

COYOTE (*CANIS LATRANS*) AND DOMESTIC DOG (*CANIS FAMILIARIS*) MORTALITY AND MORBIDITY DUE TO A *KARENIA BREVIS* RED TIDE IN THE GULF OF MEXICO

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ABSTRACT: In October 2009, during a *Karenia brevis* red tide along the Texas coast, millions of dead fish washed ashore along the 113-km length of Padre Island National Seashore (PAIS). Between November 2009 and January 2010, at least 12 coyotes (*Canis latrans*) and three domestic dogs (*Canis familiaris*) died or were euthanized at PAIS or local veterinary clinics because of illness suspected to be related to the red tide. Another red tide event occurred during autumn 2011 and, although fewer dead fish were observed relative to the 2009 event, coyotes again were affected. Staff at PAIS submitted carcasses of four coyotes and one domestic dog from November 2009 to February 2010 and six coyotes from October to November 2011 for necropsy and ancillary testing. High levels of brevetoxins (PbTx) were measured by enzyme-linked immunosorbent assay in seven of the coyotes and the dog, with concentrations up to 634 ng PbTx-3 eq/g in stomach contents, 545 ng PbTx-3 eq/g in liver, 195 ng PbTx-3 eq/g in kidney, and 106 ng PbTx-3 eq/mL in urine samples. Based on red tide presence, clinical signs, and postmortem findings, brevetoxicosis caused by presumptive ingestion of toxic dead fish was the likely cause of canid deaths at PAIS. These findings represent the first confirmed report of terrestrial mammalian wildlife mortalities related to a *K. brevis* bloom. The implications for red tide impacts on terrestrial wildlife populations are a potentially significant but relatively undocumented phenomenon.

Key words: Brevetoxin, coyote, Gulf of Mexico, harmful algal bloom, Padre Island National Seashore, red tide.

INTRODUCTION

Blooms of the red tide dinoflagellate *Karenia brevis*, which produces potent neurotoxins called brevetoxins (PbTx), have caused mortalities of millions of marine animals, including invertebrates, fish, birds, sea turtles, and mammals in the Gulf of Mexico (Landsberg, 2002; Flewelling et al., 2005). Evidence for a causal link between *K. brevis* red tides, brevetoxicosis, and marine animal die-offs has accumulated over the past decade, and the association is now well established (Landsberg et al., 2009). Brevetoxin-related illness or death of terrestrial species has been suspected; however, evidence that brevetoxins can impact terrestrial wildlife species has been lacking.

Karenia brevis red tides have been documented in Texas since 1935 (Magaña

et al., 2003), with the first reported event associated with a major fish kill off of Padre Island (Lund, 1936). Increasing in frequency since the late 1980s (Magaña et al., 2003), Texas red tides have had significant impacts on the local ecology and economy (Evans and Jones, 2001).

In October 2009, during a *K. brevis* bloom along the Texas coast, millions of dead fish washed ashore along the 113-km length of Padre Island National Seashore (PAIS), a unit of the U.S. National Park Service (NPS). In November 2009, PAIS employees began reporting sick or dead coyotes (*Canis latrans*) and domestic dogs (*Canis familiaris*) to the NPS Wildlife Health Branch. In October 2011, another *K. brevis* bloom occurred. During this event, the number of dead fish on the

shoreline was low relative to the 2009 event; however, coyotes again were affected. We describe the investigation of multiple canid deaths during two Texas *K. brevis* red tide events and our efforts to determine the potentially lethal route of exposure of terrestrial wildlife to brevetoxins.

MATERIALS AND METHODS

Information on *K. brevis* bloom densities and distribution for 2009 and 2011 was obtained from M. Byrd (pers. comm.) and the Texas Parks and Wildlife Department (2012). Information regarding domestic dogs was obtained through interviews with owners and local veterinary practitioners, with owner consent.

Carcasses of 10 coyotes ($n=4$ in 2009; $n=6$ in 2011) and one domestic dog (2009) were collected from beaches or inland areas by PAIS staff, and locations were recorded using global positioning system units. Carcasses were refrigerated or frozen and submitted to the Colorado State University Veterinary Diagnostic Laboratory, Fort Collins, Colorado, for routine necropsy, histopathology, and ancillary diagnostics. Potential causes of neurologic and gastrointestinal signs in animals that were observed prior to death were investigated using standard methods that included testing for infectious diseases (canine distemper and rabies), toxins (pesticides, brevetoxin), and heavy metals (arsenic, lead, mercury). Extensive testing was conducted on the first dog and coyote submitted; more focused analyses were conducted on subsequent carcasses. One of the submitted coyotes was killed by vehicular collision in August 2011, prior to the 2011 red tide event, and therefore served as a "negative" control.

Freshly dead and decaying fish were collected in December 2009 from six beach sites spanning approximately 50 km of PAIS. At each site, PAIS personnel collected and individually bagged sample fish of representative species found on the beach surface and also buried below the sand, where fish from the initial die-off were most likely to be found. At five sites, 250 mL of water and 250 mL of sand were collected from the surf zone and placed into glass jars. All samples were immediately placed on ice packs in coolers in the field and then stored at -20 C. Frozen samples were shipped on ice to the Florida Fish and Wildlife Conservation Commission's Fish and Wildlife Research Institute (FWC-FWRI) in St. Petersburg, Florida for analysis.

Brevetoxin analyses

Canid tissues, whole fish, water, and sand were analyzed for brevetoxins at FWC-FWRI. Canid tissues analyzed included liver, kidney, lung, and stomach contents. Urine and bile from a subset of 2011 coyotes were also submitted for brevetoxin analyses. Most of the 22 dead fish collected in 2009 were partially desiccated and decomposed, but they were identified to the lowest possible taxonomic level at FWC-FWRI. Each fish was weighed and then dissected into small pieces prior to analyses.

Canid tissues (2-g subsamples), whole fish, sand, and seawater were extracted for brevetoxins as previously described (Naar et al., 2007; Flewelling, 2008). Brevetoxins and brevetoxin-like compounds in sample extracts were quantified using a competitive enzyme-linked immunosorbent assay (ELISA) performed according to Naar et al. (2002) with modifications described in Flewelling (2008). The ELISA results were calculated by comparison to a standard curve generated using PbTx-3 (Marbionc, Wilmington, North Carolina, USA). Limit of detection was approximately 5–10 ng PbTx-3 eq/g of tissue, 1 ng PbTx-3 eq/mL of urine, 5 ng PbTx-3 eq/g of sand, and 0.03 μ g PbTx-3 eq/L of seawater. Presence of brevetoxins in canid tissues and fluids was confirmed using ultraperformance liquid chromatography (UPLC)–tandem mass spectrometry (MS/MS). Prior to analysis, tissue extracts (1 g eq) were diluted to 20% methanol and passed through preconditioned Strata-X SPE cartridges (60 mg, 3 mL, Phenomenex, Torrance, California, USA). Columns were washed with 4 mL of 20% methanol, and toxins were eluted with 4 mL of 100% methanol. SPE-cleaned tissue and body fluid extracts (0.5 mL eq) were then evaporated to dryness, redissolved in 0.5 mL 80% methanol, and passed through 0.22- μ m polyvinylidene fluoride (PVDF) filters (EMD Millipore, Billerica, Massachusetts, USA). Analyses were performed on an Acquity UPLC system coupled to a Quattro microTM API triple quadrupole mass spectrometer (Waters, Milford, Massachusetts, USA) operated in positive ionization mode. The LC separations were performed on an Acquity UPLC BEH C18 column (100 \times 2.1 mm, 1.7 μ m, Waters) using a mobile phase consisting of water (A) and acetonitrile (B) in a binary system, with 0.1% acetic acid as an additive. Elution gradient was 35% B for 1.2 min, increasing to 80% B at 8.6 min, 95% B at 10 min, holding at 95% B for 2 min, returning to 35% B at 12.5 min, and 1.5 min equilibration before the next injection. The flow rate was 0.25 mL per min and

injection volume was 10 μ l. The MS parameters were set to 3.6 kV capillary voltage, 2 V extraction cone voltage, 100 C source temperature, 500 C desolvation temperature, and 750 L N/hr desolvation gas flow. The detector was operated in multiple reaction monitoring mode using optimized cone voltages and collisions energies for each of the following transitions: PbTx-1 m/z 867.4>849.5, PbTx-2 m/z 895.5>877.4, PbTx-3 m/z 897.5>725.3, PbTx-7 m/z 869.5>779.4, PbTx-9 m/z 899.8>863.6, and oxidized PbTx-2 m/z 911.8>875.3. Additionally, two common brevetoxin metabolites were monitored: cysteine-PbTx-B m/z 1018.7>929.2 and cysteine-PbTx-B-sulfoxide m/z 1034.7>929.2. Toxins were quantified using a six-point calibration of a mixed standard of pure brevetoxins and metabolites (Marbionc).

RESULTS

K. brevis blooms

In September 2009, elevated concentrations of *K. brevis* were first noted in Port Aransas by the University of Texas Marine Science Institute, and by early-mid October Texas Parks and Wildlife Department staff began receiving reports of effects typically associated with *K. brevis* blooms (e.g., respiratory irritation in beachgoers, fish kills; Steidinger et al., 1998). The highest cell *K. brevis* concentrations ($1\text{--}20 \times 10^6$ cells/L) were observed in October and November 2009, with red tide affecting >200 km of the Texas Gulf coast between Port Aransas and the Mexico border (Fig. 1). The bloom began to dissipate in December and by January 2010 was mainly restricted to Corpus Christi Bay, where it lingered until late February 2010.

The Texas Gulf coast again experienced a widespread *K. brevis* red tide from mid-September 2011 to January 2012, with PAIS most affected from October to December 2011. Multispecies fish kills were documented along with dead sea turtles (*Cheloniidae*) and bottlenose dolphins (*Tursiops truncatus*) and several disoriented birds (B. Mase, pers. comm.).

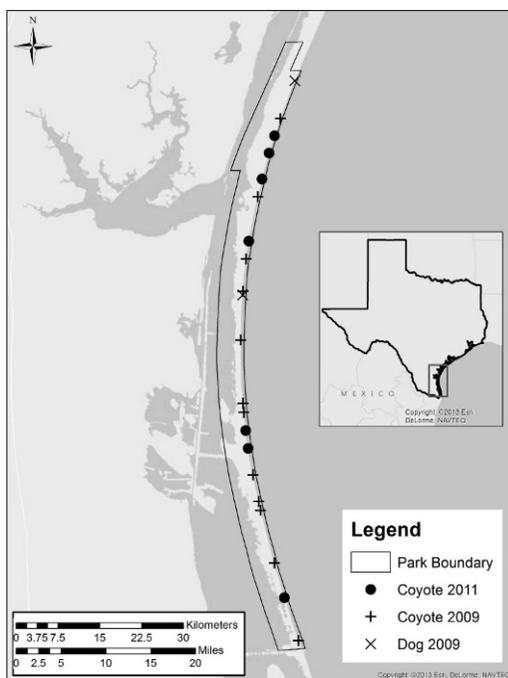


FIGURE 1. Map of Padre Island National Seashore, Gulf of Mexico, USA, depicting locations of coyotes (*Canis latrans*) and domestic dogs (*Canis familiaris*) affected by red tide events in 2009 and 2011.

Summary of affected canids

During 2009–2010, PAIS staff observed or received reports of 11 dead coyotes, and another two coyotes were humanely euthanized via gunshot because of the extent of their illness. Coyotes were found between mile markers (MMs) 0 and 59, a 95-km stretch of the 129-km (80-mi) island. Four of the coyote carcasses were submitted for necropsy and ancillary testing.

In 2009, three domestic, owned dogs died or were euthanized after becoming ill after being on a red tide-affected beach in or near PAIS. One of those dogs was submitted for necropsy and ancillary testing. In 2009–2010 two coyotes and nine dogs potentially exposed to brevetoxin were observed by the owners, veterinarians, or biologists prior to death or treatment of the animals. In each case, the animals exhibited neurologic signs,

including tremors, weakness or paralysis, or seizures. Additional signs often included anorexia (dogs), vomiting, and drooling.

In 2011, PAIS reported 11 coyotes dead or euthanized; no domestic dog illnesses were reported. As in the previous red tide, sick or dead coyotes were seen along the entire length of the island (Fig. 1). Six coyotes were submitted for necropsy, including the coyote that died in August 2011 from vehicular collision, prior to the red tide. Two of the submitted coyotes had exhibited neurologic signs prior to death.

Brevetoxin results

Brevetoxins were detected in tissues and body fluids of the dog and in nine of 10 coyotes tested (Table 1). The coyote killed by vehicular collision was the only animal whose tissues tested negative. Of the tissues examined, most often the highest concentrations of brevetoxins were found in liver (mean = 202 ng PbTx-3 eq/g), with maximum concentrations measured in the dog (545.1 ng PbTx-3 eq/g). Brevetoxin concentrations in kidney ranged from not detected (<ld) to 195 ng PbTx-3 eq/g. Low levels of brevetoxins were detected in lung in two of three coyotes tested from 2009–2010 (7.1 and 14.3 ng PbTx-3 eq/g) and in the one coyote tested from 2011 (45 ng PbTx-3 eq/g). Stomach contents were positive for brevetoxins in 73% (8/11) of animals tested. Concentrations were highly variable (<ld–633.9 ng PbTx-3 eq/g), with a mean of 138.2 ng PbTx-3 eq/g. In 2011, 100% (3/3) of urine samples from coyotes were positive, ranging from 32.9 ng PbTx-3 eq/mL to 106.2 ng PbTx-3 eq/mL. The single bile sample from a 2011 coyote contained 417.4 ng PbTx-3 eq/mL.

The liquid chromatography (LC)-MS/MS analyses confirmed PbTx-3 in 97% (33/34) of tissues samples that were positive by ELISA (Table 1). Other brevetoxin analogs (PbTx-7 and oxidized PbTx-2) and metabolites (cysteine-PbTx-B and cysteine-PbTx-B-sulfoxide) were also detected, but less frequently, and

their presence or absence was not specific to tissue type (data not shown). Parent brevetoxins PbTx-1 and PbTx-2 were not detected in any samples.

Ancillary test results

All ancillary tests for potential causes of death and the antemortem signs exhibited by dogs and coyotes were negative, except for mercury, which was abnormally elevated in liver and kidney of 6/10 coyotes (data not shown). The coyote killed by a car in August 2011 (NPS 11-121) was negative for all ancillary tests.

Fish samples

Fish carcasses ($n=22$) collected comprised six taxa, mainly eels and catfish (Table 2), and 91% (20/22) contained concentrations of brevetoxins >982 ng PbTx-3/g tissue. Concentrations, expressed as total toxin content based on the weight of the fish, ranged from 12 to 314 μ g PbTx-3 eq/fish. The three fish collected at the most northern sites (MM6.8 and MM8.4) had the lowest total brevetoxin loads: two hardhead catfish (*Ariopsis felis*) in which brevetoxin was not detected, and a sooty eel (*Bascanichthys bascanium*) (MM6.8) with 6.5 μ g PbTx-3 eq/fish. The total toxin content in fish collected at MM20 (where 78% of the fish specimens were collected) ranged from 10 μ g PbTx-3 eq/fish to 314 μ g PbTx-3 eq/fish. The only fish (an Anguilliformes eel) collected from MM40 contained 47 μ g PbTx-3 eq/fish.

The LC-MS/MS analyses were conducted on six fish from sites MM20, MM25.8, and MM40: three hardhead catfish, one sooty eel, one unidentified Anguilliformes eel, and the Atlantic bumper (*Chloroscombrus chysrurus*). Parent brevetoxins PbTx-1 and PbTx-2 were not detected in any of the fish. PbTx-3 was the dominant form detected in 83% (5/6) of the fish, ranging from 6 μ g to 24 μ g per fish and accounting for 6–13% of the total toxin measured by ELISA (data not shown). These fish also contained lower but

TABLE 1. Brevetoxin (PbTx) concentrations measured by enzyme-linked immunosorbent assay (ELISA) and brevetoxin congeners and metabolites detected by liquid chromatography–tandem mass spectrometry in tissues and fluids of coyotes (*Canis latrans*) and a domestic dog (*Canis familiaris*). Data reported in ng/g (stomach contents, tissues) or ng/mL (urine, bile).

| National Park Service animal ID (date collected) | Sample type | ELISA PbTx-3 equivalent ^a | PbTx-3 ^a |
|---|-------------------------------|--------------------------------------|---------------------|
| 10-041 (22 November 2009) | Stomach contents | 128.5 | 66.6 |
| | Liver | 216.0 | 28.4 |
| | Kidney | 169.9 | 74.6 |
| | Lung | 14.3 | 4.7 |
| Dog 10-047 (20 November 2009) | Stomach contents | 330.1 | 4.0 |
| | Liver | 545.1 | 101.6 |
| | Kidney | 142.6 | 26.7 |
| 10-081 (30 December 2009) | Stomach contents | 56.3 | 6.3 |
| | Liver | 291.4 | 48.2 |
| | Kidney | 107.9 | 21.2 |
| | Lung | 7.1 | 1.7 |
| 10-097 (10 January 2010) | Stomach contents | 17.9 | 1.1 |
| | Liver | 18.9 | 1.1 |
| | Kidney | 15.9 | 4.0 |
| | Lung | <ld | <ld |
| 10-130 (27 February 2010) | Stomach contents | <ld | <ld |
| | Liver | 18.5 | <ld |
| 11-121 (8 August 2011) ^b | Stomach contents | <ld | — |
| | Liver | <ld | — |
| | Kidney | <ld | — |
| 12-016 (23 October 2011) | Stomach contents | 633.9 | 39.7 |
| | Liver | 181.7 | 26.0 |
| | Kidney | 96.9 | 26.4 |
| | Urine | 106.2 | 11.5 |
| | Lung | 45.0 | + |
| 12-021 (1 November 2011) | Stomach contents | 294.6 | 10.2 |
| | Liver | 159.7 | 14.0 |
| | Kidney | 74.3 | 14.4 |
| 12-026 (7 November 2011) | Stomach contents ^c | 41.2 | 22.1 |
| | Liver | 314.8 | 37.1 |
| | Kidney | 160.2 | 40.8 |
| | Bile | 417.4 | 187.2 |
| 12-029 (10 November 2011) | Stomach contents ^c | <ld | 4.8 |
| | Liver | 227.9 | 33.8 |
| | Kidney | 195.0 | 76.9 |
| | Urine | 50.7 | 19.5 |
| 12-030 (10 November 2011) | Stomach contents ^c | 10.3 | 9.6 |
| | Liver | 242.8 | 67.2 |
| | Kidney | 86.3 | 15.2 |
| | Urine | 32.9 | 9.3 |

^a <ld = not detected; + = present; — = no data.

^b Coyote killed by vehicular collision outside of red tide time frame.

^c No solid stomach contents; only gastric fluids were analyzed.

measurable amounts of PbTx-7, cysteine-PbTx-B, and cysteine-PbTx-B-sulfoxide, whereas in the unidentified *Anguilliformes* eel, only the latter two brevetoxin metabolites were detected.

Water and sand samples

Brevetoxin was detected in 100% of water (6/6) and sand (5/5) samples. Concentrations measured in seawater were low, ranging from 0.09 ng PbTx-3

TABLE 2. Brevetoxin concentrations in fish collected from Padre Island National Seashore during a red tide event, 21–30 December 2009. Data reported in brevetoxin-3 equivalents (PbTx-3 eq) as ng per g or µg per whole fish.

| Species | No. | Brevetoxin | |
|---|-----|----------------|-------------------|
| | | ng PbTx-3 eq/g | µg PbTx-3 eq/fish |
| Anguilliformes eel | 4 | 1,490–12,457 | 14–115 |
| Sooty eel (<i>Bascanichthys bascanium</i>) | 8 | 2,747–17,386 | 7–314 |
| Hardhead catfish (<i>Ariopsis felis</i>) | 6 | <1d–2,811 | <1d–240 |
| Ariidae catfish | 2 | 983–2,393 | 12–40 |
| Atlantic bumper (<i>Chloroscombrus chysrurus</i>) | 1 | 4,239 | 125 |
| Mullet (<i>Mugil</i> sp.) | 1 | 1,540 | 107 |

eq/mL to 0.38 ng PbTx-3 eq/mL. Brevetoxin levels measured in three of the five sand samples were also relatively low (12–49 ng PbTx-3 eq/g), but high concentrations (1,414–1,635 ng PbTx-3 eq/g) were observed at the two most southern collection sites (MM40 and MM44).

DISCUSSION

We believe that brevetoxicosis from ingestion of dead toxic fish or other toxic biota scavenged on the beach was the likely cause of the coyote and dog deaths at PAIS. Our analyses revealed that a single dead fish can provide a toxin dose in excess of 300 µg PbTx-3 eq. Because the ELISA detects a wide range of brevetoxins and brevetoxin metabolites with varying toxicities (Naar et al., 2004; Plakas et al., 2004), the total brevetoxin-like activity may not accurately estimate toxicity. However, in the dead fish, LC-MS/MS analyses confirmed the presence of significant amounts of PbTx-3, a form whose toxicity to mammals is well established (Baden and Mende, 1982). Further, the vectoring of brevetoxic fish to higher trophic levels has been well documented in marine systems (Landsberg et al., 2009), and fish have been shown to be a significant source of lethal exposure to brevetoxins, particularly in marine mammals (Flewelling et al., 2005).

Another potential toxin source is sea foam, which can be greatly enriched with brevetoxins in the surf zone (Pierce et al.,

2003). Coyotes residing on Padre Island would also have inhaled brevetoxins during the bloom, but inhalation exposure is not believed to have caused the mortalities. Brevetoxins were measured at low levels (7–45 ng PbTx-3 eq/g) in the lung of three of the four coyotes tested, but brevetoxins measured in lung tissue are not necessarily the result of exposure via inhalation. Studies exposing laboratory rats (*Rattus norvegicus*) to brevetoxins through ingestion as well as intravenous administration report widespread distribution to all organs including the lungs (Poli et al., 1990). Because brevetoxins are lipid soluble and able to cross cell membranes, they can be distributed to any perfused tissue, regardless of the route of exposure. Although the potential for inhalation of brevetoxins to induce negative health effects, including immunosuppression, in mammals has been demonstrated (Fleming et al., 2005; Zaias et al., 2011), the concentrations of brevetoxins in aerosols reaching the shore are several orders of magnitude lower than those in the seawater (Pierce et al., 2005). Exposure to lethal toxin levels by this route is unlikely, and inhalation of brevetoxins is not known to have caused mortalities of terrestrial mammals in other areas where *K. brevis* blooms occur routinely.

Coyotes and domestic dogs continued to be affected after the harmful algal bloom (HAB)-related fish kills of October 2009 and October 2011, based upon tissue brevetoxin levels found. Within a few

weeks of the initial large-scale fish die-offs in October each year, beaches appeared normal, in terms of the amount of beached fish present; however, dried carcasses were easily excavated by PAIS staff. Canid scavenging of dead and buried toxic fish is a novel mechanism by which brevetoxin reservoirs from the marine food web can contribute to terrestrial animal exposure to high levels of toxins long after a *K. brevis* bloom and associated fish kills have ended. A similar lag effect has been described in marine systems (Flewelling et al., 2005; Landsberg et al., 2009).

Brevetoxin levels were low in coyotes collected in January and February 2010, and were nondetectable in the coyote hit by a car in August 2011, prior to the red tide event that year. Cause of death of the latter coyote cannot be attributed to brevetoxin toxicity, and the extent to which brevetoxin ingestion contributed to the deaths of the former coyotes is unknown.

Behavioral and neurologic reactions of a number of dogs and coyotes suspected of brevetoxin exposure on beaches illustrated the potential potency of the toxins, and are briefly described here. During fall–winter 2009 PAIS personnel were made aware of five dogs that became ill while visiting or soon after visiting red tide–affected beaches but survived their illness. In November 2009, a leashed dog that had not been observed contacting fish or other carcasses while visiting a red tide–affected beach became acutely ill, exhibiting vomiting, diarrhea, and seizures, and died before it could be taken to a veterinarian. In December 2009, a PAIS employee took her dog to an affected beach near PAIS. During the 20-min visit, the dog was observed eating a dead fish. Within 1.5 hr, the dog became acutely ill with neurologic signs (weakness, tremors, and seizures) and was taken to a veterinary clinic for emergency treatment and hospitalization. The dog was discharged from the hospital 4 days later, and continued to exhibit seizure activity, generalized weakness, and ataxia before making a full recovery.

In most cases, the brevetoxin concentrations measured in the tissues and fluids of canids were comparable to concentrations measured in carcasses from brevetoxin-related marine mammal mortalities (Fig. 2; Flewelling et al., 2005; FWC, 2007, 2008). In some coyotes, brevetoxin concentrations in stomach contents were low, but this reflects only recent ingestion and may not be indicative of lethal dose exposure levels. In the case of NPS12-029, there were no prey or scavenged items in the stomach and only gastric fluids were available for analysis. Despite no detectable toxin by ELISA in the stomach contents of that coyote, liver, kidney, and urine had elevated concentrations similar to those measured in tissues during acute brevetoxin-related manatee (*Trichechus manatus latirostris*) and bottlenose dolphin (*Tursiops truncatus*) mortalities (Fig. 2). Often red tide–killed marine mammals present with full stomachs. Differences between marine and terrestrial mammals in routes of exposure to and absorption of brevetoxins may lead to different proximate causes of death in those groups. For example, marine mammals may be less able to avoid exposure to HABs, and illness that prevents them from surfacing to breathe may have lethal consequences. Terrestrial mammals that do not succumb to an acute brevetoxin exposure may be able to recover, as was seen in some dogs from this report and in Florida dogs that received veterinary care (Flewelling et al., unpublished data).

There is increasing recognition that other environmental stressors, such as contaminants and infectious pathogens, can influence an animal's susceptibility to the effects of phycotoxins (Pikula et al., 2010) or vice versa (Benson et al., 2011). It is unknown whether the co-occurrence of high levels of mercury in some of the affected coyotes in this study may have altered their susceptibility to brevetoxin effects. Dog mortalities from phycotoxin exposure in marine habitats are not as common as in freshwater systems, where

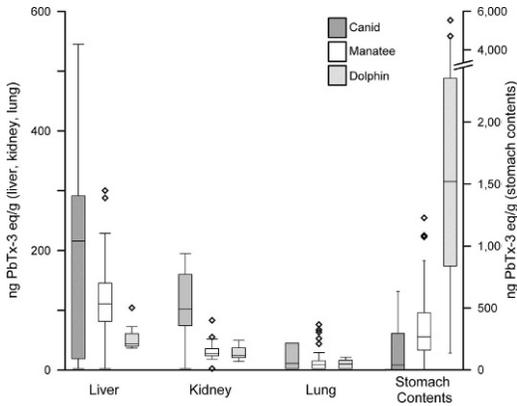


FIGURE 2. Box-and-whisker plot comparing the distribution of brevetoxin concentrations measured in 10 canids (*Canis latrans*, $n=9$; *Canis familiaris*, $n=1$) with those measured in brevetoxin-related mass mortalities of manatees (*Trichechus manatus latirostris*, $n=83$) and bottlenose dolphins (*Tursiops truncatus*, $n=39$) (FWC, 2007, 2008; Flewelling, 2008). Caps at the end of each box indicate the minimum and maximum values; the box is defined by the lower and upper quartiles; the line in the center of the box is the median. Diamonds indicate outliers (values more than $1.5\times$ the interquartile range outside of quartile 1 or quartile 3).

cyanobacteria blooms have been increasing (Puschner et al., 2008; Wood et al., 2010 and references therein). Those that have been reported have been associated with marine cyanobacteria, primarily *Nodularia* blooms producing hepatotoxic nodularins, usually following animal ingestion of water or cells (Krüger et al., 2009; Simola et al., 2012 and references therein).

Harmful marine algal blooms in Texas are becoming an increasing problem, with more diverse events from common and previously benign species occurring in the last few decades (Buskey et al., 1996; Swanson et al., 2010). *Karenia brevis* blooms have become more common, with five events occurring since 2000 (Fire et al., 2011), including 2012 (Texas Parks and Wildlife Department, 2012). There has been at least one previously undocumented anecdotal case from this region where a dog was affected with temporary blindness after swimming in a red tide in

Port Aransas in 1996 (D. Buzan, pers. comm.). This report is similar to a number of cases of brevetoxin-induced domestic dog illnesses from Florida in 2003 and 2005 (Flewelling et al., unpubl. data).

The cases described herein represent the first confirmed report of terrestrial mammalian wildlife mortality related to a *K. brevis* bloom. Further studies are warranted to better understand the impacts of brevetoxin and other marine biotoxins on canid and other terrestrial wildlife populations, as well as the potential impacts to humans and pets. One mitigation strategy may be to remove dead fish from beaches during HABs, where resources are available to do so. Although it is likely impossible to protect beachgoing wildlife from the effects of naturally occurring HABs, it may be possible to limit anthropogenic factors that contribute to the increasing number of these blooms and to limit exposure of wildlife to factors that may increase their susceptibility to marine toxins (e.g., contaminants). These factors and their interactions await further investigation.

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