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MARINE BIOTOXINS

Emergence of Harmful Algal Blooms as Health Threats to Marine Wildlife

Spencer E. Fire and Frances M. Van Dolah

Harmful algal blooms (HABs) affect aquatic ecosystems around the world, adversely affecting marine animal and human health, coastal ecosystem integrity, and economies that depend on coastal resources. Shellfish poisoning events involving humans who had ingested bivalves contaminated with HAB toxins primarily drove early scientific and social interest in HABs. More recently, research efforts have shown that HABs are often temporally and spatially correlated with the occurrence of acute morbidity or mortality of marine animals (Landsberg et al. 2005), and to date at least four classes of algal toxins have been associated with such events. Although fish, seabirds, and many other groups of marine wildlife are affected, these mortality events frequently involve marine mammals, and as such this chapter will focus primarily on the latter. In addition, since marine mammals are important sentinel species that act as barometers of ocean health and demonstrate the link between ocean and human health, the importance placed on these species in this context is warranted (Aguirre and Tabor 2004; Tabor and Aguirre 2004; Wells et al. 2004; Bossart 2006).

The frequency of associated marine mammal mortality events and HABs appears to have increased in recent years. This may be a reflection of several of factors, including (1) the increased scientific and popular attention given to marine mammal mortality events, (2) an increase in resources and observer effort dedicated to detection and study of HAB species in coastal waters, (3) an improved ability to detect algal toxins in marine mammal tissues and fluids, and (4) an apparent increase in both the frequency and the geographic distribution of HABs that has been documented over the past quarter-century (Van Dolah 2000; Hallegraeff 2003; Sellner et al. 2003). Exposure of marine mammals to algal toxins occurs via food-web transfer or directly through respiratory exposure. The susceptibility of marine mammals to algal toxins is therefore dependent not only upon the occurrence of toxin-producing algae within their habitat but, in the case of food-web transfer, on the co-occurrence of prey species at the time of a HAB to serve as vectors to higher trophic levels. Thus, management of the impacts of algal toxins on marine mammals requires an understanding of the causes and consequences of harmful algal blooms, the reasons for their apparent increase, and their dependence on large-scale oceanographic and climate changes as well as their local and regional influences.

OVERVIEW OF ALGAL TOXINS

The harmful effects of most HABs result from production of natural toxins that disrupt normal physiological function in exposed organisms (Landsberg 2002). The origins of these toxins are single-celled microalgae that, in response to favorable conditions in their environment, proliferate and/or aggregate to form dense concentrations of cells, called "blooms." In many cases, toxic species are normally occurring members of the phytoplankton community and are present in low concentrations with no evident environmental health impacts; thus, toxic effects are generally dependent on their presence in higher-than-normal cell concentrations. Toxin-producing marine algae are primarily members of two groups, the dinoflagellates and diatoms. Many species within these groups produce natural compounds that have potent biological effects on other organisms. However, less than 5% (less than 100 species) of all known dinoflagellate species and less than 25 species of diatoms are known to produce compounds that are toxic to mammals. Many of these compounds are potent neurotoxins that target ion channels or components of the cell-signaling pathways that interact with these channels. Although the ecological and physiological reasons why such toxins are produced are not fully understood, they often provide an advantage over competing algal species or function as antipredation mechanisms targeting zooplankton or small herbivores (Rue and Bruland 2001; Teegarden et al. 2008). Their impacts on higher trophic level species, such as marine mammals or humans, may thus be incidental.

Four major classes of algal toxins have been well studied worldwide (Fig. 26.1) because of their toxicity to wildlife or humans through seafood consumption: saxitoxins, brevetoxins, domoic acid, and ciguatoxins. These compounds are neurotoxins that are direct causes of, or have been associated with, marine mammal mortality events, generally as a result of dietary exposure from naturally contaminated items in the food web. In addition to these comparatively well-studied algal toxins, several novel algal toxins with adverse human health effects have been identified over the past decade, and these should be considered when investigating marine mammal mortality events with no obvious cause. These include diarrheic shellfish poisoning toxins, azaspiracid, yessotoxins, and spirolides. The following sections provide a brief review of each of these toxin classes and their documented impacts on marine mammals.

MARINE MAMMAL MORBIDITY AND MORTALITY EVENTS ASSOCIATED WITH ALGAL TOXINS

Saxitoxins

Saxitoxin (STX) and its derivatives form a suite of more than 21 water-soluble, tetrahydropurine neurotoxins. These toxin congeners are produced in varying combinations by marine dinoflagellates in three genera (*Alexandrium*, *Gymnodinium*, and *Pyrodinium*) and by several species of freshwater cyanobacteria (Landsberg 2002). In humans, STXs cause the clinical illness known as paralytic shellfish poisoning (PSP), with symptoms that include tingling and numbness of the perioral area and extremities, loss of motor control, and death by respiratory paralysis. STXs bind to site 1 of the voltage-gated sodium channel and thereby block neurotransmission (Levin 1992). However, STX is rapidly cleared from the blood (less than 24 hours in humans), so victims generally survive if they are put on life support. In the United States, STX historically posed a threat primarily on the northeast and west coasts, but it has recently been found in the Indian River Lagoon, Florida, where its occurrence is now persistent (Landsberg et al. 2002, 2006; Abbott et al. 2009).

STXs were first implicated in marine mammal deaths during a humpback whale (*Megaptera novaeangliae*) mortality event in Massachusetts between November 1987 and January 1988 (Anderson and White 1989; Geraci et al. 1989). Although baleen whales had not previously been reported to mass-strand, the unusually high frequency of strandings (14 whales within 5 weeks) in this event involved whales of robust body condition with stomachs full of undigested Atlantic mackerel (*Scomber scombrus*). In addition, since whales were observed exhibiting normal behavior 90 minutes before being found dead, death appeared to have occurred quickly, and an acutely toxic substance was suspected as the cause of death (Geraci et al. 1989). In this region, the STX-producing dinoflagellate *Alexandrium tamarense* forms blooms annually (Anderson 1997), and therefore STX was

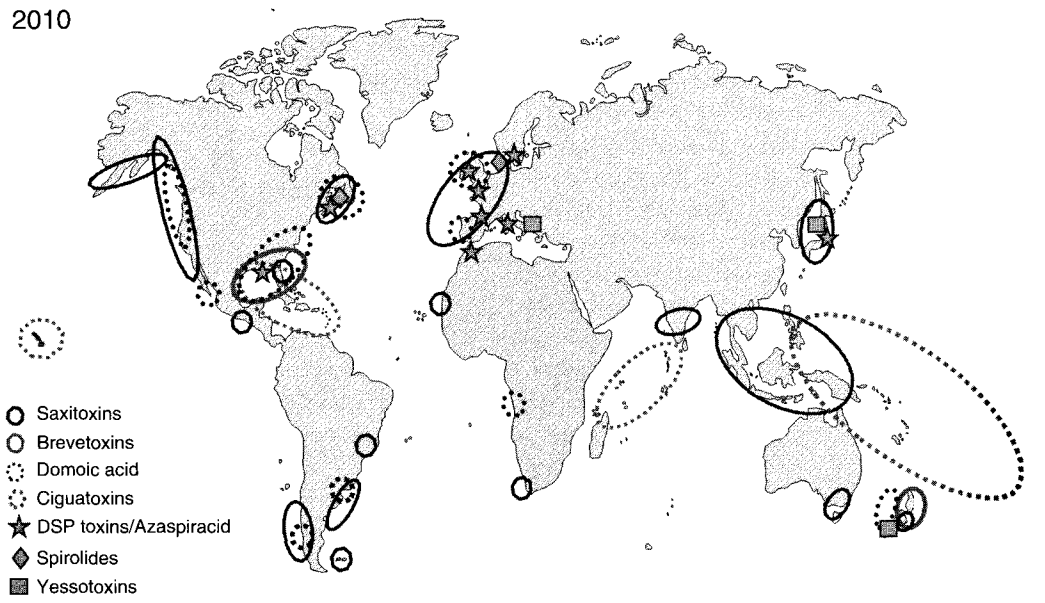

Figure 26.1:
Distribution of major classes of HAB toxins with known effects (open circles) or potential adverse effects (shapes) on marine wildlife. (Adapted from Van Dolah 2005.)

investigated as a potential causative agent. STX-like activity was detected by mouse bioassay in whale stomach contents, liver, and kidney, although the presence of toxin could not be confirmed by other analytical methods. Although identification of STX as the causative agent in these whale deaths remains circumstantial, detection of STX in planktivorous mackerel caught in local waters during the same timeframe suggested toxic exposure via the food web. Based on the STX concentration detected in the mackerel, it was estimated that a whale consuming 4% of its body weight in these mackerel daily would have ingested 3.2 μg STX/kg body weight (Geraci 1989). By comparison, the lethal dose of STX in an adult human is estimated at 6 to 24 μg/kg (Levin 1992). However, physiological differences that would make humpback whales more susceptible to the toxic effects of STX include (1) a large proportion of blubber (30% of body mass) into which the water-soluble STX would not partition, leaving these toxins more concentrated in target tissues; (2) the "marine mammal diving response," which shunts blood away from organs that function in detoxification, further concentrating the neurotoxin in sensitive organs such as the heart and brain; and (3) the sensitivity of the cetacean respiratory system to anesthetic agents (Kooyman 1985; Geraci 1989).

STX exposure was also a likely factor contributing to a mass mortality of Mediterranean monk seals (*Monachus monachus*) occurring in northwest Africa during May and June 1997. In this event, over 100 animals died along the coast of Mauritania, representing more than 70% of the local population and approximately 33% of the world population of this endangered species (Osterhaus et al. 1997; Harwood 1998; Hernandez et al. 1998). Morbilliviruses, which had previously caused mass mortalities of other marine mammal species (Gulland and Hall 2007) were detected in the monk seal carcasses and therefore were identified as a likely causative agent in this event. However, unlike previous morbillivirus-associated events, the seals were in good nutritional state, appeared to experience a rapid death, and exhibited symptoms consistent with STX exposure. In addition, abundant concentrations of three toxic dinoflagellate species were identified in waters near the seal colony, and fish collected from seal feeding grounds as well as seal liver samples were positive for STX (Hernandez et al. 1998). As with the Massachusetts humpback whales, a limited understanding of STX effects on

marine mammals prevented confirmation of STX as the cause of death, although both STX and morbillivirus were likely contributors (Harwood 1998). In any case, mass mortalities from PSP toxins may have far-reaching impacts on the population biology of long-lived mammals such as the Mediterranean monk seal (Forcada et al. 1999), considering that this event reduced the breeding population to fewer than 77 individuals and may therefore have reduced the population's genetic variability and ultimately compromised the survival of the species.

In addition to mortalities, STX exposure may also play a less obvious role in marine mammal health. Evidence of trophic transfer of STX from blooms of the toxic dinoflagellate *Alexandrium fundyense* to the North Atlantic right whale (*Eubalaena glacialis*) has raised speculation regarding its potential role in the unexplained decrease in reproduction rate of this endangered whale species. The North Atlantic right whale population currently consists of fewer than 350 individuals and appears to be declining (Kraus et al. 2001; Waring et al. 2009). Coastal waters of New England and the Bay of Fundy, which are major feeding grounds for these whales, experience *A. fundyense* blooms, associated STX production, and shellfish closures almost annually. The copepod *Calanus finmarchicus*, which dominates the right whale's diet, grazes on toxic *A. fundyense* and has been found to contain high concentrations of STX (Durbin et al. 2002). An estimation of STX accumulation in right whales during such blooms places their exposure levels between 4.73 and 9.65 μg STX/kg per day (Durbin et al. 2002), a value similar to the estimated lethal dose in humans (Levin 1992). STX detected in right whale feces from the area reached as high as 0.5 μg/g (Doucette et al. 2006), though the significance of this level of STX is not yet clear with respect to the level of STX circulating in the blood, or with regard to its effects on behavior and physiology. Thus it is possible that sublethal exposure to STX may affect whale behavior, leading to reduced feeding rate and fitness and ultimately reduced calving rates (Durbin et al. 2002).

A bottlenose dolphin (*Tursiops truncatus*) mortality involving 29 strandings occurring in Florida's Indian River Lagoon in June and July 2001 was also suspected to involve STX exposure. Dolphins recovered during this period were emaciated and displayed significant skin lesions, suggesting that multiple factors were associated with their poor health status (Bossart et al. 2003). However, a subsequent outbreak of seafood poisoning cases in humans in early 2002 led to the detection of STX in Indian River Lagoon puffer fish (*Sphoeroides* spp.) and the discovery of STX production by the dinoflagellate *Pyrodinium bahamense* var. *bahamense* in Indian River Lagoon waters (Landsberg 2002; Quilliam et al. 2002). Since neither of these organisms had previously been known to be associated with STX production or accumulation in the Indian River Lagoon, dolphins from the 2001 mortality event were re-examined for evidence of STX exposure. At least two dolphins examined had puffer fish in their stomachs, and stomach contents from these and other animals recovered from this event tested positive for low concentrations of STX (T. Leighfield personal communication 2010). However, puffer fish do not appear to be a normal component of the dolphin's diet (Barros and Odell 1990), and the levels of STX necessary to cause death in dolphins is unknown; therefore, it is not known if STX played a role in the compromised health and mortality of these animals.

Domoic Acid

Domoic acid (DA) is a water-soluble amino acid produced by certain diatoms in the genus *Pseudo-nitzschia*. DA mimics the neurotransmitter glutamate and is a potent activator of certain subtypes of glutamate receptor present in the brain. Symptoms of DA poisoning in humans (known clinically as amnesic shellfish poisoning) include nausea, vomiting, diarrhea, dizziness, disorientation, lethargy, seizures, and permanent loss of short-term memory. Neurotoxicity from DA exposure results in lesions in areas of the brain where glutamate receptors are heavily concentrated, particularly in regions of the hippocampus that are responsible for learning and memory processing (Chandrasekaran et al. 2004). The first reported toxic impacts of DA poisoning resulted from a 1987 human intoxication event in eastern Canada, when over 100 people became ill after consuming contaminated mussels (Perl et al. 1990). In the United States, DA poses a threat primarily on the West Coast, although toxin-producing diatom species are present on all coasts.

The first evidence of DA poisoning in marine mammals occurred in California in 1998, when over

400 California sea lions (*Zalophus californianus*) stranded along the central California coast during a two-month period that coincided with a bloom of the diatom *Pseudo-nitzschia australis* (Scholin et al. 2000). Similar California sea lion strandings have recurred on this coast almost annually since that time, with 1,335 cases of confirmed or suspected DA poisoning cases documented between 1998 and 2006, of which nearly half have died (Bejarano et al. 2008a; Goldstein et al. 2009). Hallmarks of DA intoxication in California sea lions (scratching behavior, disorientation, ataxia, and seizures) reflect neurological dysfunction (Gulland 2000; Silvagni et al. 2005). Histopathological examination frequently reveals damage to the brain (hippocampal atrophy and ischemic neuronal necrosis) and heart (i.e., pallor of the myocardium and fibrinous pericarditis). DA intoxication also frequently causes abortion, premature births, or death due to pregnancy-related complications because females are typically in third trimester of pregnancy at the time of the spring diatom blooms (Gulland 2000; Gulland et al. 2002; Brodie et al. 2006; Goldstein et al. 2009). Sea lion pups exposed to DA during gestation have a high frequency of abnormalities and poor survival rates (Goldstein et al. 2008; Ramsdell and Zabka 2008; Goldstein et al. 2009). Animals that survive acute DA intoxication have impaired survival and reproductive potential, as these animals experience persistent neurological dysfunction and increased likelihood of restranding (Goldstein et al. 2008). Two separate clinical syndromes are now recognized: acute DA poisoning (as described above) and a second neurological syndrome characterized by epilepsy associated with chronic consequences of previous sublethal exposure. Exposure of rats to repeated subsymptomatic doses of DA confirm the ability of DA to produce delayed epileptic seizures similar to those observed in sea lions that survive acute intoxication (Ramsdell 2010).

The complex epidemiology of DA intoxication in California sea lions raises concerns regarding the population-level consequences of repeated exposures. The increased frequency of *Pseudo-nitzschia* blooms beginning in the late 1990s coincided with a shift in the North Pacific Oscillation, an approximately 25-year multidecadal climate cycle. The current regime favors cooler ocean temperatures in the eastern Pacific, and stronger upwelling supporting larger phytoplankton blooms, with anchovies dominating the herbivore community, and is thus termed the "anchovy regime" (Chavez et al. 2003). Anchovies are efficient consumers of *Pseudo-nitzschia* and are the primary vector of DA to California sea lions. Bioenergetic modeling suggests that anchovies contribute a four-fold increase in risk of toxic effects from DA as compared with sardines (Bejarano et al. 2007), the dominant grazer during the alternate phase of the North Pacific Oscillation. If the current climate regime persists for approximately 25 years, we might expect continued severe DA impacts on this species for another decade. Although DA was not observed prior to 1998, retrospective analysis reveals that clusters of stranded animals showing symptoms similar to those described in DA-related mortality events have been reported in the past along the California coast, some of which may reflect the previous "anchovy" regime (Gilmartin 1979; Beckman et al. 1995).

Other marine mammals on the California coast experience similar DA intoxications. Several weeks following the peak of sea lion strandings in 1998, an increase in southern sea otter (*Enhydra lutris nereis*) mortalities was observed in the same region (Trainer et al. 2000; Kreuder et al. 2003). The time delay likely reflects the route of exposure, as otters feed primarily upon benthic invertebrates that become toxic following the sinking-out of toxic algal material typical of a terminating bloom (Ferdin et al. 2002; Kvitek et al. 2008; Sekula-Wood et al. 2009). DA toxicosis in sea otters is associated with a high prevalence of cardiac lesions similar to those observed in California sea lions, and exposure to DA increases the probability of myocarditis and cardiomyopathy with recurring or prolonged exposure to DA (Kreuder et al. 2005). It is estimated that DA-exposed otters are 55 times more likely to die from myocarditis than unexposed otters (Kreuder et al. 2005).

Cetaceans have also suffered DA toxicosis and deaths. In 2002, the second largest marine mammal mass mortality event in history occurred in southern California and involved multiple cetacean species, including short-beaked (*Delphinus delphis*) and long-beaked (*Delphinus capensis*) common dolphins, minke (*Balaenoptera acutorostrata*) and humpback (*Megaptera novaeangliae*) whales, and harbour (*Phocoena phocoena*) and Dall's porpoises (*Phocoenoides dalli*), in addition to extensive sea lion, harbour seal (*Phoca vitulina*), and southern sea otter mortalities (Heyning 2003; Torres de la Riva et al. 2009). In 2004, a similar

DA-associated multi-species stranding event occurred in the Gulf of California, which involved common dolphins (*D. delphinus, D. capensis*) and sea lions (Sierra-Beltran et al. 2005). DA intoxication via an anchovy vector was also recently demonstrated to be a cause of death of a minke whale (*Balaenoptera acutorostrata*) stranding during an intense *Pseudo-nitzschia* bloom in southern California in 2007 (Fire et al. 2010).

The impacts of DA on wildlife are not limited to marine mammals. The first confirmed report of DA on the West Coast of the United States occurred in 1991 and involved 95 Brandt's cormorants (*Phalacrocorax penicillatus*) and 43 brown pelicans (*Pelecanus occidentalis*) that were reported dead after ingesting anchovies containing *Pseudo-nitzschia* frustules (Fritz et al. 1992; Work et al. 1993). Surviving animals exhibited typical DA-induced behavioral clinical signs, including head weaving, scratching, and vomiting (Fritz et al. 1992). Similarly, in 1996 brown pelican mortalities occurred in Baja California, causing over 150 bird deaths and decimating 50% of the colony (Ochoa et al. 1996; Sierra-Beltran et al. 1997). The 1998 *Pseudo-nitzschia* bloom in central California may likewise have contributed to the reduced 1998–99 interannual survival of the marbled murrelet (*Brachyramphus marmoratus*) (Peery et al. 2006). Invertebrates and fish are generally viewed as vectors for DA intoxication to higher trophic levels (Bejarano et al. 2008b), but reports of mass sardine mortalities associated with the 2004 Gulf of California event (Sierra-Beltran et al. 2005) and DA-associated mortalities of Humboldt squid (*Dosidicus gigas*) in 2003 and salmon sharks (*Lamna ditropis*) suggest that its toxic impacts are not limited to birds and mammals (NOAA Marine Biotoxins Program, unpublished data 2003).

Brevetoxin

Brevetoxins (PbTx) are a suite of polyether toxins produced by the dinoflagellate *Karenia brevis*, best known as the causative organism of Florida red tides. PbTx or PbTx-like compounds are also produced by *K. brevis*-like species in New Zealand (Haywood et al. 1996) and raphidophytes of the genus *Chattonella* in Japan (Khan et al. 1995a; Khan et al. 1995b) and have been identified in a fish-killing *Chattonella* bloom in Delaware Bay (Bourdelais et al. 2002). Like STX, PbTx target the voltage-gated sodium channel but bind to site 5, causing opening of the channel under conditions in which it is normally closed and resulting in inappropriate neuronal transmission (Ramsdell 2008). In humans, PbTx exposure causes the clinical illness known as neurotoxic shellfish poisoning (NSP), and symptoms in mammals include nausea, tingling, and numbness around the mouth, severe muscular aches, loss of motor control, and in severe cases, seizures. PbTx-containing aerosols are also a route of exposure, since fragile *K. brevis* cells are easily lysed by wind or surf action, and human exposure to aerosolized PbTx results in coughing, gagging, and a burning sensation in the upper respiratory tract (Backer et al. 2003; Pierce et al. 2003; Fleming et al. 2005).

Florida's Gulf of Mexico coast experiences PbTx-producing blooms of *K. brevis* almost annually, and these blooms may persist for several months. As evidenced by the conspicuous fish kills associated with *K. brevis* blooms, finfish are particularly sensitive to PbTx, likely because these lipophilic toxins in the water column pass across the gill epithelium and directly into the bloodstream. However, in large blooms, the effects of PbTx occur at all trophic levels, from invertebrates to birds and turtles, to marine mammals. Particularly severe *K. brevis* blooms have led to massive die-offs of polychaetes, amphipods, and gastropods (Simon and Dauer 1972; Roberts 1979; Landsberg et al. 2009) and negative sublethal effects in copepods and bivalves (Huntley et al. 1986; Summerson and Peterson 1990). Cormorants (*Phalacrocorax auritus*), ducks (*Aythya affinis*), and other seabird species are reported to sustain heavy mortalities during *K. brevis* blooms (Shumway et al. 2003; Landsberg et al. 2008). Sea turtle stranding frequencies increased four-fold during a particularly severe mortality event in 2005–06, and red tide intoxication was determined as the cause of death in over 90% of these individuals (D. Fauquier, personal communication).

Manatees (*Trichechus manatus*) and bottlenose dolphins are the marine mammal species most severely affected by *K. brevis*. The first reported association between *K. brevis* blooms and manatee deaths occurred in 1963 near Fort Myers (Layne 1965). A subsequent epizootic in 1982 involved 38 manatee deaths coinciding with a persistent *K. brevis* bloom and was associated with fish kills and cormorant

deaths (O'Shea et al. 1991). Behavior of the affected manatees included disorientation, the inability to submerge or maintain a horizontal position, listlessness, and labored breathing. Most animals had stomachs full of seagrasses and associated filter-feeding tunicates (*Molgula* spp.), indicating recent feeding. However, analysis of tunicates by mouse bioassay did not detect PbTx, and the involvement of toxins as the causative agent remains circumstantial. Mass mortalities similar to the 1982 event have occurred in 1996, 2002, 2003, and 2005 (FWC 2007), all between the months of February and April, along approximately a 100-mile stretch of Florida coast centered on the mouth of the Caloosahatchee River. *K. brevis* blooms, which typically develop offshore, make landfall during fall and winter, and then dissipate (Tester and Steidinger 1997), made unusual spring appearances in these embayments during these years. These blooms coincided with large numbers of manatees wintering at warm water refuges and low-salinity areas in the Caloosahatchee River region while beginning their migration northward from the river during the early spring (Reynolds and Wilcox 1986). Thus, the co-occurrence of *K. brevis* blooms in embayments where manatees are concentrated results in a high likelihood of PbTx exposure that may precipitate the observed mass mortality events (Landsberg and Steidinger 1998; Landsberg 2002). It was not until the 1996 event, when 149 manatees died in association with a *K. brevis* bloom, that the presence of PbTx was confirmed as a factor in mortality. Stomach contents from several animals were positive for PbTx, consisting of seagrasses, tunicates, and other epifauna that were suspected in the 1982 event (Landsberg and Steidinger 1998). Evidence of PbTx exposure and severe congestion was also observed in the respiratory tract, liver, kidney, lung, and brain, as well as in lymphocytes and macrophages in these tissues (Bossart et al. 1998). Thus, PbTx exposure and possible immunosuppression in manatees may also result in part from chronic inhalation in addition to neurotoxic effects from ingestion (Baden 1996; Bossart et al. 1998). Based on extrapolation from the human symptomatic dose of 24 μg/kg body weight, a manatee dietary intake of 7% body weight per day, and PbTx concentrations detected in stomach contents, the estimated oral dose would be sufficient to cause symptoms in a 700-kg manatee (Baden 1996). In the 2002 mass mortality, manatee deaths began not during but several weeks after termination of a February *K. brevis* bloom in the region, raising questions about the source of toxin in that event. Seagrasses collected from the area contained high levels of PbTx, both on the grass blades and in associated filter-feeding organisms, several months after termination of the bloom (Flewelling et al. 2004).

Bottlenose dolphin mortalities also have a long history of circumstantial association *K. brevis* blooms. The earliest reported association between these "red tides" and mass dolphin mortalities was a 1946–47 bloom occurring between Florida Bay and St. Petersburg that persisted for eight months (Gunter et al. 1948). At the time, the identity of *K. brevis* as the causative organism was tenuous, and the toxin was unidentified. PbTx was also proposed as a causative agent in an unprecedented die-off of over 740 bottlenose dolphins occurring between New Jersey and Florida in 1987–88 (Geraci 1989). Strandings coincided with a rare bloom of *K. brevis* originating in the Gulf of Mexico and carried via the Gulf Stream into Atlantic coastal waters, resulting in toxic shellfish and human NSP intoxications (Tester et al. 1991). Although PbTx was reported in the stomach contents and liver of several dolphins (Baden 1989), evidence of PbTx involvement remains equivocal due to inadequate confirmatory analytical methods available at the time, co-occurring morbillivirus infection, and a temporal mismatch in several PbTx-positive animals and the presence of the observed *K. brevis* bloom. A subsequent Florida bottlenose dolphin mortality event occurred in 1999–2000 coinciding with a persistent *K. brevis* bloom along the Florida panhandle near St. Joe Bay and Choctawhatchee Bay. In this event, PbTx was confirmed in stomach contents, liver, and/or kidney samples in 41% of the animals (Twiner et al. 2009). Stomach contents, consisting primarily of finfish, had the highest PbTx concentrations, and most individuals that stranded were in good body condition, indicating acute poisoning via oral PbTx exposure (Mase et al. 2000). Histopathological examination of two animals showed significant lesions in the upper respiratory tract and PbTx-specific antibody staining in lung and spleen tissue (Van Dolah 2005).

A prominent mass mortality of 107 bottlenose dolphins occurred in the spring of 2004 in the Florida panhandle, highlighting the importance of identifying food web vectors in PbTx-associated die-offs.

Although no *K. brevis* bloom was observed at the time, extremely high concentrations of PbTx were detected in dolphin stomach contents, which were full of large numbers of undigested menhaden (*Brevoortia* spp.), a planktivorous fish (Flewelling et al. 2005). Prior to this event, it was suspected that the ichthyotoxic effects of PbTx would kill finfish before they could accumulate sufficient toxin to transfer it up the food web to predators. Subsequent experimental and field work showed that PbTx could accumulate in multiple species of finfish despite a wide variety of feeding habits, indicating multiple sources of PbTx in the Florida coastal ecosystem (Naar et al. 2007; Fire et al. 2008).

In perhaps the most severe *K. brevis*-related marine mammal mortality event observed to date, large numbers of bottlenose dolphins, along with several other cetacean, finfish, and invertebrate species, died as a result of a *K. brevis* bloom occurring along the central west Florida coast during 2005–06 (Landsberg et al. 2009). At least three large peaks in dolphin strandings were observed during this time, and the presence of PbTx was confirmed in tissues and fluids from over 80 individual animals (Twiner et al. 2009). PbTx was also detected in multiple species of finfish known to be major prey items for the dolphins, showing exposure to PbTx from multiple sources in the food web. In addition, impacts of PbTx exposure on fish stocks in this region differed across trophic guilds, resulting in a shift in relative abundance of available dolphin prey species, and a dominance of clupeids, which may act as more efficient PbTx vectors to upper trophic levels (Gannon et al. 2009).

Ciguatoxin

Ciguatoxins (CTX) are a suite of polyether toxins produced by the dinoflagellates *Gambierdiscus* spp., which cause the clinical illness in humans known as ciguatera fish poisoning (CFP). CTX is similar to PbTx in chemical structure, pharmacological target, and clinical signs; however, the potency of CTX is much greater, and neurotoxic symptoms often persist for longer periods and can include reversal of temperature sensation, tachycardia, hypertension, paralysis, and death (Lewis 2001). In the United States, ciguatera-producing *Gambierdiscus* populations primarily occupy tropical and subtropical waters of Hawaii and southern Florida. Since *Gambierdiscus* spp. are generally benthic epiphytes that grow on filamentous algae associated with coral reefs and reef lagoons, CTX typically enters the food web via herbivorous fishes and invertebrates, and can bioaccumulate in high trophic-level reef fishes such as grouper and barracuda (Lehane and Lewis 2000; Cruz-Rivera and Villareal 2006).

Although much is known about the effects of CTX on humans, evidence of involvement of CTX as a factor in mortality and disease of marine mammals is limited and speculative. It has been proposed as one of several potential factors in the decline of Hawaiian monk seals (*Monachus schauislandi*) in the tropical Pacific. A 1978 Hawaiian monk seal mortality event occurring in Laysan Island resulted in the deaths of over 50 animals, and high levels of CTX-like activity were estimated by bioassay in the liver of two animals (Gilmartin et al. 1980). However, inconsistent additional assay results prevented unequivocal confirmation of CTX as the causative agent. A 1992–93 recovery effort that relocated a severely depleted monk seal stock from Midway Island to French Frigate Shoals resulted in only 11% of the translocated animals surviving beyond one year, and one hypothesis is that the reefs at Midway support a high incidence of CTX in the food web (Gilmartin and Antonelis 1998). Surveys of monk seal prey species in Midway lagoon in 1986 and 1992 detected CTX in over half of all fish tested (Wilson and Jokiel 1986). Again, these results were equivocal due to high false-positive rates reported by the detection method employed (Dickey et al. 1994; Wong et al. 2005).

NOVEL AND EMERGENT CONCERNS FOR HAB TOXINS AND MARINE ANIMAL HEALTH

DSP Toxins

Okadaic acid (OA) and its derivatives, the dinophysistoxins (DTX), are groups of polyether compounds that cause the human illness diarrhetic shellfish poisoning (DSP), and are thus collectively referred to as DSP toxins. Although DSP toxins are protein phosphatase inhibitors that cause a relatively mild intoxication resolving within a few days, OA-like toxins have been identified as potential tumor promoters in sea turtles (Landsberg et al. 1999). Produced by the dinoflagellates *Dinophysis* spp. and *Prorocentrum* spp., the

first reported DSP outbreak in North America occurred in 1990 in Nova Scotia (Marr et al. 1992), but cases have also been reported in Europe, Japan, South America, South Africa, New Zealand, Australia, and Thailand. Effects of OA on marine mammals have not been reported in the literature; however, OA was detected for the first time in several bottlenose dolphins from a mortality event occurring in Texas in February through April 2008 (Fire et al. 2011). The event co-occurred with a bloom of *Dinophysis ovum* and associated shellfish closures in the region (Deeds et al. 2010; Swanson et al. 2010), but the OA concentrations detected in dolphin feces and gastric contents were very low relative to analytical detection limits, and the role of DSP toxins as a factor in the mortality of these animals remains unclear.

Azaspiracid

Azaspiracids (AZA) are a newly identified nitrogen-containing polyether toxin, reported for the first time in 1995 in association with an outbreak of gastrointestinal illness in humans following the consumption of shellfish from Ireland (Furey et al. 2003). Toxic symptoms due to AZA poisoning are similar to DSP poisoning and include nausea, vomiting, diarrhea, and stomach cramps (Twiner et al. 2008). Production of AZA is associated with the dinoflagellate *Azadinium spinosum* and has been reported in Europe, North America, and Africa (James et al. 2003; Twiner et al. 2008; Tillmann et al. 2009). The mechanism of toxicity for AZA is still unclear, and although documented to induce teratogenic effects in fish (Colman et al. 2005), no impacts on marine mammals have been reported.

Yessotoxins

Yessotoxin (YTX) is a sulfated polyether toxin produced by dinoflagellates belonging to the genera *Gonyaulax* (=*Protoceratium*) and *Lingulodinium*. Originally thought to be one of the DSP toxins, it has been shown to have no diarrhetic activity and little toxic potency when administered orally to mice (Tubaro et al. 2008). However, it has potent lethality to mice when injected, inducing neurological symptoms. Blooms of both *Lingulodinium polyedrum* and *Gonyaulax grindleyi* have been implicated in fish and shellfish mortality events, although the role of YTX in those events was not investigated. The impacts of YTX on marine mammals are not known.

Spirolides

Spirolides are a group of macrocyclic amines produced by the dinoflagellate *Alexandrium ostenfeldii* and implicated in shellfish toxicity in northern Europe (Cembella et al. 2000). The mode of action of this toxin is not clear, but injection into mice causes rapid death following neurological symptoms. The distribution of *A. ostenfeldii* in North America is limited to eastern Canadian waters and the Gulf of Maine. Health impacts from spirolide exposure in marine mammals are unknown.

NON-ACUTE EXPOSURE, MULTIPLE TOXINS, AND NOVEL IMPACTS

Acute effects of HAB toxin exposure in marine mammals are often quite conspicuous, resulting in dramatic die-offs of large numbers of animals and associated marine fauna. Dense blooms and high concentrations of toxins present in seawater, prey items, and marine mammal tissues and fluids typically precipitate these events. However, variability in the severity of HABs and the frequency in which they occur can result in repeated, sublethal exposures to one or more toxins with negative impacts on marine mammal health. Repeated nonlethal DA exposure in California sea lions has significant long-term effects such as degenerative heart disease, chronic epileptic syndrome, and *in utero* toxicity resulting in reproductive failure (Brodie et al. 2006; Goldstein et al. 2008; Ramsdell and Zabka 2008; Zabka et al. 2009). Florida bottlenose dolphins and their fish prey have been shown to contain PbTx several months to over a year following termination of a *K. brevis* bloom (Fire et al. 2007; Naar et al. 2007), suggesting year-round toxin exposure, but long-term impacts on these populations are unknown. The effects of annual STX-producing blooms or potential CTX exposure in marine mammals likely includes negative impacts to growth of already depleted populations (Gilmartin and Antonelis 1998; Durbin et al. 2002), but few data exist to support this hypothesis, in part due to insufficiently sensitive detection methods, confounding factors such as infectious disease, or a lack of longitudinal data.

As HAB observation efforts become more widespread, toxic species are increasingly reported in new regions, resulting in increased awareness of marine mammal exposure to multiple HAB toxins. Northern right whales in their summer feeding grounds in New England and Bay of Fundy waters experience frequent exposure to both STX and DA (Doucette et al. 2006; Leandro et al. 2010). Biomonitoring efforts have detected PbTx and DA simultaneously in Florida bottlenose dolphins for several years (Twiner et al. 2011), including live animals as well as stranded carcasses (NMFS 2004). Low levels of DA, PbTx, and OA were all detected in various bottlenose dolphins stranding during a mortality event occurring near Galveston, Texas, in 2008 (Fire et al. 2011). Currently, it is not known whether exposure to multiple HAB toxins results in additive or synergistic effects that increase the potency of one or more of the toxins present (Twiner et al. 2008).

Notwithstanding the advent of successful HAB monitoring efforts and technologies, evidence of HAB toxin exposure is seen in marine mammals even in the absence of observed toxic blooms. In a 1997–2009 survey of pygmy and dwarf sperm whales (*Kogia* spp.) stranding between North Carolina and the Atlantic coast of Florida, feces from nearly 90% of all individuals sampled were positive for DA, despite the fact that no DA-producing HABs were associated with any of the DA-positive animals (Fire et al. 2009). This may be an indication that undetected HAB activity occurring in remote regions can affect marine mammals, and highlights the importance of continued efforts to investigate such associations.

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