Limnol. Oceanogr., 55(6), 2010, 2299–2304

doi:10.4319/lo.2010.55.6.2299

Photodegradation of the brevetoxin PbTx-2 in coastal seawater

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Abstract

The photodegradation rate of the brevetoxin analog PbTx-2 was investigated in a variety of natural water matrices. The observed first-order photodegradation rate coefficient of the logarithmic-transformed dissolved PbTx-2 concentrations vs. irradiation time was 0.20 ± 0.04 h^{-1} in coastal seawater, corresponding to a half-life of approximately 3 h based on 10 separate photolysis experiments. No loss of PbTx-2 occurred in dark controls, indicating that this was primarily a photo-mediated process. Photodegradation rate coefficients in samples in which dissolved organic matter was removed by ultraviolet oxidation prior to photolysis resulted in significantly slower rates of PbTx-2 photodegradation (0.08 ± 0.03 h^{-1}). When trace metals were also removed prior to photolysis, no loss of PbTx-2 occurred, indicating that direct photolytic loss of PbTx-2 is insignificant in coastal seawater. The proposed mechanism of PbTx-2 decay is a photosensitized pathway involving organic matter and trace metals that is significantly accelerated by decreasing oxygen concentrations. The influence of molecular oxygen on the rate of toxin loss has important ramifications for the fate of PbTx-2 during *Karenia brevis* blooms, as in situ dissolved O_2 concentrations fluctuate widely during bloom development and decay. Sunlight-mediated reactions are therefore a significant, yet previously unrecognized, sink of dissolved PbTx-2 in seawater under environmentally relevant conditions.

Harmful algal blooms (HABs) are a worldwide phenomenon caused by microalgae or phytoplankton that reach extremely high concentrations. Naturally occurring toxins are often secreted during these blooms, engendering a variety of economic, environmental, and human health effects that have reinforced the idea that these HABs are events of global concern (Hallegraef 1993; Trainer and Baden 1999). Eutrophication and natural processes have been hypothesized as contributing factors in HAB occurrence. These processes include river runoff, pollution, aeolian dusts, ocean temperature, ocean circulation, upwelling, and pycnocline structure (Walsh and Steidinger 2001; Sellner et al. 2003; Kirckpatrick et al. 2004). The observed increase in HAB events has been speculated to result from interactions of a variety of natural and anthropogenic events occurring over a broad range of temporal and spatial scales (Donaghay and Osborn 1997).

Blooms known as red tides occur annually in the Gulf of Mexico off the western Florida coast, resulting in large economic losses in fisheries and serious public health issues. These blooms are caused by the toxigenic dinoflagellate *Karenia brevis*, with typical bloom cell densities of 10^7–10^8 cells L^{-1} (Steidinger et al. 1978; Tester and Steidinger 1997). In addition to water discoloration, *K. brevis* blooms are responsible for respiratory illness in humans and other mammals (Kirckpatrick et al. 2004), marine mammal mortalities (Bossart et al. 1998), and contamination of marine food webs, with subsequent trophic transfer (Tester et al. 2000). The relationship between *K. brevis* blooms and fish kills and respiratory illness has been known for some time (Woodcock 1948). The responsible toxins produced by *K. brevis* comprise a family of over 10 lipophilic *trans* fused cyclic polyethers with *syn* relative stereochemistry known as brevetoxins. They are released into the surrounding water column following cell lysis, with PbTx-2 and PbTx-3 as the major toxins detected in most blooms (Bourdelais et al. 2005; Pierce et al. 2008; Tester et al. 2008).

Very little is known regarding the abiotic degradation of the dissolved fraction of brevetoxins in natural waters, despite the well-documented deleterious effects to public health and marine life. This is particularly significant for brevetoxins because recent studies indicate that water column concentrations can reach as high as 73 μg L^{-1} during bloom events (Tester et al. 2008). One potentially important sink for brevetoxins in coastal waters involves sunlight-mediated photochemical transformations. Earlier studies have revealed that photodegradation is an important sink for other algal toxins in natural waters, including microcystins (Welker and Steinberg 2000) and domoic acid (Bouillon et al. 2006). Khan et al. (2010) recently reported that TiO_2 effectively degrades relatively high concentrations (μmol L^{-1}) of PbTx upon exposure to sunlight and suggested that addition of the photocatalyst may be an effective remediation strategy for the toxin in surface waters where blooms of *K. brevis* occur. Photodegradation of brevetoxins has important ramifications with respect to the biogeochemistry of coastal waters because it may significantly reduce the lifetime of these powerful and bioaccumulative neurotoxins in the milieu in which they are produced. Photodegradation of brevetoxins has further implications for the health of coastal ecosystems because it generates photoproducts with unknown toxicity, fate, and potential for trophic transfer.

The aim of the present study was to carry out a detailed kinetic study of the photodegradation of extracellular (dissolved) PbTx-2 in order to quantify the importance of this removal process in coastal seawater. A variety of controlled irradiation experiments were conducted to assess both the rate and mechanism of photodegradation under environmentally relevant conditions. The influence

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of potentially important physical and chemical parameters, including chromophoric dissolved organic matter (CDOM), trace metals, and dissolved oxygen, on the photodegradation of PbTx-2 is also discussed.

Methods

Reagents and standards—All solvents were obtained from Fisher Scientific and were chromatography-grade products, unless otherwise stated. A Milli-Q Plus water system (Millipore) provided the deionized water (DIW; 18.2 MΩ cm^{-1}) used for sample extraction and mobile phases. PbTx-2 (packaged under inert gas, −20°C; > 95% purity; 100 μg) was obtained from World Ocean Solutions. Each vial was rinsed with 1 mL acetone, transferred to a high-performance liquid chromatography (HPLC) screw-cap vial, and stored at 4°C as a stock solution at a final concentration of 100 μg mL^{-1}. This stock was spiked as an acetone extract into seawater, which was subsequently apportioned into vials for irradiation.

Photolysis experiments—Seawater was collected from Wrightsville Beach, North Carolina (34.208°N, 77.796°W) in an acid-cleaned 1-liter glass container. Filtered (0.2 μm) Wrightsville Beach seawater (WBSW) was stored in the dark at 5°C until use. Irradiation solutions of filtered WBSW were spiked with PbTx-2, and 25-mL aliquots were apportioned into each of the 17 screw-cap quartz cells (30 cm long, 30 mL in volume; Spectrocell). Twelve cells were placed vertically in a circular carrier and submerged in a temperature-controlled water bath (25°C) under a solar simulator (Spectral Energy 153 lamp housing, 1-kW Xe arc lamp with an AM1 filter to remove wavelengths of < 290 nm). The spectral quality and intensity of the solar simulator are very similar to those of midsummer, noontime solar irradiance for 34°N latitude. Light measurements at individual cell locations were measured before and after photolysis with an Ocean Optics spectrophotometer connected to fiber-optic cable terminated with an ultraviolet (UV) cosine collector. Three cells were kept dark as controls. Duplicate irradiated samples were removed from the solar simulator and extracted for PbTx-2 analysis every hour for 6 h, whereas dark controls were extracted at 0, 3, and 6 h.

Sample extraction—Extraction and analysis of PbTx-2 were carried out using a modification of an earlier technique (Cheng et al. 2005). Water samples were extracted with C_{18} reverse-phased cartridges prewashed with 6 mL of acetone and 10 mL of DIW. Samples were passed directly from irradiation cells through the cartridge to mitigate loss of toxin. Samples were drawn dropwise through cartridges by vacuum (~ 1 mL min^{-1}). Each cartridge was washed with 10 mL of DIW to remove salts; this was followed by elution with 6 mL of acetone. This procedure gave nearly quantitative recovery, as demonstrated in experiments using 180 nmol L^{-1} PbTx-2 in 0.2 μm–filtered WBSW (92% ± 2%; $n = 3$). Eluants were vacuum-dried for 6 h, reconstituted with 1.5 mL of acetone, and transferred to screw-capped HPLC vials.

Liquid chromatography–mass spectrometry—PbTx-2 samples were analyzed by positive ion electrospray liquid chromatography–mass spectrometry (LCMS; Applied Biosystems) at *m/z* 895.6. An optimized gradient elution was used (mobile phase A: 98% Milli-Q water, 2% acetonitrile; mobile phase B: 98% filtered acetonitrile, 2% DIW, both with 0.1% formic acid and 0.01% trifluoracetic acid) through a reverse-phase C_{18} column (2.0 × 150 mm; 5 μm; Phenomenex), with an injection volume of 4.0 μL. Triplicate injections of each sample were alternated with a blank injection. Concentrations of PbTx-2 were quantified at *m/z* 895.6 using standard curves prepared from PbTx-2 standards for each set of runs.

Experimental treatments—Dissolved organic matter (DOM) and trace metals were removed from filtered WBSW in a series of experiments to determine their role in PbTx-2 photodegradation. Dissolved organic carbon was determined with a Shimadzu TOC 5000 carbon analyzer equipped with an ASI 5000 autosampler. DOM was removed by high-energy UV oxidation in quartz tubes for 3 h (1200-W mercury vapor lamp; Ace Glass). Trace metals were removed from UV-oxidized WBSW by batch treatment (20 g L^{-1}) with Chelex-100 (BioRad). The trace metal–clean seawater was decanted after overnight treatment on a shaker table, 0.2-μm filtered to remove any resin particles, and UV oxidized in quartz tubes for 3 h to remove any organics leached from the resin. Treated seawater was stored refrigerated before photolysis. Chelex-100 resin was cleaned before use by successive soakings in 6 mol L^{-1} HCl and 2 mol L^{-1} HCl (trace metal grade) and high-purity 1 mol L^{-1} NH_4OH (Fisher Optima), with repeated rinsing of the resin with DIW between steps.

Filtered WBSW was deoxygenated with nitrogen gas to determine the effect of oxygen and reactive oxygen species on the photodegradation of PbTx-2. Filtered WBSW (0.2 μm) was placed in a nitrogen-filled glove bag and bubbled with nitrogen gas (filtered, high purity) overnight. The pH of the seawater increased from 7.95 to 8.45 after bubbling, but was returned to its original pH by addition of dilute HCl. A deoxygenated sample of WBSW was spiked with PbTx-2 inside the N_2-filled glove bag. Aliquots of solutions were poured into quartz cells (30 cm long, 30 mL in volume) to minimize headspace and were capped. Samples were irradiated as in previous WBSW experiments, extracted, and analyzed by LCMS.

Results

Photodegradation of PbTx-2 in filtered coastal seawater—A series of 10 controlled photolysis experiments was carried out with PbTx-2 in filtered WBSW to determine the rate constant for photodegradation of the toxin in seawater. A representative plot of the change of PbTx-2 concentration as a function of irradiation time is presented in Fig. 1. There was a significant loss of PbTx-2 over the 6-h incubation period in the light-exposed sample, with concentrations decreasing exponentially as a function of irradiation time from near 100 nmol L^{-1} to less than 30 nmol L^{-1}. No loss of PbTx-2 was observed in the dark

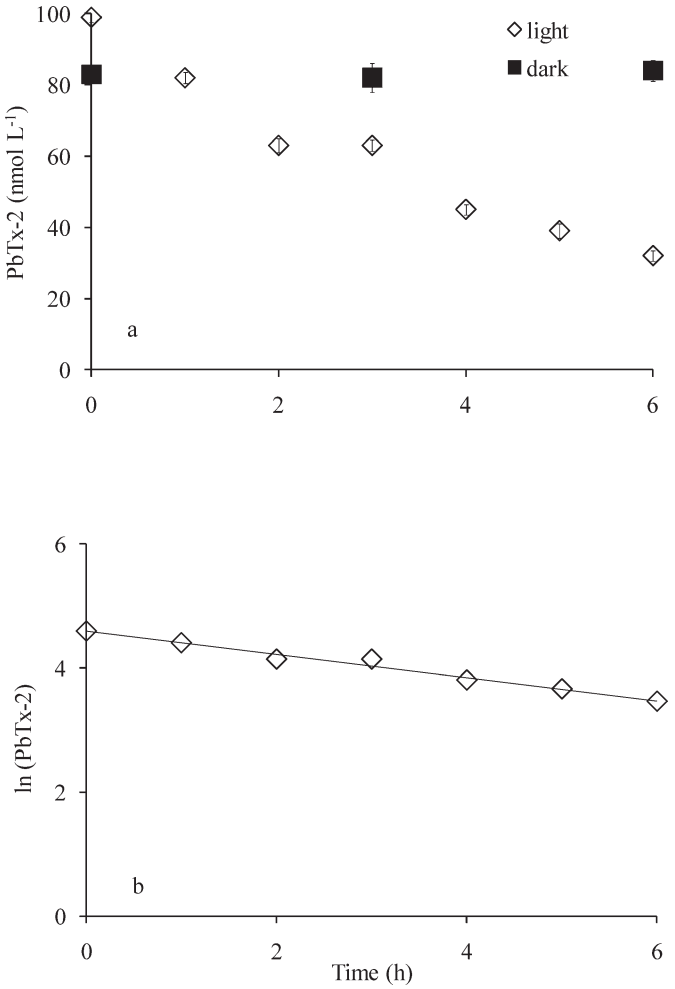

Fig. 1. (a) Concentration of PbTx-2 (nmol L^{-1}) in light-exposed and dark control samples as a function of irradiation time (h) in 0.2 μm–filtered WBSW. Error bars represent the standard deviation of triplicate LCMS injections per sample. (b) Natural logarithm of PbTx-2 concentration (nmol L^{-1}) as a function of irradiation time (h). The line represents a best-fit linear regression for light-exposed samples.

control (Fig. 1), indicating that light was responsible for the loss of PbTx-2. Observed first-order rate coefficients (k_{obs}) of PbTx-2 degradation in 10 filtered WBSW samples were obtained by linear regression of the logarithmic-transformed PbTx-2 concentrations vs. irradiation time (Fig. 1; Table 1). The average first-order rate coefficient in filtered WBSW was 0.20 ± 0.04 h^{-1} (Table 1).

Role of environmental factors on the kinetics of PbTx-2 photodegradation—A second series of controlled photodegradation experiments was conducted to determine the effect of sample matrix on the rate of PbTx-2 photodegradation in seawater. Seawater containing 125 μmol L^{-1} dissolved organic carbon (DOC) was first UV irradiated by a 1.2-kW high-pressure mercury vapor lamp for 6 h to remineralize the DOC present in samples prior to irradiation. DOC analysis performed before and after UV irradiation indicated that organics were photo-oxidized, resulting in seawater DOC concentrations below the detection limit of 5 μmol L^{-1}. The resulting k_{obs}, measured in 10 separate photolysis experiments, for the photochemical loss of PbTx-2 measured 0.08 ± 0.03 h^{-1} (Table 2), with no loss observed in dark controls. The results presented in Table 2 indicate that PbTx-2 degrades at a significantly (p = 0.0001; Mann–Whitney test; Systat 10.2) slower rate in UV-oxidized WBSW relative to nonirradiated seawater. This indicates that some fraction of the ambient DOM pool in seawater significantly enhances the rate of PbTx-2 photodegradation in seawater. Khan et al. (2010) reported humic acid–mediated photodegradation of PbTx in seawater, indicating that these macromolecular chromophoric molecules may play a central role in the environmental fate of the toxin.

A second experiment exploring the role of sample matrix was performed with UV-oxidized WBSW that was also treated with Chelex-100 to determine the effect of trace metals on the photodegradation of PbTx-2. There was no loss of PbTx-2 in UV-oxidized, trace metal–clean, filtered WBSW in both light-exposed and dark samples (Fig. 2). This indicates that trace metals are involved in the

Table 1. Pseudo first-order rate coefficient of PbTx-2 photodegradation in 0.2 μm–filtered WBSW obtained by linear regression of the logarithmic-transformed PbTx-2 concentrations vs. irradiation time. All samples were irradiated for 6 h under simulated sunlight at 25°C. SD, standard deviation.

Trial	First-order rate constant (h^{-1})
1	0.14
2	0.13
3	0.24
4	0.21
5	0.22
6	0.26
7	0.18
8	0.23
9	0.20
10	0.19
Mean±SD	0.20±0.04

Table 2. Pseudo first-order rate coefficient of PbTx-2 photodegradation in UV-oxidized, 0.2 μm–filtered WBSW obtained by linear regression of the logarithmic-transformed PbTx-2 concentrations vs. irradiation time. All samples were irradiated for 6 h under simulated sunlight at 25°C. SD, standard deviation.

Trial	Pseudo first-order rate constant (h^{-1})
1	0.10
2	0.07
3	0.05
4	0.07
5	0.05
6	0.10
7	0.05
8	0.07
9	0.09
10	0.12
Mean±SD	0.08±0.03

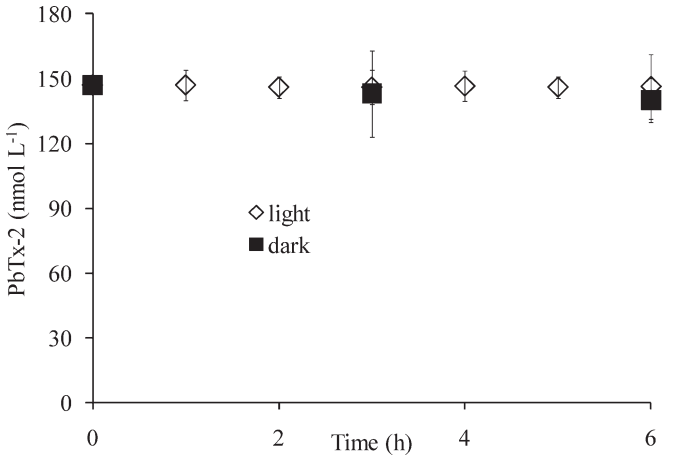

Fig. 2. Concentration of PbTx-2 (nmol L^{-1}) in light-exposed and dark control samples as a function of irradiation time in UV-oxidized, trace metal–clean, 0.2 μm–filtered WBSW. Error bars represent the standard deviation of triplicate LCMS injections per sample.

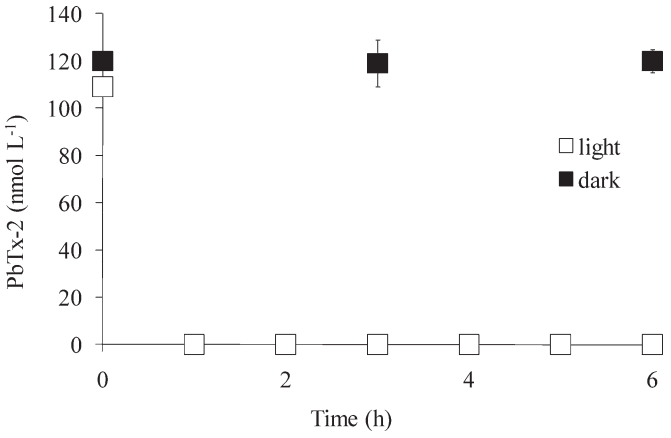

Fig. 3. Concentration of PbTx-2 (nmol L^{-1}) in light-exposed and dark control samples as a function of irradiation time in deoxygenated, 0.2 μm–filtered WBSW. Error bars represent the standard deviation of triplicate LCMS injections per sample.

photodegradation of PbTx-2 in seawater. Results from Fig. 2 also indicate that direct photodegradation of PbTx-2 is most likely not an important sink for the dissolved fraction of the toxin in seawater, a finding that is in agreement with those of an earlier study (Khan et al. 2010) in which no direct photodegradation of PbTx-3 was observed upon exposure to 350-nm light.

A solution of WBSW was purged with N_2 for 4 h prior to irradiation in order to evaluate the role of dissolved oxygen on the photodegradation of PbTx-2 in seawater. PbTx-2 in the reduced-O_2 sample photodegraded rapidly to undetectable levels after 1 h of sunlight exposure and remained at nondetectable levels for the entirety of the 6-h irradiation (Fig. 3). There was no significant change in deoxygenated dark controls during the 6 h, indicating that the loss in irradiated flasks was a light-mediated process.

Discussion

Implications and mechanism of photodegradation—The average observed first-order photodegradation rate coefficient of PbTx-2 was 0.2 ± 0.05 h^{-1} in filtered untreated WBSW exposed to simulated sunlight. A series of experiments was conducted to assess which environmental factors affect the photolysis rate in order to evaluate the mechanism of PbTx-2 photodegradation. The first treatment removed DOM by UV oxidation prior to photolysis, resulting in significantly slower rates of PbTx-2 photodegradation (0.08 ± 0.03 h^{-1}). When trace metals were also removed, no photodegradation of PbTx-2 occurred, indicating that direct photolysis of the toxin is not a significant sink in natural waters.

The following mechanism involving metal-complexed chromophoric DOM (CDOM-m) is proposed for the photosensitized degradation of PbTx-2 based on the results presented in Figs. 1–3:

$$\text{CDOM-m} + h\nu \rightarrow (^3\text{CDOM-m})^* \quad (1)$$

$$\text{PbTx-2} + (^3\text{CDOM-m})^* \rightarrow \text{CDOM-m} + \text{PbTx-2}^* \quad (2)$$

$$\text{PbTx-2}^* \rightarrow \text{products} \quad (3)$$

Absorption of solar radiation by CDOM-m in natural waters results in formation of a singlet excited species that decays, in part, by undergoing intersystem crossing to a much longer-lived triplet excited state complex (^{3}CDOM-m)*, depicted by Eq. 1 in the proposed mechanism above. These excited triplet state molecules can rapidly increase light-induced transformation of compounds that are otherwise relatively stable in sunlight (Zepp et al. 1985). Compounds containing conjugated double-bond moieties are particularly good triplet energy acceptors. The lactone ring contained at one end of PbTx-2 and the unsaturated aldehyde functional group at the other end (Fig. 4) would therefore likely participate in photosensitized reactions with the (^{3}CDOM-m)*, as described in reaction sequence 2. The removal of both trace metals and CDOM prior to photolysis resulted in no photodegradation of PbTx-2 in WBSW, indicating the importance of a trace metal (m) complexed or otherwise associated with CDOM in the proposed mechanism sequence (CDOM-m). It is likely that the reaction between PbTx-2 and (^{3}CDOM-m)* depicted in Eq. 2 occurs within a hydrophobic microregion of CDOM rather than in bulk solution, as predicted for hydrophobic compounds transformed by excited state triplet species (Latch and McNeill 2006).

The effect of molecular oxygen on the photodegradation of PbTx-2 lends additional support to the importance of the mechanism presented in reaction sequences 1–3, although electron transfer from triplet excited CDOM to PbTx-2 may also be occurring. The results outlined in Fig. 3 demonstrate that a decrease in the excited triplet state quencher dissolved oxygen significantly increased the rate of photodegradation of PbTx-2, indicating that sensitized photodegradation involving energy transfer from triplet-excited CDOM to PbTx-2 may play an important role in the removal of this toxin in seawater. The results

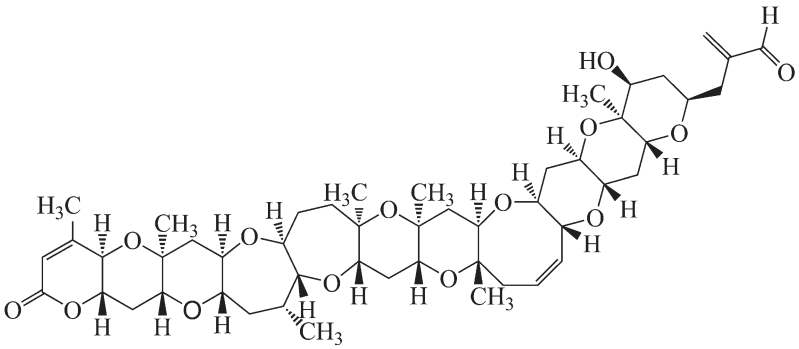

Fig. 4. Structure of PbTx-2.

presented in Fig. 3 also indicate that the (^{3}CDOM-m)* produced in Eq. 1 can transfer its energy to dioxygen, in addition to PbTx-2, via Eq. 4, thus:

$$(^3\text{CDOM-m})^* + {}^3O_2 \rightarrow \text{CDOM-m} + {}^1O_2 \qquad (4)$$

Molecular oxygen quenches sensitizers such as (^{3}CDOM-m)* by transferring energy to triplet-state oxygen-generating singlet state oxygen as a byproduct (Turro 1978). When oxygen was removed prior to photolysis, PbTx-2 degraded very rapidly upon exposure to simulated sunlight, to nondetectable levels after 1 h (Fig. 3). Dark controls prepared in deoxygenated WBSW showed no degradation, indicating that this result represented a light-induced effect. The results indicate that triplet state oxygen is in direct competition, via Eq. 4, with PbTx-2 for the excited state photosensitizer (^{3}CDOM-m)*. The removal of oxygen from this system prior to photolysis allows more (^{3}CDOM-m)* to photosensitize PbTx-2, rapidly increasing the photodegradation of the toxin via the pathway presented in reaction sequences 2 and 3.

The findings of this study are significant because they demonstrate that degradation of PbTx-2 via an indirect photochemical pathway is an important sink for the dissolved phase of the toxin, with a half-life of approximately 3 h in surface seawater under environmentally relevant conditions. Further studies are required to evaluate the quantum yield or efficiency of PbTx-2 photodegradation as a function of irradiation wavelength in order to model the temporal variability of the photochemical loss rate at multiple locations and depths in the water column. Future studies must also identify byproducts of PbTx-2 photodegradation (Eq. 3) in order to ascertain the environmental significance of this process in coastal seawater. The photolability of other brevetoxin analogs and their associated byproducts should be quantified in order to assess the role of sunlight-mediated processes in the biogeochemical fate of these related toxins in seawater. Finally, future studies should elucidate the role of trace metals in the sensitized photodegradation of other organic pollutants.

Acknowledgments

This work was supported by the National Science Foundation (Atmospheric Chemistry 0646153 and Chemical Oceanography 0825538). Members of the Marine and Atmospheric Chemistry Research Laboratory group at the University of North Carolina Wilmington assisted with sampling and analyses. We would also like to thank the reviewers for their insightful comments.

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Associate editor: Stephen P. Opsahl

Received: 09 February 2010
Accepted: 26 May 2010
Amended: 15 June 2010