Utility of Red Tide (*Karenia brevis*) Monitoring Data as a Predictive Tool to Estimate Brevetoxin Accumulation in Live, Free-Ranging Marine Mammals

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Exposure of common bottlenose dolphins (*Tursiops truncatus*) to brevetoxins (PbTx) produced by blooms of the toxic phytoplankton *Karenia brevis* frequently results in severe health impacts, including illness and large-scale mortality events. Although PbTx accumulation in dead-stranded dolphins is well documented, there are limited data for corresponding brevetoxin exposure in live dolphins. In addition, the severity of impacts on living survivors of such toxic blooms is difficult to assess due to a lack of data on the relationship between *K. brevis* bloom severity and corresponding PbTx concentrations in exposed animals. Here we present results of PbTx analysis of urine, serum, milk, gastric fluid, and feces samples collected from live, free-ranging dolphins ($n = 253$) from Sarasota Bay, Florida during 2000–2018, and investigate the relationship between PbTx concentrations detected and corresponding *K. brevis* cell abundances that are temporally (within 30 days) and spatially (within 16 km) associated with each individual. We found that 28% of dolphins were associated with elevated *K. brevis* abundances (10,000–60,000,000 cells/L), with 41% ($n = 104$) of dolphins testing positive for PbTx in at least one sample type. The proportion of PbTx-positive animals was significantly greater in animals exposed to elevated cell abundances vs. those exposed to background cell abundances (<10,000 cells/L), with 60 and 34% testing positive, respectively ($p < 0.001$). PbTx was detected most frequently in feces (57%, $n = 38$), followed by gastric (35%, $n = 37$), urine (32%, $n = 55$), and blood (7%, $n = 17$). PbTx concentrations by sample type were highest in feces (2–231 ng/g; mean 46), followed by urine (0.8–90 ng/g; mean 7.2), gastric (0.8–61 ng/g; mean 12), and blood (0.3–5 ng/g; mean 1.3). Regression analyses of *K. brevis* cell abundance as an index of exposure vs. corresponding PbTx concentration found no statistically significant relationship for feces ($p = 0.120$), gastric ($p = 0.349$), urine ($p = 0.053$), or blood ($p = 0.729$) samples.

PbTx concentrations typically ranged over two orders of magnitude between minimum and maximum values and did not scale with corresponding indices of exposure, which ranged over three orders of magnitude or more. Our results indicate that *K. brevis* cell abundance alone is a poor predictor of brevetoxin accumulation in bottlenose dolphins, and suggest that alternative methods (e.g., endocrine or immunological biomarkers) should be investigated as more appropriate methods for determining the severity of health impacts due to red tides.

INTRODUCTION

Harmful algal blooms (HABs) are frequently associated with morbidity and large-scale mortalities of marine mammals, due to exposure to potent neurotoxins produced during such events (Broadwater et al., 2018; NOAA, 2020). Assessing the impacts of HAB toxins on marine mammals is often problematic, and is complicated by complex trophic interactions, time lags between blooms and observable impacts, and logistical constraints inherent in sampling free-ranging animals. Important marine sentinel species such as the common bottlenose dolphin (*Tursiops truncatus*) are apex predators that are typically distanced from toxic phytoplankton by several trophic links, with the organisms in each link having distinct rates of toxin accumulation and/or depuration (Landsberg, 2002; Doucette et al., 2006; Naar et al., 2007). Should a strong correlative relationship exist between the toxin concentration detected in an animal and the severity of the HAB to which it was exposed, this would serve as a useful tool to predict toxin accumulation in individuals or back-calculate toxic phytoplankton abundances associated with mortality events. Previous work has suggested that the abundance of toxic phytoplankton alone is a poor predictor of toxin accumulation rates and likelihood of exposure in dead-stranded marine mammals (Fire et al., 2007, 2020a; Landsberg et al., 2009). This is primarily due to the fact that apex predator marine mammals do not graze on phytoplankton nor ingest large amounts of seawater, accumulate negligible amounts of HAB toxins transdermally or *via* inhalation, leaving dietary intake (through large fish and invertebrates) as the only feasible route of biologically significant exposure. However, the relationship between phytoplankton abundance and toxin accumulation in exposed animals has not been specifically demonstrated for live, free-ranging marine mammals.

Bottlenose dolphins endemic to the west coast of Florida are frequently and severely impacted by blooms of the red tide organism *Karenia brevis*, which occur on a nearly annual basis (Steidinger et al., 1998; Steidinger, 2009) and produce a suite of neurotoxins known as brevetoxins (PbTx). These blooms typically occur over very large spatial areas (~3,000 km^2), can last for several months, and often result in mass mortality events of multiple species of marine megafauna (Zhao et al., 2013; Broadwater et al., 2018). This is of particular concern for populations of non-migratory, resident marine mammals that do not travel long distances and therefore reside in red tide-affected areas for the entire duration of each bloom. Although high PbTx tissue loads and acute PbTx exposure have been assigned as the cause of several large-scale dolphin mortality events in Florida waters, corresponding information for the impacts on living dolphins that survive such events is limited (Twiner et al., 2012; Litz et al., 2014). The present study addresses this lack of knowledge by (1) providing a long-term dataset of PbTx loads in resident dolphins from a region that frequently experiences severe *K. brevis* blooms, and (2) by investigating the relationship between *K. brevis* abundance associated with individual dolphins sampled in the field and their degree of PbTx exposure. Here we present PbTx data from live, free-ranging bottlenose dolphins resident to Sarasota Bay, Florida from 2000–2018, and test the utility of *K. brevis* abundance data as an index of exposure that corresponds to PbTx concentrations detected in various toxicologically important sample matrices.

METHODS

Study Site and Target Population

Sarasota Bay, Florida (27°N, 82°W), a coastal barrier island estuary of approximately 125 km^2, was selected as the location for the present study since it (1) experiences frequent, severe *K. brevis* blooms, (2) is a region of continuous HAB monitoring by multiple phytoplankton ecology research programs, and (3) is inhabited by a population of about 150 bottlenose dolphins that have been intensively studied for over 50 years (Scott et al., 1990; Wells, 2003; FWC, 2019; MML, 2019). Through long-term study of this population, the majority of these animals are individually identifiable in the field, have known sexes, ages, and spatial use and ranging patterns, and are therefore known to have high site fidelity to Sarasota Bay (Wells, 2014; Tyson and Wells, 2016). As a result of multiple years of photographic mark/recapture efforts on members of this population, the home ranges of most individuals confirm that they are year-round residents of Sarasota Bay, and therefore will be exposed to any *K. brevis* blooms present within the study area (Fire et al., 2020b).

Sample Collection

Samples of urine, feces, blood, milk, and gastric fluid were collected from bottlenose dolphins sampled during health assessments occurring between June 2000 and June 2018, following methods described in Wells et al. (2004). Briefly,

dolphins were captured by encircling them with a 500 m × 4 m seine net deployed in shallow (<2 m) waters and secured by a team of veterinarians, trained dolphin handlers, and biologists to ensure the safe restraint of the animals. Each sampled individual was transferred to a padded, shaded platform onboard a 9 m veterinary examination boat where body measurements and various health samples and data were collected, and then the dolphin was released on-site. Whole blood was collected *via* venipuncture of the fluke vasculature and was stored using blood spot collection cards as described by Maucher et al. (2007), or separated by centrifugation and the serum fraction collected for analysis. Urine samples were collected *via* the urethra using a sterile catheter. Gastric samples were collected using a small tube inserted *via* the esophagus into the stomach, from which stomach fluid was drained and stored. Milk samples were collected *via* negative pressure applied using a modified catheter-syringe device placed over the expressed mammary gland. Feces samples were taken during processing using a sterile catheter. All samples were collected into sterile polypropylene vials and frozen (−20°C for blood cards, −80°C for all other samples) until toxin analysis was conducted. Several dolphins were sampled multiple times during the study period, and duplicate data for animals sampled more than once during the same health assessment were excluded from the dataset. Sample sets from the same individual but collected during separate health assessments (≥1 year apart) were treated as independent sampling events for this study.

PbTx Extraction and Analysis

Samples were extracted according to methods described in Twiner et al. (2011) and Fire et al. (2019). Fluid samples (urine, serum, milk, or gastric fluid) were centrifuged at $10,000 \times g$ for 10 min. The supernatant was collected and filtered using 0.45 μm syringe-driven glass fiber filters. Feces samples and sections of blood spot cards were mechanically disrupted using a handheld tissue homogenizer in three volumes of acetone (1:3 w/v). The resulting slurry was sonicated for 120 s, transferred to conical polypropylene tubes, and centrifuged at $3,400 \times g$ at 15°C for 15 min. The pellet was re-extracted in an additional three volumes of acetone, centrifuged and the combined supernatants were filtered *via* a 0.45 μm 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), following procedures outlined by the manufacturer (Abraxis LLC, Warminster, PA, United States). 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. A subset of samples was also analyzed using a PbTx radioimmunoassay (RIA) and/or liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods described in Twiner (Twiner et al., 2011). The limit of detection varied among assays but was approximately 1.0 ng PbTx per gram of sample.

Phytoplankton Data

Quantitative cell abundance data from statewide *K. brevis* monitoring efforts occurring in Florida were 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 Florida state waters for the period from 01 January 1999 to 31 December 2018 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, >0–10,000 cells/L; low, >10,000–100,000 cells/L; medium, >100,000–1,000,000 cells/L; high, >1,000,000 cells/L).

Comparative Analyses of Bloom Exposure Groups

Dolphin sampling dates and locations were cross-referenced with HABSOS *K. brevis* abundance data to assign each individual to one of two bloom exposure groups, based on the following criteria: (1) the "Exposed" group included animals that were temporally and spatially associated with any *K. brevis* abundance value of ≥10,000 cells/L and (2) the "Baseline" group included all remaining animals, including those for which no corresponding *K. brevis* abundance values were available. Since the *K. brevis* dataset is the result of primarily event-response sampling, it was assumed for this study that an absence of *K. brevis* data represented background cell abundances. A custom script to match *K. brevis* abundance data to each individual's stranding date 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, United States). 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 16-km radius of a given dolphin's sampling location. This subset of data was further restricted to include only values occurring within the 30-day window prior to the dolphin sampling date. The 16-km radius was selected based on estimated maximum travel distances for resident bottlenose dolphins whose movements are known to be restricted to Sarasota Bay, which has a maximum linear distance of approximately 32 km. The 30-day date range criterion for the Exposed group was 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 (Naar et al., 2007; Fire et al., 2008a; Hinton and Ramsdell, 2008). 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. PbTx concentrations by sample type (blood, urine, gastric, and feces) were also compared between exposure groups, using Welch's *t*-test. Due to the wide range of values detected, PbTx concentrations were log-transformed prior

to analysis (ln + 1) and concentrations reported as below the assay limit of detection (<dl) were assigned zero values.

Karenia brevis abundance data were used to investigate any dose-response relationship between *K. brevis* exposure and PbTx concentrations detected for each sample type. The maximum cell abundance value from each animal's set of filtered values 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 the PbTx concentration in each sample type as the dependent variable. Animals for which no corresponding *K. brevis* data were found were assigned zero values for their index of exposure. 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. In addition, a Spearman correlation was performed between the index of exposure and PbTx concentration for each sample type. 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 α = 0.05.

RESULTS

In total, 253 dolphins were tested for PbTx between 2000 and 2018, yielding 599 samples of urine, feces, gastric, milk or blood. Twenty-eight percent of dolphins ($n = 70$) were associated with *K. brevis* abundance values of ≥10,000 cells/L, and were therefore assigned to the Exposed group; the remaining 72% ($n = 183$) were assigned to the Baseline group. Maximum *K. brevis* cell abundances associated with each dolphin ranged from background levels (≤1,000 cells/L) to 60,000,000 cells/L (**Supplementary Table 1**). Overall, 41% ($n = 104$) of dolphins tested positive for PbTx in at least one sample type (**Table 1**). Comparing between exposure groups, the proportion of PbTx-positive animals was significantly greater in the Exposed group relative to the Baseline group, with 60% and 34% testing positive, respectively ($p < 0.001$).

PbTx was detected most frequently in feces (57%, $n = 38$), followed by gastric (35%, $n = 37$), urine (32%, $n = 55$), and blood (7%, $n = 17$) (**Table 1**). PbTx concentrations by sample type were highest in feces (2–231 ng/g; mean 46), followed by urine (0.8–90 ng/g; mean 7.2), gastric (0.8–61 ng/g; mean 12), and blood (0.3–5 ng/g; mean 1.3). PbTx was not detected in milk samples, which were therefore excluded from statistical analyses.

PbTx concentrations were significantly higher in the Exposed group relative to the Baseline group for feces ($p < 0.001$), gastric ($p = 0.032$), and urine ($p = 0.013$) samples, but were not significantly different for blood samples ($p = 0.133$; **Figure 1**).

Simple regression analyses of *K. brevis* cell abundance as an index of exposure vs. corresponding PbTx concentration found no statistically significant relationship for feces ($p = 0.120$), gastric ($p = 0.349$), urine ($p = 0.053$), or blood ($p = 0.729$)

TABLE 1 | Proportions of PbTx-positive dolphins and sample types by exposure category, and PbTx concentrations detected.

|	Exposed		Baseline		Combined	
% (*n*/total) testing positive						
Dolphins	60%	(42/70)	34%	(62/183)	41%	(104/253)
Feces	94%	(15/16)	45%	(23/51)	57%	(38/67)
Gastric	48%	(15/31)	29%	(22/76)	35%	(37/107)
Urine	59%	(26/44)	23%	(29/126)	32%	(55/170)
Blood	14%	(9/65)	4%	(8/181)	7%	(17/246)
Mean (min–max) concentrations detected, ng/g						
Feces	85.7	(9.2–231)	20.2	(2.0–123)	46	(2.0–231)
Gastric	19.8	(0.8–61)	6.1	(1.0–14)	12	(0.8–61)
Urine	3	(0.8–8.0)	11.1	(0.8–90)	7.2	(0.8–90)
Blood	0.9	(0.3–1.3)	1.7	(1.0–5.0)	1.3	(0.3–5.0)

samples. Spearman correlation analyses indicated that while correlation was statistically significant for all sample types, correlation coefficients were close to zero for all sample types except feces (rho = 0.715 feces; 0.365 gastric; 0.399 urine; 0.256 blood; **Figure 2**). Regression analysis of index of exposure vs. presence/absence of PbTx in individual dolphins indicated no statistically significant relationship ($p = 0.580$; data not shown).

DISCUSSION

Overall, our findings indicate that the majority of live, free-ranging bottlenose dolphins exposed to *K. brevis* cell abundances of >10,000 cells/L accumulate detectable levels of PbTx, and do so at approximately twice the frequency of individuals sampled at background *K. brevis* abundances. Similarly, the concentrations of PbTx detected in three of four sample types were also higher in dolphins sampled during elevated levels of *K. brevis* vs. those sampled during baseline conditions. This complements earlier work demonstrating large differences in the PbTx concentrations detected and proportion of PbTx-positive dolphin carcasses recovered during red tides vs. those recovered during non-bloom conditions (Fire et al., 2007). However, our results contrast with previous findings demonstrating that live dolphins sampled during non-bloom conditions show no evidence of PbTx exposure (Fire et al., 2008b). These conflicting results are likely due to differences in sampling design and sample size, as the previous preliminary effort included only a handful of non-bloom individuals, all of which were sampled during the same 2-week health assessment. We suggest that the results of the present study are a more accurate representation of degree and frequency of PbTx exposure in live dolphins from this region.

Our results indicate that *K. brevis* is a poor predictor of detectable PbTx concentrations in gastric, urine and blood samples, and a weak predictor of PbTx concentration in feces samples. While analysis of some sample types showed significant correlation with index of exposure, correlation coefficients approaching zero indicate that these relationships were not

**FIGURE 1 |** Comparison of PbTx concentrations by sample type.
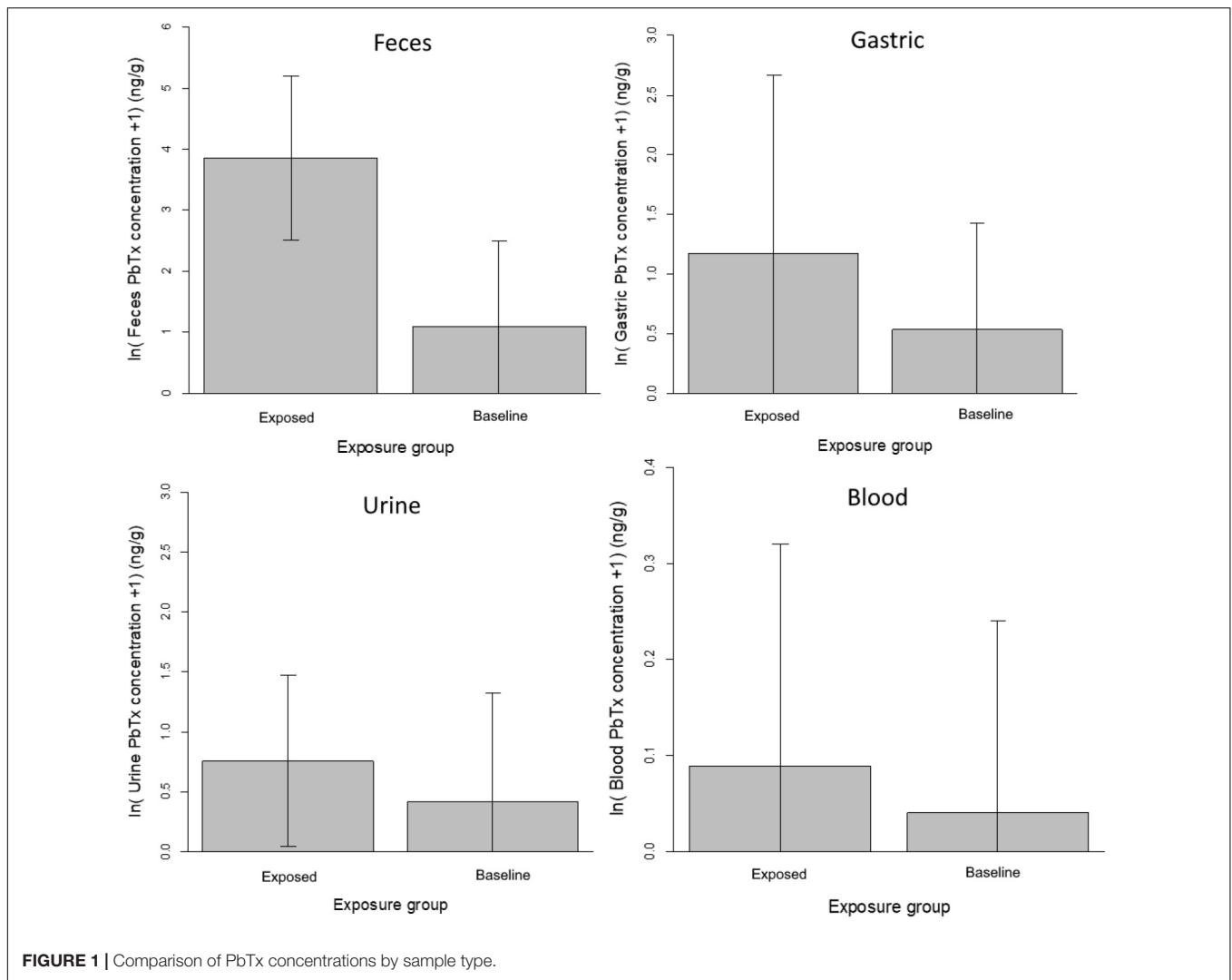
biologically meaningful. We found relatively low prevalence of PbTx in blood samples, and narrow ranges of PbTx concentrations in feces, gastric, and urine samples despite high variability in their associated *K. brevis* abundance values. PbTx concentrations typically ranged over two orders of magnitude between minimum and maximum values and did not scale with corresponding indices of exposure which ranged over three orders of magnitude or more.

These findings indicate that despite the feasibility of collecting feces, urine, gastric, and blood samples from live, free ranging dolphins, and their utility in determining presence/absence of PbTx in exposed individuals, they have limited use in estimating the degree of exposure to *K. brevis* blooms. This is likely due to the uncertainty with which *K. brevis* exposure levels were assigned to the animals in the present study, as the 30-day window used to assign indices of exposure to each dolphin was based on limited information on estimated PbTx transfer rates through the food web (Landsberg, 2002; Doucette et al., 2006; Naar et al., 2007). Likewise, the 16-km buffer used to assign *K. brevis* cell counts to each dolphin sampling event may be insufficiently

precise to account for the actual movements of individuals used in this study. In addition, the spatial distribution of *K. brevis* is highly heterogeneous at scales of a few kilometers, and the discontinuous, patchy nature of these blooms may account for much of the variation observed in the indices of exposure used herein (Tester and Steidinger, 1997; Zhao et al., 2013). The type of sample able to be collected safely from living, wild dolphins may also be a limitation in estimating exposure. Ideally, liver tissue is very useful for addressing PbTx detoxification and depuration rates in experimental dosing models, as well intestinal tissue for determining how much PbTx is assimilated from the gastrointestinal tract, however, these samples were not available from the live animals in this study. While PbTx concentrations in blood are theoretically at equilibrium with the rest of the tissues in an exposed organism, serum PbTx values in bottlenose dolphins are typically at concentrations near the limit of detection for most PbTx analytical methods, and may often go undetected.

Further efforts to assess the relationship between *K. brevis* exposure and PbTx accumulation in live dolphins may benefit from more physiologically relevant biomarkers of toxin exposure,

FIGURE 2 | PbTx concentration vs. index of exposure.
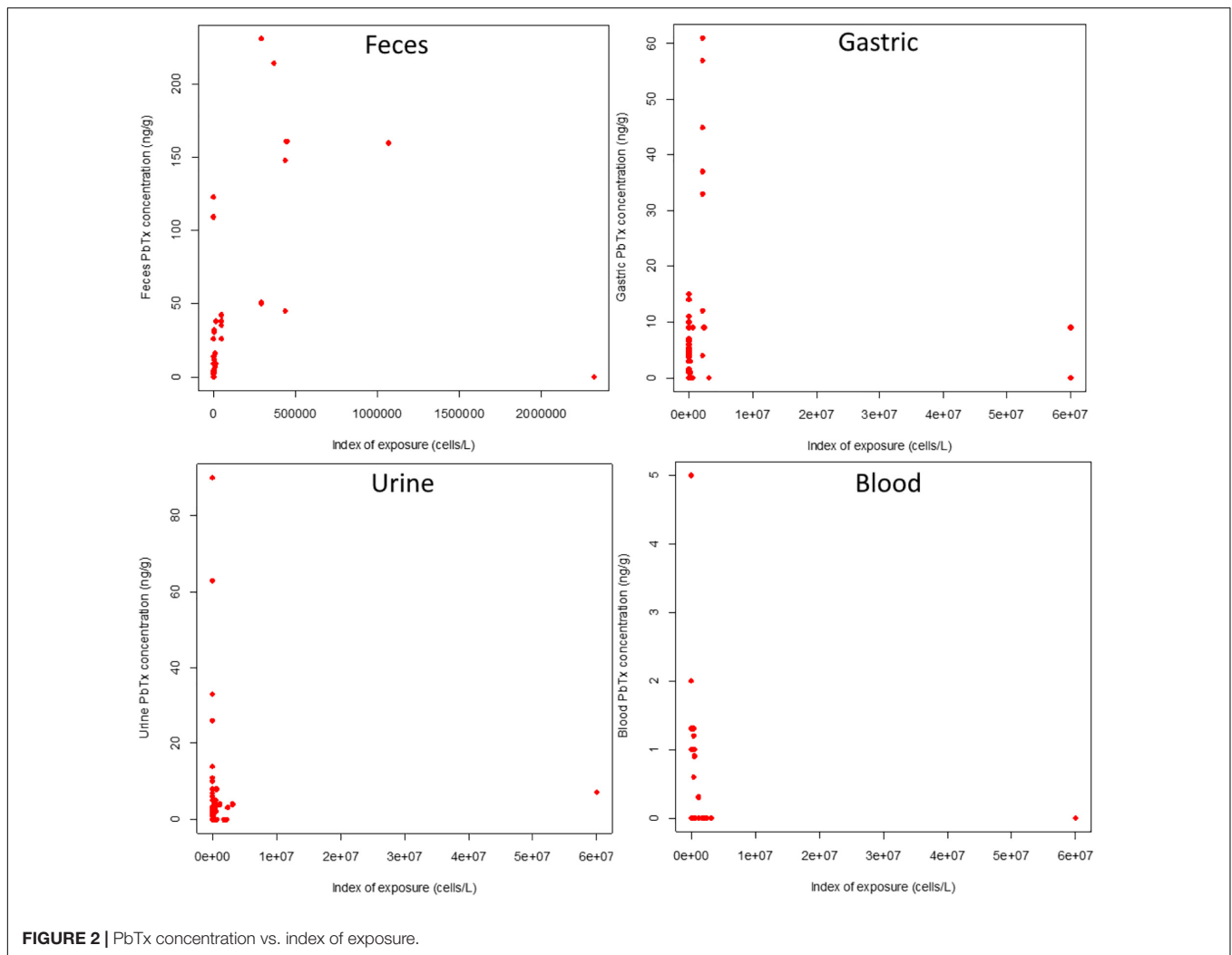
potentially including plasma cortisol concentration or immune response. Feces and gastric samples are only proxies for uptake and assimilation of toxins, and feces and urine as indicators of detoxification and depuration *via* renal and biliary routes do not provide information on real-time health impacts. *In vitro* exposure of dolphin peripheral blood cells to PbTx is known to increase proliferation of multiple immune cell types, and this merits investigation as a complementary data set to those used in the present study (Gebhard et al., 2015). In addition, targeted fine-scale seawater sampling combined with tracking movements of live dolphins immediately prior to health assessments may give more accurate estimates of individual exposure to *K. brevis* cells and reduce the impact of patchy cell distribution on future analyses.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The animal study was reviewed and approved by Mote Marine Laboratory annual Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

SF designed and oversaw all aspects of the study. GM provided statistical guidance and analyses, and assisted with development of the manuscript. ES carried out laboratory analyses and assisted with development of the manuscript. RW provided field logistical support, field sampling design, sample collection, and assisted with development of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars. 2021.611310/full#supplementary-material

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