Association between red tide exposure and detection of corresponding neurotoxins in bottlenose dolphins from Texas waters during 2007-2017

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ABSTRACT

Harmful algal blooms produced by the phytoplankton species *Karenia brevis* and its associated neurotoxin, brevetoxin (PbTx), occur throughout the Gulf of Mexico and have had devastating impacts on co-occurring populations of bottlenose dolphins (*Tursiops truncatus*), an important marine sentinel species. The majority of documented impacts, however, are from the eastern Gulf of Mexico, with a critical lack of information on the degree and frequency of PbTx exposure in bottlenose dolphins from Texas coastal waters. This study documents PbTx exposure in Texas bottlenose dolphins between 2007 and 2017 and their association with co-occurring *K. brevis* blooms. PbTx was detected in 60% (*n*=112) of the animals tested. Liver tissue samples had the highest frequency of detection (62%), followed by feces (41.4%) and gastric contents (30.4%). PbTx was not detected in urine or intestinal tissue. The

concentration ranges of PbTx detected in feces (1.2-216, mean 38.4 ng/g), gastric contents (3.3-1016, mean 158 ng/g) and liver (0.6-52.4, mean 8.5 ng/g) samples were an order of magnitude less than values reported for Florida dolphins for the same sample types. The proportion of dolphins recovered within 4 weeks of a bloom that tested positive for PbTx ('Bloom' group; 75%) was significantly higher compared to those that were recovered 5-8 weeks after termination of a bloom ('Post-Bloom' group; 36%; p=0.004). The proportion of PbTx-positive animals with no observed bloom association ('Baseline' group; 60%) was also significantly greater than the Post-Bloom group (p=0.012). No significant difference in proportion of PbTx-positive animals was detected between Bloom and Baseline groups (p=0.242). No significant differences in liver PbTx concentrations were observed between any pairwise combinations of the 3 exposure groups (p=0.261). Overall, these findings suggest persistent PbTx exposure for many individuals in these populations, although the health impacts of such exposure are not known.

INTRODUCTION

Harmful algal blooms (HABs) caused by the toxic phytoplankton species *Karenia brevis* have a long history of severe negative impacts on the coastal ecosystems and human populations of the Gulf of Mexico. This species naturally produces a suite of potent neurotoxins called brevetoxins (PbTx) that cause neurological impairment, morbidity and death in exposed animals, and can be widely distributed throughout the food web during such blooms (Landsberg et al., 2005; Landsberg, 2002). Prominent among the harmful impacts of brevetoxin exposure are mass mortality events (MMEs) involving marine mammals with deaths at times exceeding 100 animals during a single event. The majority of documented *K. brevis* blooms and marine mammal MMEs in the Gulf of Mexico, however, are concentrated along the central west coast and panhandle regions of Florida, with fewer observed impacts in the states westward (NOAA/NCEI, 2020; Steidinger et al., 1998). The bottlenose dolphin (*Tursiops truncatus*) is the most abundant marine mammal species found throughout the Gulf of Mexico and is a

critical marine sentinel species for evaluating marine ecosystem health, including the impacts of *K. brevis* blooms on marine organisms (Bossart, 2006; MMC, 1982; Wells et al., 2004). Several studies have described the magnitude of PbTx exposure and dolphin mortality during *K. brevis*-induced MMEs as well as during baseline conditions, however, corresponding data for the Gulf of Mexico west of Florida are lacking (Fire et al., 2007, 2015; Flewelling et al., 2005; Litz et al., 2014; Mase et al., 2000; Twiner et al., 2012).

Although not occurring with the same frequency as in Florida waters, the Texas coast has experienced several sporadic *K. brevis* blooms since monitoring efforts have been in place (Magaña et al., 2003). The observed impacts of these blooms on bottlenose dolphins however, are much less-well studied relative to Florida populations, and the corresponding lack of data for exposure levels in this species presents a challenge to resource managers tasked with managing protected marine mammal species in this region. Several bottlenose dolphin MMEs have occurred in Texas over the past three decades, the majority of which do not have a known cause, despite taking place in waters where HABs are frequent and often severe (NOAA, 2020a; Worthy, 1998; Zimmerman, 1998). A large-scale bottlenose dolphin MME that occurred in Texas coastal waters in early 2008 was associated with elevated levels of three common HAB species, each of which is a known toxin producer (Fire et al., 2011). However, the role of HABs in this event was undetermined, since multiple HAB toxins were detected in dolphin tissues, including PbTx, which was present in the absence of a reported *K. brevis* bloom. The cause of a subsequent Texas bottlenose dolphin MME in 2012 was attributed to biotoxin exposure, however multiple HAB toxins (including PbTx) and HAB species were also associated with this event (Gulland, 2006; NOAA, 2020a).

The purpose of the present study is to assess the overall degree of PbTx exposure to bottlenose dolphins recovered along the Texas coast as part of a decade-long survey, including data from MME-response efforts and periods with no reported HAB activity. Here we present data for accumulation of PbTx in various dolphin tissues from 2007-2017 and compare toxin concentrations detected during *K.*

brevis blooms to those detected during the absence of such events. We draw comparisons between the degree of PbTx exposure in Texas dolphin populations to that of Florida dolphin populations that are frequently and repeatedly exposed to *K. brevis* blooms. As an initial effort to model the dose-response relationship between *K. brevis* exposure and its corresponding PbTx accumulation in individual dolphins, we further compare the abundance of *K. brevis* cells at the time and location of each individual's stranding to the distribution of toxin in its tissues, as well as compare exposures between groups of animals sampled before, during and after blooms. These results represent the entire known dataset for reported PbTx values in dolphins occurring in Texas waters, and will therefore be of use for future studies drawing comparisons between PbTx exposure in multiple U.S. regions where *K. brevis* occurs. The findings produced herein can be used to inform management decisions on marine mammal stranding and health response efforts during future *K. brevis* blooms along the Texas coast.

METHODS

Animal sample collection - Samples were collected from 112 dead-stranded bottlenose dolphins recovered from estuaries and nearshore coastal beaches along the Texas coastline between 2007 and 2017. All carcasses were processed by trained and authorized stranding responders following established federal protocols for cetacean necropsy and sampling for marine biotoxin analysis (Geraci and Lounsbury, 2005). Standardized data collection for each individual dolphin included stranding date, stranding location, state of decomposition, morphometrics, sex, age class, gross pathology, and evidence of human interaction. Feces, gastric contents, intestinal tissue, liver, and/or urine (approx. 1-5 g or ml each) was collected from each of the dolphins sampled, for a total of 148 samples used in toxin analyses. These sample matrices were selected for their high likelihood to test PbTx-positive in an exposed animal and/or typically represent the best indicator sample types for HAB toxin exposure (Fire and Van Dolah, 2012; Flewelling et al., 2005). Although logistical constraints inherent in stranding response often precluded a full suite of samples from being collected from each individual, at least one sample was

available from each animal for analysis. Following tissue sample collection at necropsy, all samples were stored frozen at −20 °C or −80 °C in labeled Whirl-Pak freezer bags or polypropylene centrifuge tubes until extraction.

Toxin extraction and analysis - Samples were extracted according to methods described in Fire et al. (2019). Briefly, fluid samples (urine or gastric fluid) were centrifuged using an Allegra X-30R benchtop centrifuge (Beckman-Coulter, Brea, CA) at 10,000 x *g* for 10 minutes. The supernatant was collected and filtered using 0.45 µm Acrodisc GF/F syringe-driven filters (Pall Life Sciences, Port Washington, NY). Feces, liver and solid gastric contents were mechanically disrupted using a PRO 250 tissue homogenizer and 7 mm probe (PRO Scientific Inc., Oxford, CT) in 3 volumes of acetone (1:3 w/v). The resulting slurry was sonicated for 120 s, transferred to conical polypropylene tubes, and centrifuged at 3400 × *g* at 15 °C for 15 min. The pellet was re-extracted in an additional 3 volumes of acetone, centrifuged and the combined supernatants were filtered via a 0.45 µm Acrodisc syringe filter, evaporated, and resuspended in 6 mL of 80% aqueous methanol. This solution was solvent partitioned twice with 6 mL hexane, and the methanolic fraction collected, evaporated and resuspended in 2 mL of 100% methanol. Extracts were stored at -20°C until analysis.

The extracts and filtered samples were analyzed for PbTx using a commercially available enzyme-linked immunosorbent assay (ELISA; Eurofins Abraxis, Warminster, PA), following methods outlined in Fire et al. (2015) and Fire et al. (2019). The ELISA measures competition between free PbTx molecules and PbTx -enzyme conjugates for binding sites on anti-PbTx antibodies immobilized on a microtiter plate, in order to determine the total PbTx binding activity of the sample extract to the antibodies. Spectrophotometric absorbance (450 nm) endpoint values for unknown sample extracts (in duplicate) were calculated against those measured for a 10-point calibration curve ranging in concentration from 0.005 to 2.0 ng PbTx/mL. A sigmoidal dose-response curve was fitted to the data using Prism 4.0 (GraphPad Software, San Diego, CA), and the EC_{70} value was used as the in-assay limit of detection. Negative control samples from managed care bottlenose dolphins were used for sample

spikes and for determining the minimum dilution for each sample matrix necessary to eliminate false positive results due to non-specific binding of anti-PbTx antibody. Extracts of feces, liver, urine and gastric samples were diluted with phosphate-buffered saline at 1:50 prior to analysis, and intestinal tissue extracts were diluted at 1:20. Brevetoxin extraction efficiency for samples spiked with PbTx-3 standard reference material (MARBIONC, Wilmington, North Carolina) was 91±6 for solid samples and 95±4% for fluid samples. The limit of detection varied with each individual assay calibration curve, and was typically between 0.6 and 1.8 ng PbTx per gram of sample.

Phytoplankton data - Data for reported *K. brevis* blooms for the state of Texas were obtained from the National Oceanic and Atmospheric Administration (NOAA) Gulf of Mexico Harmful Algal Bloom Forecast ("HAB Bulletin data" hereafter) archives (NOAA, 2020b). These summary reports are released on a weekly basis, and the qualitative data therein were used to assign individual dolphins to bloom exposure categories based on their presence/absence in the county where a bloom was reported (see below).

Quantitative *K. brevis* cell abundance data was also obtained from the Harmful Algal BloomS Observing System (HABSOS) database maintained by the NOAA National Centers for Environmental Information (NOAA/NCEI, 2020). A subset of queried data corresponding to *K. brevis* cell counts (# cells/L of seawater) from Texas state waters for the period between 01 January 2005 to 31 December 2017 was used to assign numerical proxy values of bloom exposure to individual dolphins in the sample set. Associated metadata included date/time of sampling, latitude/longitude, county and categorical abundance descriptions (not observed, 0 cells/L; very low, >0 to 10,000 cells/L; low, >10,000 to 100,000 cells/L; medium, >100,000 to 1,000,000 cells/L; high, >1,000,000 cells/L).

Comparative analyses of bloom exposure groups - Dolphin stranding date and location data were cross-referenced with weekly HAB Bulletin data reports to assign each individual to one of three bloom exposure groups, based on the following qualitative criteria: 1) the "Bloom" group included animals that stranded in the same county as a reported *K. brevis* bloom, and either during the bloom or

up to 4 weeks after termination of the bloom, 2) the "Post-Bloom" group included animals that stranded in the same county as a previously reported bloom and between 5-8 weeks following termination of that bloom, and 3) the "Baseline" group included all remaining animals, including those for which no HAB bulletin data were reported. The 4-week date range criteria for the Bloom group were selected based on previous studies describing the persistence of lipophilic toxins such as PbTx in animal dose-response models, suggesting a residence time/lag time of up to several weeks in exposed prey items of bottlenose dolphins (Fire et al., 2008; Hinton and Ramsdell, 2008; Naar et al., 2007). Frequencies of PbTx-positive animals (presence/absence of PbTx in at least one sample type per individual) between exposure groups were compared using logistic regression. As liver tissue was the most abundant sample type in the dataset and also the site of PbTx detoxification in mammals, liver PbTx concentrations were also compared between exposure groups, using ANOVA or Welch's t-test. PbTx concentrations were log-transformed prior to analysis ($\log_{10} + 1$) and concentrations reported as below the assay limit of detection (<dl) were assigned zero values.

Quantitative cell abundance data was used to investigate any dose-response relationship between *K. brevis* exposure and PbTx concentrations detected in liver tissue. A custom script to match HABSOS *K. brevis* cell abundance data to each individual's stranding data and location was generated using Google Visualization API Query Language (version 0.7) and a corresponding query in a Google Sheets worksheet (Google LLC, Mountain View, CA). The script utilized the Haversine formula for determining the distance between two latitude/longitude points (Robusto, 1957) to select only those *K. brevis* data points that fell within a 55-km radius of a given dolphin's stranding location. This subset of data was further restricted to include only cell abundance values occurring within the 30-day window prior to the dolphin stranding date. The 55-km radius was selected based on published maximum estimates of home ranges and/or travel distances for bottlenose dolphins occurring along the central and upper coasts of Texas (Lynn and Würsig, 2002; Maze and Würsig, 1999). The 30-day window was selected based on the criteria for PbTx residence time in the marine food web described previously. The

maximum cell abundance value from this filtered dataset was assigned as an index of exposure for each animal, and compared using logistic regression as above, with the index of exposure as the independent variable and liver PbTx concentration as the dependent variable. Animals for which no corresponding *K. brevis* data were found within the 55-km and 30-day restriction criteria were assigned zero values for their index of exposure. Liver PbTx concentrations reported as <dl were assigned zero values for these analyses.

A logistic regression model was used to determine if the probability of PbTx detection in an animal can be predicted using the index of exposure, with the index (cells/L) as the independent variable and presence/absence of PbTx in the same individual as the dependent variable. A simple linear regression model comparing the number of days since an individual's last exposure to *K. brevis* (for blooms of ≥10,000 cells/L) to its corresponding liver PbTx concentration was also performed. In addition, a Spearman correlation was performed between the index of exposure and liver PbTx concentration. Index of exposure values and PbTx concentrations used to determine presence/absence in these tests were assigned using the criteria above. Analyses were conducted using R 3.6.3 (R Core Team, 2020). All significance values were set at $\alpha=0.05$.

RESULTS

Distribution of PbTx, dolphin and* K. brevis *data - Overall, 60% (67 of 112) of the animals in this study tested positive for PbTx, with PbTx-positive individuals widely distributed across the Texas coastline (Figure 1). Dolphin stranding locations ranged from near the Texas-Louisiana border to the Texas-Mexico border but were mainly concentrated near Galveston Bay and Corpus Christi Bay. The majority of strandings (77%) occurred on the Gulf of Mexico-facing beaches, with the remainder taking place in estuarine or riverine waters. Approximately half (76 of 148) of the samples tested positive for PbTx, with liver samples having the highest frequency of detection (62%), followed by feces (41.4%) and gastric samples (30.4%; Table 1). PbTx was not detected in any of the urine or intestinal tissue

samples. Despite liver tissue yielding the highest proportion of toxin-positive samples, the minimum, average and maximum PbTx concentrations (0.6, 8.5, and 52.4 ng/g) were all lower in liver relative to feces (1.2, 38.4, and 216 ng/g) and gastric (3.3, 157.6, 101.6 ng/g) samples. The PbTx concentrations detected in toxin-positive samples also varied widely for feces, gastric and liver, with maximum values exceeding minimum values by three orders of magnitude in each of these sample types.

Figure 1. Study area and bottlenose dolphin stranding/sampling locations, 2007-2017.

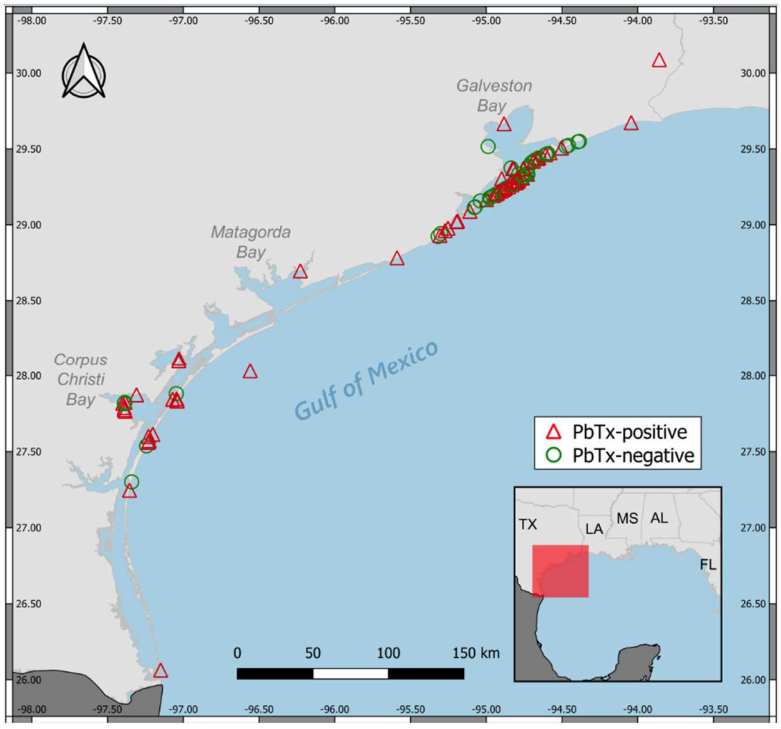


Table 1. PbTx concentrations detected (ng/g or ng/mL) in tissue samples and proportion of samples testing positive, by sample type.

Sample type	*n* pos.	% pos.	PbTx conc. min	avg	max
Feces	12/29	41.4%	1.2	38.4	216
Gastric	7/23	30.4%	3.3	157.6	1016
Urine	0/4	0%	-	-	-
Liver	57/92	62%	0.6	8.5	52.4
Intestinal tissue	0/1	0%	-	-	-
Total	76/149	51%			

Reports from weekly NOAA HAB bulletins and available HABSOS cell abundance data indicate that *K. brevis* blooms were distributed across most of the Texas coast at least once during the study period, with the exception of waters east of Galveston Bay having no abundance data available (Figure 2). At least seven *K. brevis* blooms occurred in the study area between 2006-2017, with maximum cell abundances for each bloom ranging from >200,000 to >189,000,000 cells/L (Figure 3). However the temporal distribution of dolphins tested for PbTx was not uniform, due to the event response-focused nature of marine mammal stranding response, with the majority of sampled animals recovered after January 2011.

Figure 2. Phytoplankton sampling locations and *K. brevis* cell abundance values, 2006-2017 HABSOS data.

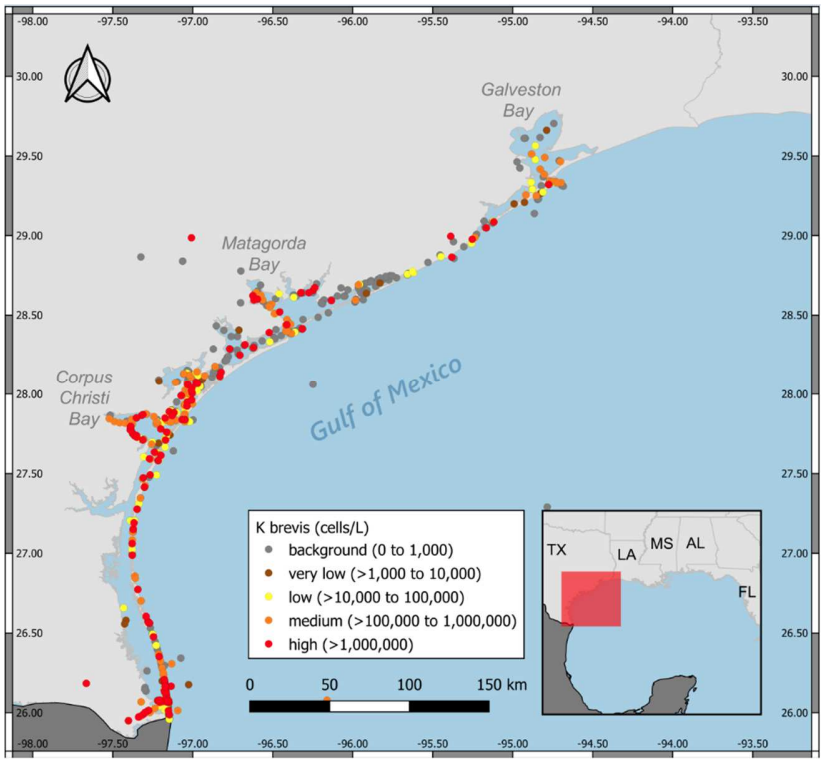
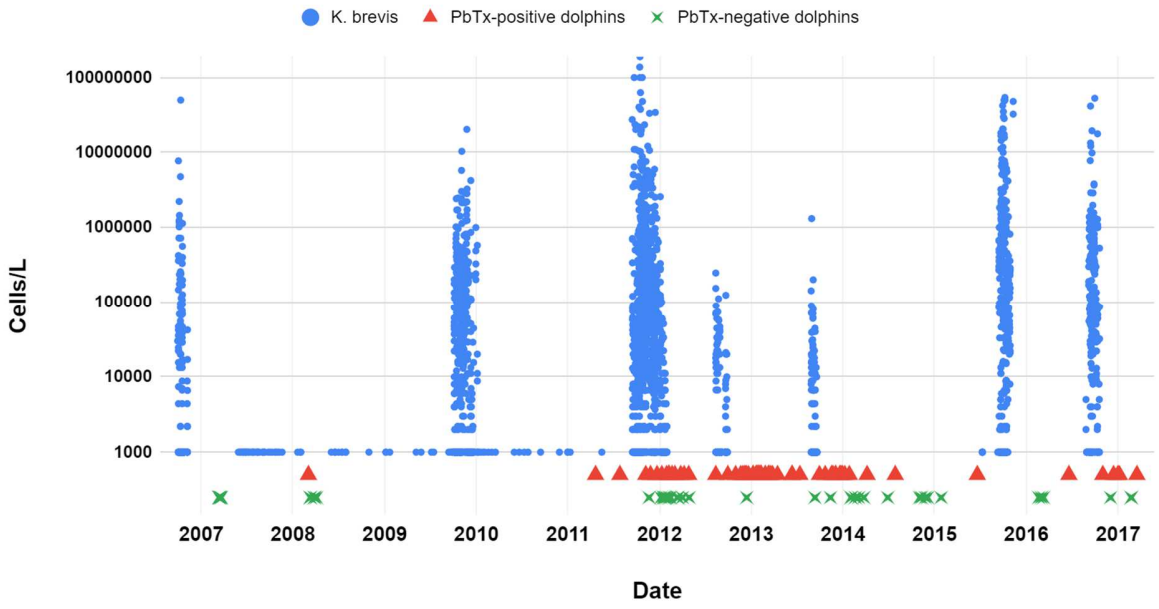


Figure 3. Temporal distribution of *K. brevis* abundance values and bottlenose dolphins tested for PbTx, 2006-2017.



Comparative analysis of exposure groups - Individual animals were assigned to exposure groups described previously as Bloom, Baseline, and Post-bloom. When comparing these exposure groups with the presence of PbTx, the Bloom group had the highest frequency of PbTx-positive animals (75%, n=12), followed by the Baseline group (60%, n=90) and Post-Bloom group (36.4%, n=11; Table 2). Bloom and Baseline animals were 6.8 (p=0.004) and 4.0 (p=0.011) times more likely to test positive relative to Post-Bloom animals, respectively. No significant difference in frequencies between Bloom and Baseline dolphins was observed (p=0.261).

Liver samples were the most common in the dataset, and also represent an internal dose of PbTx. For individuals with liver PbTx data available, the range of log-transformed concentrations detected was compared between each exposure group (Figure 4). Concentration ranges were highly variable, and no significant differences in PbTx values were observed among the pairwise combinations of exposure groups (p=0.261). Post-hoc recategorization of dolphins into only two exposure groups (combining Bloom and Post-Bloom groups into "Non-baseline"), similarly yielded no significant difference when comparing frequency of detection by animal, or when comparing liver PbTx concentration ranges (p=0.953; Figure 5).

Table 2. Frequency of detection, liver PbTx concentration ranges (ng/g), and index of exposure ranges for bloom exposure groups.

			Liver PbTx conc.				Index of exposure		
Exposure group	*n* pos.	% pos.	min	avg	max	% pos.	min	avg	max
Baseline	54/90	60%	0.6	6.7	34.3	66%	0	0	0
Bloom	9/12	75%	1.4	19.4	38	60%	0	3,797,092	34,276,000
Post-bloom	4/11	36.4%	2.5	33.0	52.4	25%	0	51,990	133,300

Figure 4. Liver PbTx concentration values (mean+SD; ng/g) for Baseline, Bloom and Post-Bloom exposure groups; p=0.261.

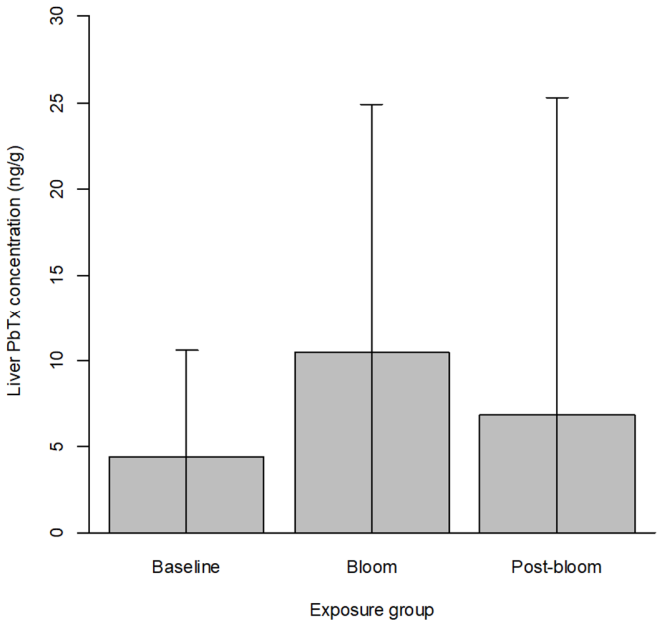
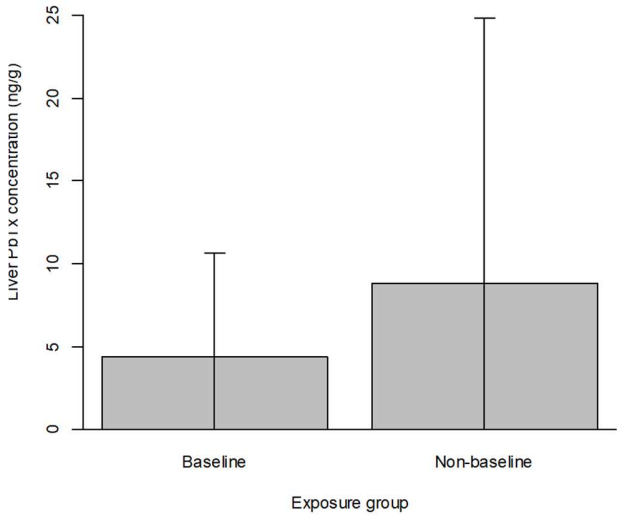


Figure 5. Liver PbTx concentration values (mean+SD; ng/g) for Baseline and Non-baseline exposure groups; p=0.953.



Dose-response relationship - Quantitative cell abundance values were used as a continuous variable to test relationships between *K. brevis* cell counts (as a proxy for PbTx dose) associated with a dolphin stranding and the resulting liver PbTx concentration in that individual. No relationship between index of exposure and PbTx concentration was detected (r_s=0.448, p=0.071; Figure 6). Similarly, in

logistic regression models of index of exposure values versus presence/absence of PbTx in the corresponding dolphin (any sample type), no relationship was observed (p=0.364; Figure 7). When modeling the number of days since each individual's last *K. brevis* bloom exposure to its corresponding liver PbTx concentration, a statistically significant relationship was observed (p=0.023) but due to the low coefficient of determination (0.046) and shallow slope (-0.005) the trend is not biologically meaningful (Figure 8): PbTx concentration = 7.00 – 0.005*(days since bloom)

Figure 6. Index of exposure versus detected PbTx in liver tissue; r_s=0.448; p=0.071.

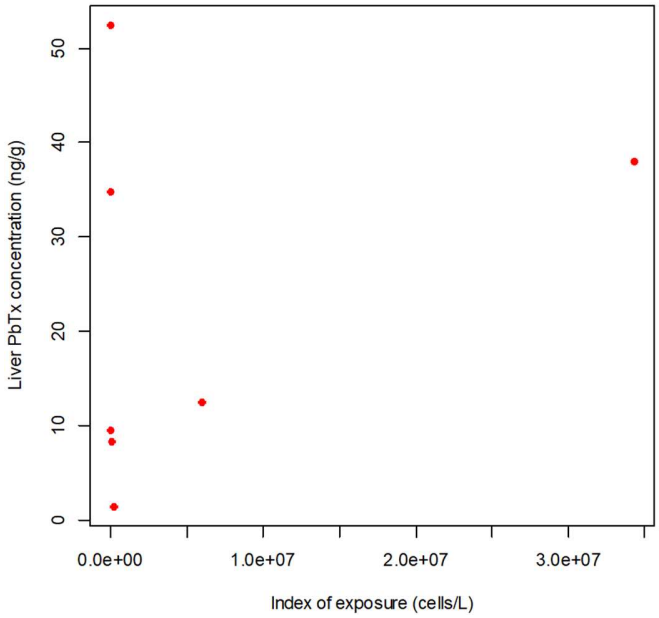


Figure 7. Index of exposure vs presence/absence of PbTx in individual bottlenose dolphins; p=0.364.

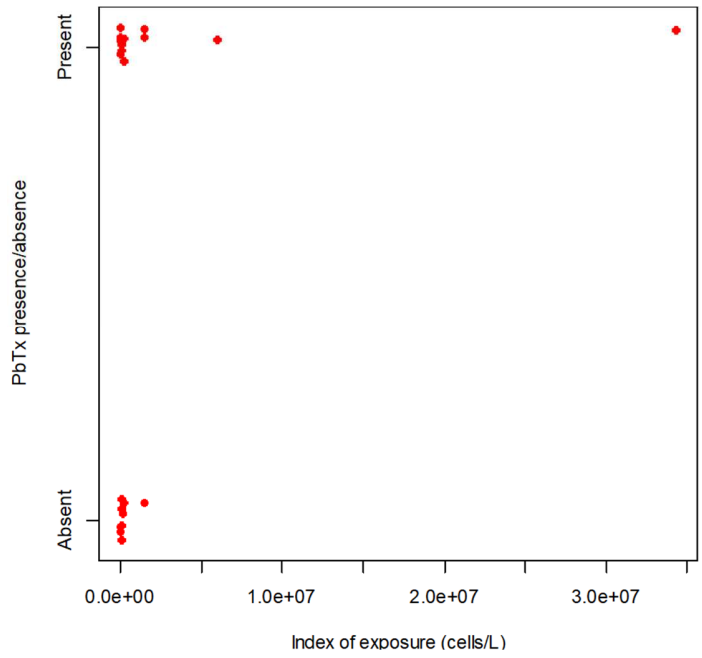
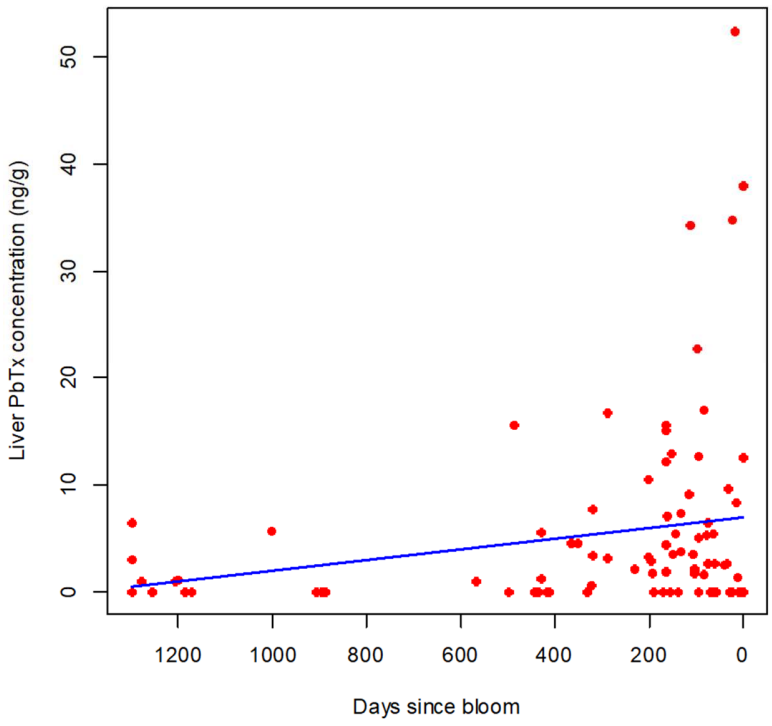


Figure 8. Number of days since last bloom exposure vs corresponding liver PbTx concentration. The best linear fit line is PbTx concentration = 7.00 – 0.005*(days since bloom); p = 0.023; adjusted R^2 = 0.046. Due to the low coefficient of determination and shallow slope (-0.005) the trend is not biologically meaningful.



DISCUSSION

Overall, the findings from the present study indicate a high prevalence of PbTx exposure in beach-stranded bottlenose dolphins from Texas, regardless of their apparent association with *K. brevis* blooms. Neither the frequency of detection (% of animals testing positive) nor degree of PbTx accumulation (liver PbTx concentrations) were statistically different between Baseline and Bloom groups. This suggests that either 1) PbTx is sufficiently persistent in the tissues of exposed dolphins to be detectable several months or more than a year post-exposure, or 2) PbTx remains available in the Texas dolphin food web in the absence of *K. brevis* blooms. The first scenario, while possible, had not been tested in dolphins due to obvious constraints in repeated sampling of wild, free-ranging marine mammals, and because the regions where these animals may be exposed to PbTx experience *K. brevis* blooms on a nearly annual basis (Steidinger et al., 1998). However, experimental animal dosing models demonstrate that PbTx is a lipophilic toxin with slow elimination rates (fish $t_{1/2}$ = 24 d, rodent $t_{1/2} \cong$ 6-7 d), and several studies have failed to document complete PbTx elimination within observation periods of up to 8 weeks (Cattet and Geraci, 1993; Hinton and Ramsdell, 2008; Kennedy et al., 1992; Leighfield et al., 2014; Poli et al., 1990). It is therefore reasonable to assume that some degree of persistence of PbTx in dolphin tissues post-exposure occurs and is detectable using the detection methods described here, but may still require a limited amount of exposure to PbTx in the absence of a bloom. The second scenario is also likely since *K. brevis* is a common part of the phytoplankton community and produces PbTx even at background cell abundances, causing this toxin to accumulate in a wide variety of potential dolphin prey species. As the only significant route of PbTx exposure in bottlenose dolphins is via ingestion of prey items, finfish represent the primary dietary source of PbTx during a *K. brevis* bloom and non-bloom conditions (Flewelling, 2008; Flewelling et al., 2005). High levels of PbTx in multiple species of finfish have been documented several months (and in some cases, over a year) following the termination of a *K. brevis* bloom, suggesting year-round exposure to the dolphins that consume them (Fire et al.,

2008; Naar et al., 2007). It is likely that these findings represent a mixture of the proposed exposure scenarios, given the frequency of reported *K. brevis* blooms along the Texas coast. An additional possibility is the limitations of performing statistical tests on unequal group sizes. The Baseline animals (n=90) far outnumbered the Bloom animals (n=12) and the analysis methods used to compensate for this inequality resulted in reduced statistical power.

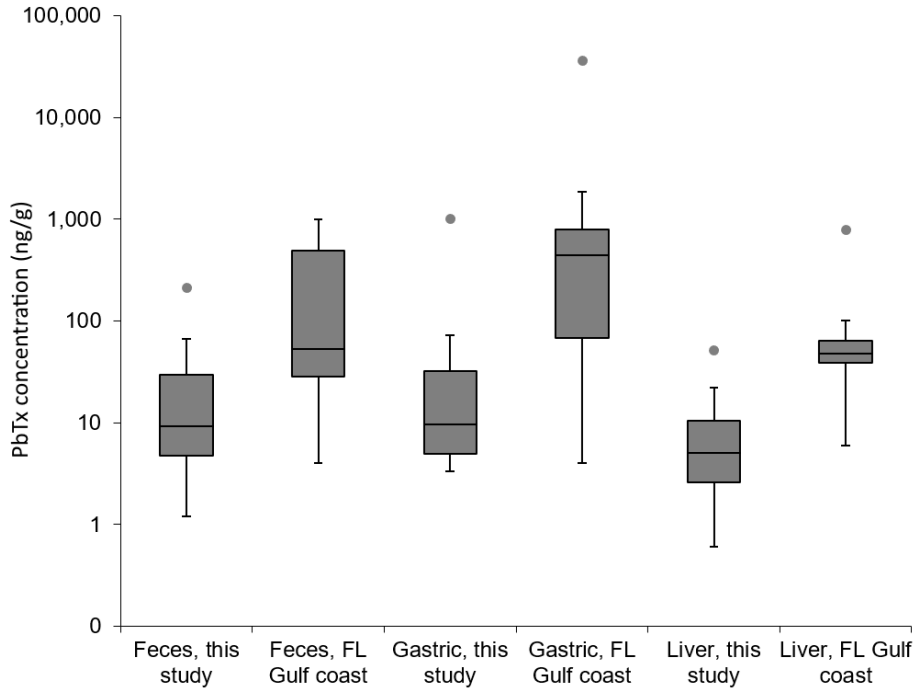
Post-Bloom dolphins were PbTx-positive significantly less frequently relative to individuals from the Bloom or Baseline groups. While further study is required to account for this difference, several ecological factors may influence these observations. For example, major disruptive shifts are observed in prey fish during and following severe Florida *K. brevis* blooms, in which entire finfish communities experience decreased densities and species richness, while increasing the dominance of some fish guilds at the expense of others (Gannon et al., 2009). PbTx accumulation varies greatly by fish species in *K. brevis*-affected communities, and this may influence which prey species act as the main PbTx vector to dolphins during a toxin-producing event (Fire et al., 2008). In addition (and likely due to these disruptions), dolphin feeding habits are altered by *K. brevis* blooms, causing increases in dolphin depredation on commercial and recreational fishing gear (Berens McCabe et al., 2010; Powell and Wells, 2011). The combination of these disruptions may result in dolphins feeding on fish species after a bloom that accumulate PbTx to a lesser degree than their non-bloom preferred prey. An alternate explanation for these results may involve changes in behavior and movement patterns that have been observed in bottlenose dolphins affected by *K. brevis* blooms in Florida. Juvenile dolphins in Sarasota Bay, Florida spent significantly less time actively foraging during *K. brevis* blooms, and exhibited expanded ranging behavior, possibly in response to lack of suitable prey (McHugh et al., 2011). If dolphin behavioral changes lead to consumption of less toxic prey in alternate foraging grounds, this may account for the decrease in PbTx-positive animals observed post-bloom in the present study.

Our tests investigating relationships between quantitative *K. brevis* metrics (index of exposure) and concentrations or presence/absence of PbTx also yielded no significant results, nor did tests using

the time since last exposure as a predictor of PbTx concentration in dolphin tissues. This confirms previous work suggesting that the abundance of toxic phytoplankton alone is a poor predictor of the accumulation rates and likelihood of exposure of HAB toxins in apex predators such as marine mammals (Fire et al., 2007). We suggest that future studies investigating measurable impacts of *K brevis* in bottlenose dolphins incorporate more robust analyses of food web toxin transfer routes and toxicokinetic models in order to better understand this dose-response relationship.

In terms of the degree of toxin accumulation in the present study relative to previous reports, Texas bottlenose dolphins have lesser detectable PbTx concentrations compared to the same sample matrices tested in dolphins from the Florida Gulf of Mexico coast (Figure 9). For feces, gastric and liver samples collected during *K. brevis* blooms and during non-bloom conditions, the ranges of PbTx concentrations in Texas animals are approximately an order of magnitude lower than their Florida counterparts for all three sample types (Fire et al., 2007; Flewelling et al., 2005; Twiner et al., 2012). This may be a reflection of the lower frequency and intensity of Texas red tides compared to those occurring in Florida waters, where blooms occur on average once per year (Steidinger et al., 1998). Alternatively, it may also be a function of generally smaller home ranges and foraging habitats of dolphins in Sarasota Bay compared to Texas dolphin populations, allowing for greater flexibility in prey choice in the latter group (Lynn and Würsig, 2002; McHugh, 2010).

Figure 9. PbTx concentration ranges in feces, gastric and liver samples from the present study, relative to published data for Florida Gulf of Mexico bottlenose dolphins.



In summary, our findings demonstrate evidence of long-term, repeated exposure of Texas bottlenose dolphin populations to toxins produced by *K .brevis* blooms. We further suggest that year-round PbTx exposure is likely for many individuals in these populations, although the health impacts of such exposure are not known. Since both *K. brevis* blooms and large-scale bottlenose dolphin MMEs are common occurrences along much of the Texas coast, these findings inform protected resource managers in their ability to respond to HAB-associated marine mammal morbidity and mortality events and aid in determining the causative role of HABs. In addition to informing investigations of impacts in dead-stranded animals and associated resource management policies, these data comprise a useful frame of reference for studying the health impacts on living survivors of *K. brevis* blooms. It is currently not known to what degree chronic or repeated exposure to PbTx impairs dolphin foraging ability, immune response and/or general ability to thrive post-bloom, partly due to an underlying lack of available data for "background" exposure in bottlenose dolphins in Texas. The findings presented here are a first step in providing these data in order to set the groundwork for more comprehensive studies of ecological impacts in this region.

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