karenia brevis* under CO₂ limitation of growth rate: Evolutionary implications and potential effects on bloom toxicity. *Limnology and Oceanography*, 59(2): 560-577.

Hill, P.S., Milligan, T.G. and Geyer, W.R., 2000. Controls on effective settling velocity of suspended sediment in the Eel River flood plume. *Continental Shelf Research*, 20(16): 2095-2111.

Hitchcock, G.L., Fourqurean, J.W., Drake, J.L., Mead, R.N. and Heil, C.A., 2012. Brevetoxin persistence in sediments and seagrass epiphytes of east Florida coastal waters. *Harmful Algae*, 13(0): 89-94.

Jackson, M.L., 1973. *Soil Chemical Analysis-Advanced Course*, 2nd ed. M.L. Jackson, Madison, WI, 893 p

Khan, U., N. Benabderrazik, A. J. Bourdelais, D. G. Baden, K. Rein, P. R. Gardinali, L. Arroyo, and K. E. O'Shea. 2010. UV and solar TiO(2) photocatalysis of brevetoxins (PbTxS). *Toxicon* 55: 1008–1016.

Kieber, R. J., J. Pitt, S. A. Skrabal, and J. L. C. Wright. 2010. Photodegradation of the brevetoxin PbTx-2 in coastal seawater. *Limnol. Oceanogr.* 55: 2299–2304.

Kieber, R. J., R. F. Whitehead, and S. A. Skrabal. 2006. Photochemical production of dissolved organic carbon from resuspended sediments. 51: 2187–2195.

Leonard, L., C. Hine, M. E. Luther, R. P. Stumpf, and E. E. Wright. 1995. Sediment Transport Processes in a West-Central Florida Open Marine Marsh Tidal Creek - the Role of Tides and Extra-Tropical Storms. *Estuar. Coast. Shelf Sci.* 41: 225–248.

Mayer, L. M., Schick, L.L., and Skorko, K., 2006. Photodissolution of particulate organic matter from sediments. *Limnol. Oceanogr.* 51: 1064-1071.

Maze, G., Olascoaga, M.J. and Brand, L., 2015. Historical analysis of environmental conditions during Florida Red Tide. *Harmful Algae*, 50: 1-7.

Mead, R. N., E. E. Probst, J. R. Helms, G. B. Avery, R. J. Kieber, and S. A. Skrabal. 2014. Enhanced detection of the algal toxin PbTx-2 in marine waters by atmospheric pressure chemical ionization mass spectrometry. *Rapid Commun. mass Spectrom.* 28: 2455–60.

Mendoza, W. G., R. N. Mead, L. E. Brand, and D. Shea. 2008. Determination of brevetoxin in recent marine sediments. *Chemosphere* 73: 1373–1377.

Pierce, R. H., M. S. Henry, P. C. Blum, S. E. Osborn, Y.-S. Cheng, Y. Zhou, C. M. Irvin, A. J. Bourdelais, J. Naar, and D. G. Baden. 2011. Compositional changes in neurotoxins and their oxidative derivatives from the dinoflagellate, *Karenia brevis*, in seawater and marine aerosol. *J. Plankton Res.* 33: 343–348.

Pierce, R.H., Henry, M.S., Blum, P.C., Lyons, J., Cheng, Y.S., Yazzie, D., and Zhou, Y., 2003. Brevetoxin concentrations in marine aerosol: Human exposure levels during a *Karenia brevis* harmful algal bloom. *Bull. Environ. Contam. Toxicol.* 70: 161–165.

Pierce, R.H., Henry, M.S., Higham, C.J., Blum, P., Sengco, M.R., and Anderson, D.M., 2004. Removal of harmful algal cells (*Karenia brevis*) and toxins from seawater culture by clay flocculation. *Harmful Algae* 3:141-148.

Pierce, R., M. Henry, and P. Blum. 2007. Brevetoxin abundance and composition during ECOHAB-Florida field monitoring cruises in the Gulf of Mexico. *Cont. Shelf Res.* 28: 45–58.

Probst, E. E. 2013. Improved Analysis of PbTx-2 and its sedimentary photorelease. UNC Wilmington. Master of Science. Thesis

Redshaw, C.H., Sutter, D., Myers, T.L., Naar, J., and Kubanek, J., 2010. Tracking losses of brevetoxins on exposure to phytoplankton competitors: Mechanistic insights. *Aq. Toxicol.* 100: 365-372.

Riggsbee, J. A., C. H. Orr, D. M. Leech, M. W. Doyle, and R. G. Wetzel. 2008. Suspended sediments in river ecosystems: Photochemical sources of dissolved organic carbon, dissolved organic nitrogen, and adsorptive removal of dissolved iron. *J. Geophys. Res. Biogeosciences* 113: 1–12.

Schoellhamer, D. H. 1995. Sediment Resuspension Mechanisms in Old Tampa Bay, Florida. *Estuar. Coast. Shelf Sci.* 40: 603–620.

Shetty, K.G., Huntzicker, J.V., Rein, K.S. and Jayachandran, K., 2010. Biodegradation of polyether algal toxins—Isolation of potential marine bacteria. *J. Environ. Sci. Health A* 45: 1850-1857.

Skoog, Douglas A., F. James Holler, and Stanley R. Crouch. Principles of Instrumental Analysis. Sixth ed. Belmont: Brooks/Cole, 2007. 958-959. Print.

- Southwell, M.W., Kieber, R.J., Mead, R.N., Avery, G.B. and Skrabal, S.A., 2010. Effects of sunlight on the production of dissolved organic and inorganic nutrients from resuspended sediments. *Biogeochemistry*, 98(1-3): 115-126.
- Watkins, S. M., A. Reich, L. E. Fleming, and R. Hammond. 2008. Neurotoxic shellfish poisoning. *Mar. Drugs* 6: 431–455.
- Wetterauer, A. 2012. Photodissolution of copper from resuspended estuarine sediments. UNC Wilmington. Master of Science. Thesis
- Wörmer, L., M. Huerta-Fontela, S. Cirés, D. Carrasco, and A. Quesada. 2010. Natural photodegradation of the cyanobacterial toxins microcystin and cylindrospermopsin. *Environ. Sci. Technol.* 44: 3002–3007.
- Xia, X.M. et al., 2004. Observations on the size and settling velocity distributions of suspended sediment in the Pearl River Estuary, China. *Continental Shelf Research*, 24(16): 1809-1826.

1.6 References

Table 1: Surface sediment collection sites and concentrations of PbTx-2. FWC represents sediment collection from the Florida Fish and Wildlife Conservation Commission. Mote represents Mote Marine Lab.

Sample Site ID	Date Collected	Collection method (collected by)	Concentration PbTx-2 ng g⁻¹ dry wt⁻¹
North Florida 1 (NF1)	Sept. 22, 2014	Shipek (FWC)	0.21
North Florida 2 (NF2)	Sept. 22, 2014	Shipek (FWC)	0.05
North Florida 3 (NF3)	Sept. 22, 2014	Shipek (FWC)	0.61
North Florida 4 (NF4)	Sept. 22, 2014	Shipek (FWC)	0.41
North Florida 5 (NF5)	Sept. 22, 2014	Shipek (FWC)	0.36
Central Florida 1 (CF1)	Dec. 2012	Collected by hand (FWC)	3.60
Central Florida 2 (CF2)	Jan. 2013	Ponar (Mote)	5.80
Central Florida 3 (CF3)	Jan. 2013	Ponar (Mote)	25.0
Central Florida 4 (CF4)	Dec. 2012	Collected by hand (FWC)	38.0
Central Florida 5 (CF5)	Dec. 2012	Ponar (Mote)	4.40
South Florida 1 (SF1)	Jan. 2013	Ponar (Mote)	0.23
South Florida 2 (SF2)	Jan. 08, 2014	Collected by hand (Mickler)	0.59

Table 2: Calculated concentration of PbTx-2 released from resuspended sediments after a six hour full spectrum solar irradiation. Total suspended solid are from Leonard (2007).

Size Fractionated PbTx-2 photorelease (pm g⁻¹)	Total Suspended Solid Load (mg L⁻¹)	Calculated PbTx-2 Concentration (pm)
247	140	34.6
247	25	7.3
52	140	6.2
52	25	1.3

Figure Captions:

Figure 1. Map of sampling sites. North Florida sites are abbreviated NF, Central Florida sites are abbreviated CF, South Florida sites are abbreviated SF.

Figure 2. Mean aqueous phase PbTx-2 release from bulk sediment suspensions after 6 h of full spectrum simulated solar irradiation. Error bars represent standard deviations of quadruplicate samples. Asterisks denote statistically significant photorelease of PbTx-2 (t-test, $p = 0.05$). Initial sample concentrations were measured prior to experiment and were not subjected to simulated solar irradiation. Dark control samples were wrapped in aluminum foil and placed into the solar simulator for the duration of the irradiation.

Figure 3. Mean aqueous phase PbTx-2 release from size fractionated ($<10-20\ \mu\text{m}$) sediments after six hours of full spectrum simulated solar irradiation. Error bars represent standard deviations of quadruplicate samples. Asterisks denote statistically significant photorelease of PbTx-2 (t-test, $p = 0.05$). Initial sample concentrations were measured prior to the experiment and were not subjected to simulated solar irradiation. Dark control samples were wrapped in aluminum foil and placed in the solar simulator for the duration of the irradiation.

Figure 4. Mean aqueous phase PbTx-2 release from size fractionated and bulk sediments after 6 hours of PAR simulated solar irradiation. Experiments using bulk sediments included NF3 and CF2. The experiment using SF1 used size fractionated sediment. Error bars represent standard deviations of quadruplicate samples. Asterisks denote statistically significant photorelease of PbTx-2 (t-test, $p = 0.05$).

Figure 5. Mean aqueous phase PbTx-2 release from autoclaved bulk sediments after 6 hours of full spectrum simulated solar irradiation. Error bars represent standard deviations of quadruplicate samples. Asterisks denote statistically significant photorelease of PbTx-2 (t-test, $p = 0.05$).

Figure 6: (+) APCI 895.5 m/z plus 897.5 m/z extracted ion chromatograms of aqueous phase bulk sediment site North Florida 3. Mass spectra correspond to annotated peaks in the chromatogram. Peak E represents PbTx-2. Based upon high resolution mass spectrometry neutral molecular formulas and associated error is given as well.

Figure 1:

Figure 2:

Figure 3:

Figure 4:

Figure 5:

Figure 6: