

Trophic Transfer of the Harmful Algal Toxin Domoic Acid as a Cause of Death in a Minke Whale (*Balaenoptera acutorostrata*) Stranding in Southern California

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Abstract

Unusually high concentrations of the neurotoxin domoic acid (DA) were detected in a minke whale (*Balaenoptera acutorostrata*) carcass recovered during a severe harmful algal bloom (HAB), which occurred in southern California in April 2007. Cell fragments of the toxigenic diatom *Pseudo-nitzschia australis* were observed in whale gastric fluid and feces, corresponding to a dominance of *Pseudo-nitzschia* spp. in the phytoplankton community at the time of stranding. A high abundance of otoliths from a prominent DA vector, the northern anchovy (*Engraulis mordax*), were recovered in whale stomach contents, indicating trophic transfer of DA via the food web. Whale feces contained 258 µg DA per gram sample, exceeding DA concentrations reported for any marine mammal. DA intoxication was identified as the cause of mortality of this animal, expanding on the limited understanding of the impacts of DA-producing HABs on large whales.

Key Words: domoic acid, harmful algal bloom, algal toxin, stranding, marine mammal, minke whale, *Balaenoptera acutorostrata*

Introduction

Exposure to marine biotoxins produced by harmful algal blooms (HABs) is the leading attributable cause of large marine mammal die-offs in the U.S. Between 1991 and 2008, 50% of all die-offs declared by the National Oceanic and Atmospheric Administration's (NOAA) Office of Protected Resources (2010) as unusual mortality events (UMEs) with a known cause were a result

of exposure to HAB toxins, with the majority of affected species being cetaceans. Domoic acid (DA), a neurotoxin produced by the toxic phytoplankton *Pseudo-nitzschia* spp., is responsible for frequent large-scale marine mammal mortalities in California coastal waters due to its accumulation in marine food webs and subsequent acute exposure in top predators (Van Dolah, 2005; Ramsdell, 2010). In addition to mortality from acute exposure, long-term, sublethal effects of DA have also been reported, including degenerative heart disease, chronic epileptic syndrome, and reproductive failure (Brodie et al., 2006; Goldstein et al., 2008; Zabka et al., 2009).

These dramatic negative impacts of DA-producing blooms are primarily manifest in numerically abundant species such as the California sea lion (*Zalophus californianus*), common dolphins (*Delphinus* spp.), and sea birds via consumption of known DA vectors such as small, schooling planktivorous fish (Work et al., 1993; Lefebvre et al., 1999; Scholin et al., 2000). As these fish filter and ingest suspended biological material from the water column during feeding, they accumulate and concentrate the DA made available to their predators (Lefebvre et al., 2001). Unfortunately, little is known about the effects of DA exposure in the less-abundant baleen whales, such as the minke whale (*Balaenoptera acutorostrata*), that occupy these same habitats in which DA-producing HABs frequently occur. Although baleen whales also feed on these prominent DA fish vectors, their diet also may include a large proportion of prey items that occupy lower trophic compartments, such as zooplankton (e.g., krill, copepods), which graze directly on toxic marine algae (Turner & Tester, 1997; Pauly et al., 1998). However, the paucity of data for large whale strandings associated with

DA-endemic regions makes any estimation of harmful impacts due to HABs difficult.

DA has been reported in northern anchovies (*Engraulis mordax*) and krill collected during toxic *Pseudo-nitzschia* blooms, and the high concentrations detected in their tissues make them important toxin vectors for higher vertebrates such as large whales that feed on dense concentrations of these prey (Lefebvre et al., 2001; Bargu et al., 2002). Still, baleen whale strandings are only infrequently observed in association with *Pseudo-nitzschia* blooms (M. Berman, unpub. data, 1998-2010), and scant data exist documenting food web exposure to DA. DA has been reported in fecal samples from humpback (*Megaptera novaeangliae*) and blue whales (*Balaenoptera musculus*) in association with toxic *Pseudo-nitzschia* blooms, krill, and fish occurring in Monterey Bay, California, and these whales were observed feeding on krill that were later shown to be positive for this toxin (Lefebvre et al., 2002a). However, these samples were collected from live animals that were actively feeding with no indication of debilitating neurologic effects, and little other data are available describing DA exposure in large whales. Further, given the lack of evidence of DA as a direct cause of death in baleen whales, they have often been described as “potentially impacted” (Van Dolah et al., 2003, p. 255) or “potential victims of DA toxicity” (Lefebvre et al., 2002a, p. 975).

The present study seeks to address this gap by presenting data documenting the co-occurrence of an intense, DA-producing harmful algal bloom with the death and stranding of a minke whale recovered from southern California waters during a 2007 marine mammal mortality event. Although data are limited for known HAB impacts on large whales in this region, the effects of *Pseudo-nitzschia* blooms occurring along the California coast have been the subject of much research over the last decade (Trainer et al., 2000; Anderson et al., 2006; Bargu et al., 2008; Jester et al., 2009). Ongoing statewide HAB monitoring efforts combined with independent research investigating California HABs and marine mammal health are abundant and useful in providing the framework for documenting potential impacts on baleen whales in our study region. Herein, we present toxin data from environmental monitoring efforts, minke whale samples, and sediment trap samples, as well as prey item analyses identifying the presence of known DA vector species. These results provide evidence of trophic transfer of DA at multiple points in the food web leading to the mortality of a large whale during a highly toxic *Pseudo-nitzschia* bloom occurring in California waters.

Materials and Methods

On 16 April 2007, a dead minke whale carcass (animal ID SBMNH 2007-07) was first observed floating near the south end of San Buenaventura State Beach in Ventura, California (34.26668° N, 119.27793° W; Figure 1), during a period of increased marine mammal strandings co-occurring in that region. Stranding and subsequent examination occurred 17 April 2007, during which samples were collected. Samples of feces, urine, gastric fluid, and other stomach contents were collected for biotoxin analysis. Biotoxin samples were placed in capped polypropylene tubes or sealed plastic bags and stored frozen (-20° C) until shipped overnight on dry ice to the NOAA Marine Biotoxins Program in Charleston, South Carolina. Samples were stored at -20° C prior to analysis. Organ tissue samples (heart, kidney, uterus, bladder, lymph node, liver, intestine, ovary, and lung) were also harvested for histopathological analysis. The whole stomach was collected and sealed at the esophagus and caudal to the pyloric sphincter for subsequent prey item analysis. Data from other marine mammal carcasses recovered in association with this stranding were also collected as part of long-term monitoring efforts conducted by the U.S. Marine Mammal Health and Stranding Response Program.

Seawater and shellfish samples were collected as part of ongoing monitoring efforts conducted by the California Department of Public Health. Collection and analysis of these samples followed methods described by Langlois (2008). Over 30 shellfish sampling stations and 55 phytoplankton

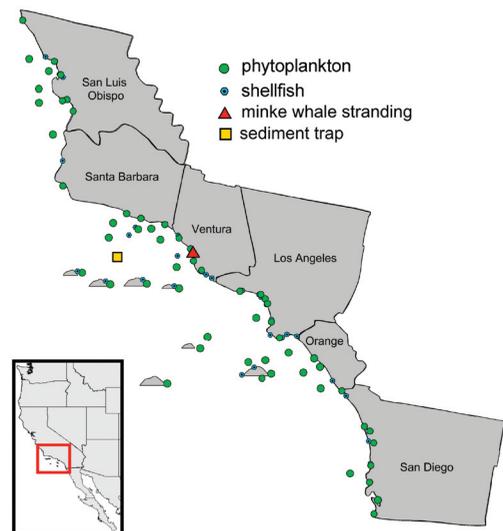


Figure 1. Collection sites for shellfish, phytoplankton, sediment, and minke whale strandings

sampling stations were utilized, distributed along the Southern California coast from San Luis Obispo to San Diego Counties (Figure 1). *Pseudo-nitzschia* cells were observed via light microscopy and their abundance quantified according to two metrics: (1) percent composition, a percentage estimate of each species identified in a sample relative to all other phytoplankton species present; and (2) relative abundance index (RAI), a normalization of cell data based on an estimate of cell mass as determined by settled cell volume, the percent composition of each species, and the sampling effort as determined by the total net tow length.

Marine sinking particles were collected from a sediment trap mooring stationed near the center of the Santa Barbara Basin (SBB) (34.23° N, 120.03° W), a bathymetric feature bounded by California's Channel Islands and Ventura and Santa Barbara Counties (Figure 1) that is known for frequent and intense *Pseudo-nitzschia* blooms (Anderson et al., 2006; Sekula-Wood et al., 2009; Pitcher et al., in press). The mooring consisted of a deep-moored Mark VI sediment trap positioned between 490 and 540 m water depth, with trap cups fixed with 10% sodium azide and 1% sodium borate as preservatives. The trap was deployed throughout 2007 with each sample collection container collecting material continuously for ~2 wks.

DA was extracted from whale feces and gastric samples by adding four volumes of 50% aqueous methanol to the homogenized sample, followed by 2 min of probe sonication (450 W; Sonifier S-450A; Branson Ultrasonics Corp., Danbury, CT, USA) in an ice bath. Samples were centrifuged (IEC Centra CL2; Thermo Scientific, Waltham, MA, USA) at $3,400 \times g$ for 10 min. The supernatants were collected, filtered through 0.45 μm hydrophilic polypropylene (GHP/GxF) syringe-driven filter disks (Acrodisc; Pall Life Sciences, East Hills, NJ, USA) and stored in 20 mL glass vials at -20°C . Urine samples and extracts of feces and gastric contents were centrifuge-filtered at $10,000 \times g$ using 0.22 μm centrifugal filter devices (Nanosep MF; Pall Life Sciences) in preparation for analysis. Dried, ground sediment trap samples were extracted in 50% aqueous methanol following methods described in Sekula-Wood et al. (2009).

Filtered urine samples, extracts of feces, and gastric fluids were analyzed for the presence of DA using tandem mass spectrometry coupled with liquid chromatographic separation (LC-MS/MS), following methods outlined by Wang et al. (2007). This method utilized an HP1100 LC system (Agilent Technologies, Inc., Palo Alto, CA, USA) and an Applied Biosystems/MDS Sciex API 4000 triple quadrupole mass spectrometer equipped with a Turbo V™ source (Applied Biosystems, Foster City, CA, USA). Chromatographic separation was performed

on a Phenomenex Luna C18(2), 5 μm , 150×2 mm column. The mobile phase consisted of water and acetonitrile in a binary system, with 0.1% formic acid as an additive. The elution gradient was 2 min of 95% water, with a linear gradient to 60% water at 15 min, 95% water at 17 min, held for 5 min, then returned to initial conditions at 23 min and held for 5 min before the next injection. Retention time of DA in samples was determined based on the retention time of a certified DA reference standard from the Institute for Marine Biosciences, NRC Canada (Halifax, NS, Canada). To reduce mass spectrometer contamination, a diverter valve sent the LC eluant to waste except for the 6-min elution window bracketing the DA retention time. Four MRM transitions from protonated DA were monitored: m/z 312 \rightarrow 266, m/z 312 \rightarrow 248, m/z 312 \rightarrow 193, and m/z 312 \rightarrow 161. DA analysis of the sediment trap samples was performed using similar LC-MS methods as described in Burns & Ferry (2007).

Prey item analysis of minke whale stomach contents was performed following methods adapted from Fitch & Brownell (1968). Briefly, stomach contents were sorted by hand, soft tissues were discarded, and fish otoliths and other hard parts were cleaned in 70% ethyl alcohol and dried. Sagittal otoliths were then identified using the Santa Barbara Museum of Natural History otolith reference collection, sorted by species, and each otolith was counted and the condition was recorded. The minimum number of individuals present for each prey species was determined by dividing the total otolith count by two.

Sediment trap and fecal samples were examined by light and scanning electron microscopy (SEM) to identify frustules of *Pseudo-nitzschia* species known to produce DA. Prior to microscopic examination, samples were prepared using Simonsen's method for cleaning diatom frustules (Hasle, 1978). Fecal samples were rinsed with distilled water and added to an equal volume of KMnO_4 . After oxidation for 24 h, an equal volume of HCl was added, and the solution was heated until the sample color changed from purple to clear. Sediment trap samples were prepared by resuspending un-ground, freeze-dried particulate material in deionized water. The cleaned frustules were examined by light microscopy using an Olympus BX51 with differential interference contrast and phase contrast optics. For SEM, preparations were dehydrated using a graded acetone series (10 to 100%) and a series of hexamethyldisilazane (HMDS) (25 to 100%) treatments. The samples were coated with approximately 1.5 nm of platinum using a Denton sputter-edge coater (Moorestown, NJ, USA). Samples were examined with a JEOL 5600LV (Tokyo, Japan) SEM.

Results

Beginning on 1 April 2007, a rapid increase in the abundance of *Pseudo-nitzschia* spp. cells was observed in the water column from San Luis Obispo (SLO), Santa Barbara (SB), Ventura (VU), and Los Angeles (LA) Counties (Figure 2). *Pseudo-nitzschia* were the dominant algal group (> 80% relative abundance) observed between 8 April and 6 May. *Pseudo-nitzschia* cells reached maximum abundance on 29 April, during which time they comprised 90% of the observed phytoplankton community and RAI values peaked at 21.0 (Figure 2). A rapid increase in DA concentrations was also detected in mussels in the same geographical region, and high DA concentrations were tightly coupled with the observed *Pseudo-nitzschia* bloom (Figure 3). DA concentrations in LA and SLO samples reached maximum values just above the 20 $\mu\text{g/g}$ FDA regulatory limit for seafood (26 $\mu\text{g/g}$ at LA; 30 $\mu\text{g/g}$ at SLO), while concentrations at VU reached a maximum of 237 $\mu\text{g/g}$ (Food and Drug Administration [FDA], 2010). One SB sample collected on 24 April reached a DA concentration of 610 $\mu\text{g/g}$ —a concentration over 30 times the FDA regulatory limit and the highest concentration ever recorded in this region. Sediment trap collections began to detect particulate DA during the 13 through 31 March time frame, with concentrations increasing to just below 2 $\mu\text{g/g}$ by 14 April. An intense peak in sediment trap DA concentrations was detected during the 15 April through 4 May time frame, with corresponding DA concentrations exceeding 100 μg DA per g sediment (Figure 4). SEM analysis of sediment trap samples collected during this peak period detected a high abundance of *Pseudo-nitzschia* frustules (Figure 5). A sharp increase in

the frequency of cetacean strandings (including that of the 16 April minke whale) occurring in southern California coastal waters was observed between 8 and 29 April, also corresponding to the peak of the *Pseudo-nitzschia* bloom (Figure 2).

The minke whale was an immature female in overall good body condition with blubber depths over 30 mm. The retention of light muscle tissue and the measured body length of 408 cm indicate a 6- to 8-mo-old newly weaned individual. The minimal degree of decomposition of this fresh dead carcass allowed for suitable morphological and histopathological analyses that reported no significant pathological findings in the heart, kidney, uterus, bladder, lymph node, liver, intestine, ovary, or lung (D. Rotstein, unpub. data, 2007). External morphology of the carcass also indicated no evidence of ship strike or entanglement (M. Berman, unpub. data, 2007). Blubber toxicology for persistent organic pollutants was relatively low compared to other cetacean values from this region (M. Berman, unpub. data, 2010).

The presence of domoic acid was confirmed by LC-MS/MS in the minke whale gastric fluid, urine, and feces samples at concentrations of 2.35, 2.93, and 258.67 μg DA per g of sample, respectively (Figure 6). During SEM analysis of the whale feces, extremely high numbers of *Pseudo-nitzschia* frustules were observed and identified as *P. australis* (Figure 5). *P. australis* frustules were also observed in gastric fluid from this animal, although they were documented in much fewer numbers.

Stomach content analysis performed on the whale revealed a high abundance of fish otoliths. No soft tissue or other hard parts were present in the stomach, and the majority of the otoliths were in identifiable condition. A total of 560 northern anchovy otoliths were observed, indicating a

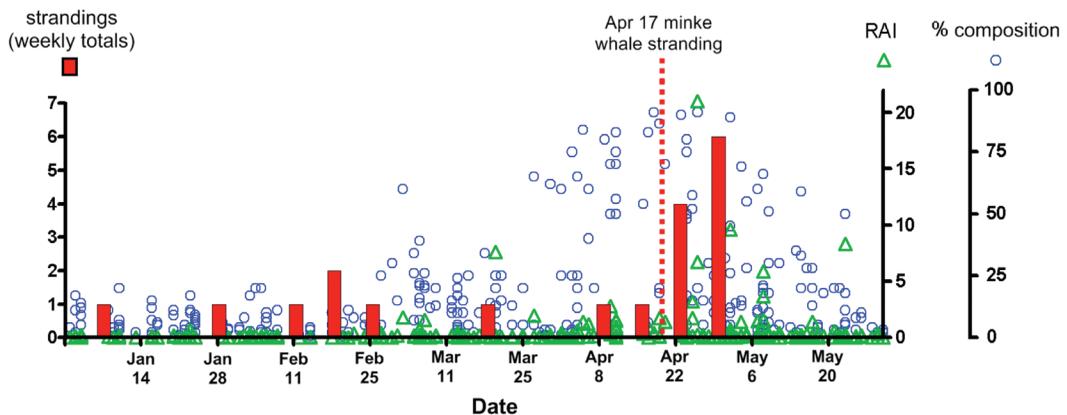


Figure 2. Time series of *Pseudo-nitzschia* spp. abundance and reported cetacean strandings, January to May 2007; RAI = relative abundance index.

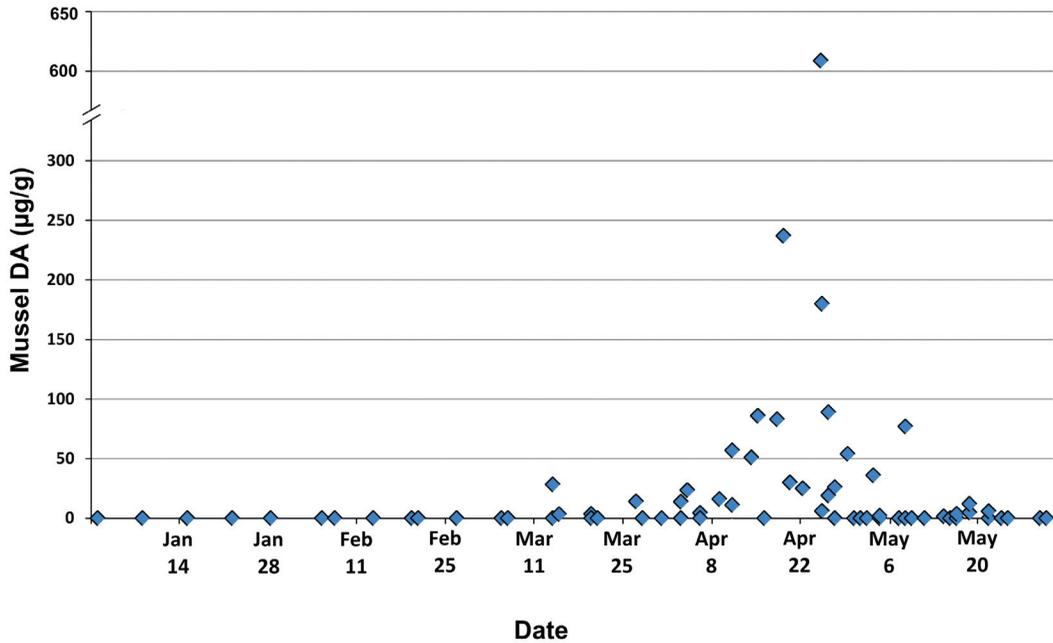


Figure 3. DA concentrations detected in mussels collected from San Luis Obispo, Santa Barbara, Ventura, and Los Angeles Counties

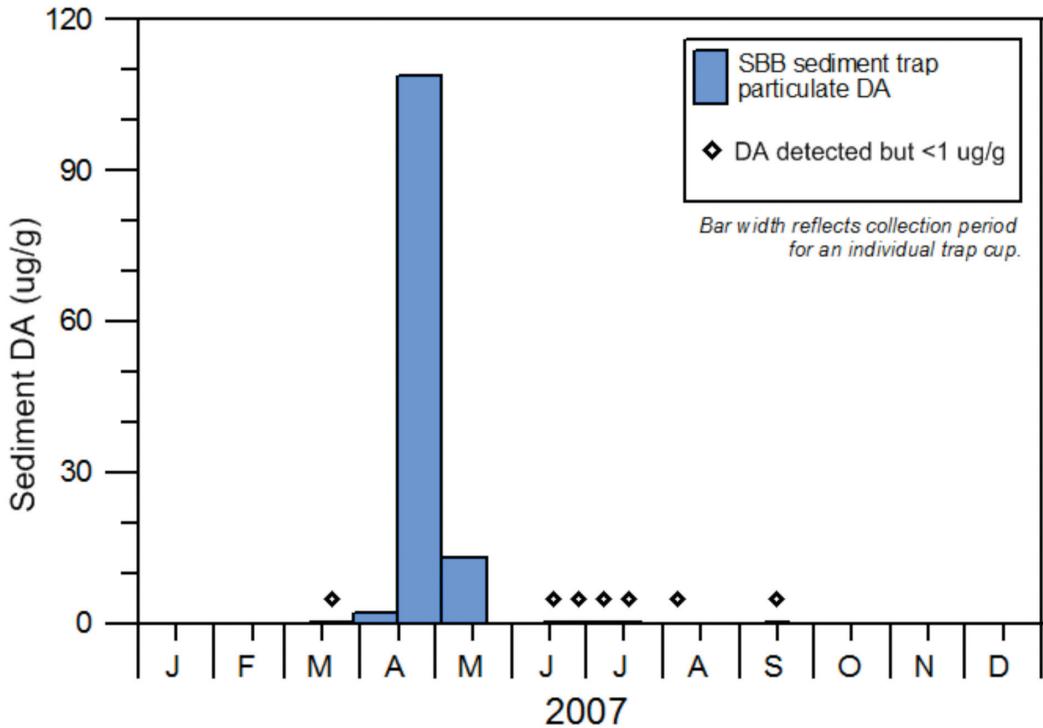


Figure 4. Domoic acid concentrations detected in marine sediment samples from the Santa Barbara Basin. All data points (e.g., bars, markers) are from 2-wk integrated samples; all 2-wk intervals shown were sampled and tested for DA.

minimum of 280 anchovies had been consumed and digested prior to death. Based on digestion rates and otolith clearance rates for baleen whales and other piscivorous marine mammals, the otoliths remaining in the stomach were likely from a feeding bout occurring less than 15 h prior to death (Bigg & Fawcett, 1985; Vikingsson, 1997).

Discussion

In April 2007, an intense *Pseudo-nitzschia* bloom occurred throughout much of southern California as evidenced by the high *Pseudo-nitzschia* spp. cell abundance, dominance of *Pseudo-nitzschia* spp. in the phytoplankton community, and very high levels of DA in sentinel mussels. Concurrently, an intense peak in cetacean mortalities was associated with the *Pseudo-nitzschia* bloom peak, during which time marine mammal stranding organizations in southern California were reporting approximately one cetacean stranding per day during a 3-wk period (7 to 29 April) (M. Berman, unpub. data, 2007).

The presence of a large number of anchovy otoliths in the stomach contents of the minke whale is direct evidence that the whale was feeding heavily on schools of this known DA vector (Lefebvre et al., 2001). In addition, *Pseudo-nitzschia* frustules observed in both gastric fluid and feces from the whale showed a very high abundance of *P. australis*, which is considered the most toxic of the *Pseudo-nitzschia* spp. commonly found in California waters (Trainer et al., 2000). Furthermore, the abundance of these frustules observed by SEM in the feces sample was similar to *P. australis* frustule abundance observed by SEM in the sediment trap samples (Figure 5) and similar to that observed by SEM in *P. australis* culture samples (S. Morton, unpub. data, 2010).

The morphological and histopathological findings indicating good body condition and rapid death are a further indication of acute neurotoxicosis consistent with other marine mammal mortalities due to HAB toxin exposure (Geraci et al., 1989; Gulland, 2000). Finally, the detection of DA in gastric fluid and urine and at extremely high concentrations in feces confirms not only exposure to, but metabolism and clearance of, the toxin in this whale. The DA concentration detected in feces is the highest reported in any marine mammal to date. Cause of stranding was determined based on guidelines described in Greig et al. (2005), using a combination of results from gross necropsy, histopathological examination of tissues, biotoxin analysis, and temporal and spatial association with a domoic acid-producing harmful algal bloom. Based on biotoxin and necropsy findings, the observed healthy body condition,

and an absence of pathology, we can conclude this animal died from acute domoic acid toxicity.

Based on fish weights and maximum DA concentrations reported for field-collected anchovies (Lefebvre et al., 2001, 2002b) and daily feeding rates and body weight of the minke whale (Sergeant, 1969; Horwood, 1990), an estimated DA dose for a minke whale consuming 4% of its body weight in DA-contaminated anchovies daily is approximately 6.8 mg/kg body weight. By comparison, symptoms of oral DA toxicity in humans

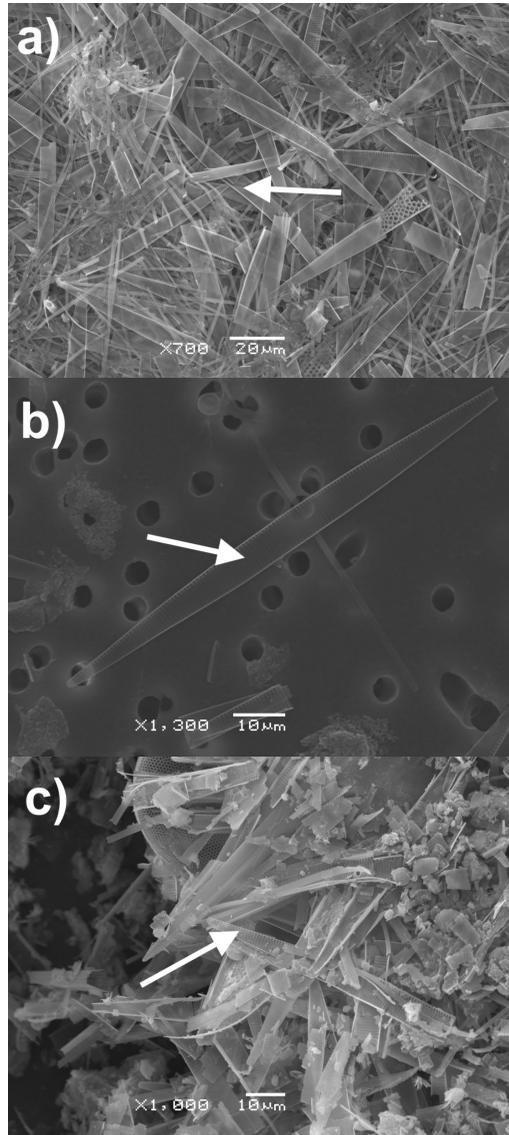


Figure 5. SEM images of *P. australis* frustules (indicated by arrows) identified in a) minke whale feces, b) gastric fluid, and c) sediment samples

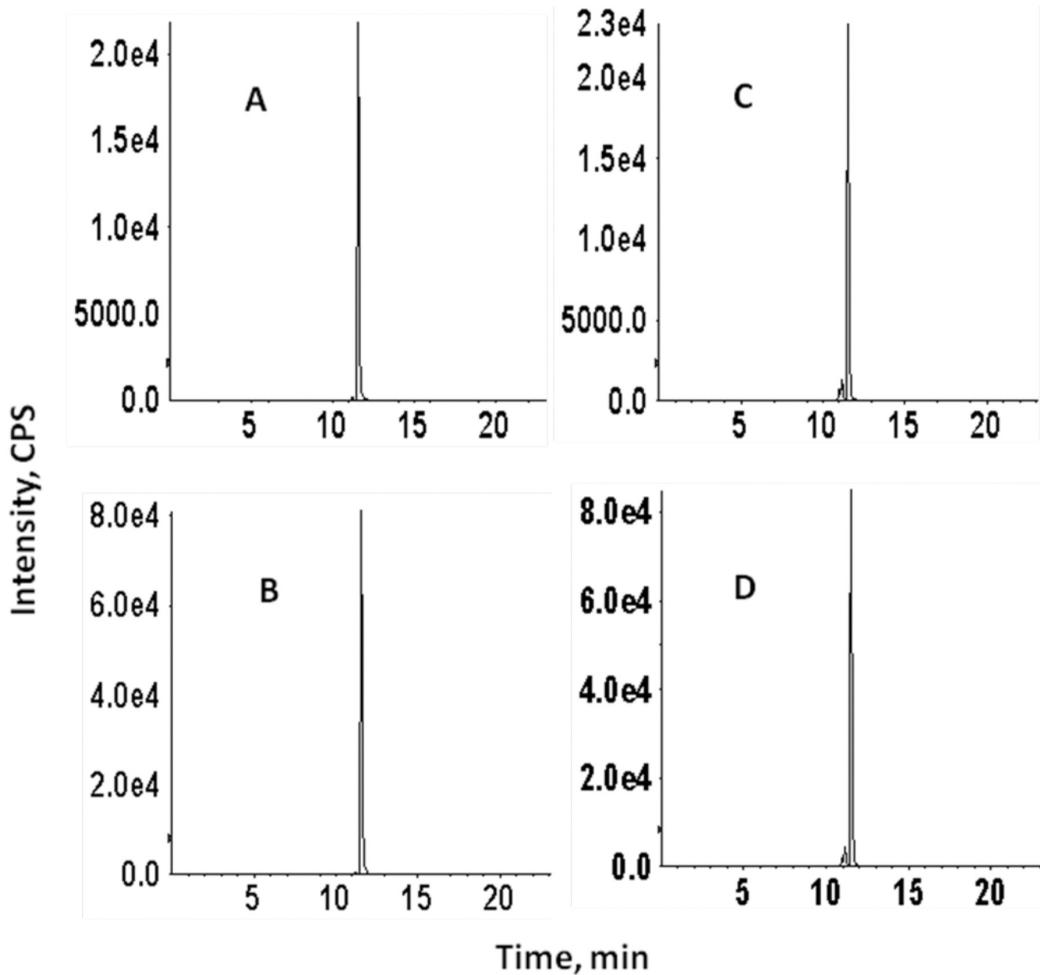


Figure 6. LC-MS/MS extracted ion chromatograms of the two major MRM transitions of DA: a DA NRC standard (50 ng/ml) with MRM transitions at m/z 312 \rightarrow 161 (A) and m/z 312 \rightarrow 266 (B), and a 1,000-fold diluted extract of whale feces with MRM transitions at m/z 312 \rightarrow 161 (C) and m/z 312 \rightarrow 266 (D)

and cynomolgus monkeys are manifest at 0.7 and 1.0 mg/kg body weight, respectively, with lethal oral DA doses in humans estimated at 4 mg/kg (Perl et al., 1990; Truelove et al., 1997; Wekell et al., 2004). The symptomatic and lethal DA dose thresholds for baleen whales are not known; however, a given minke whale, if feeding exclusively on anchovies containing the DA concentrations reported above, would exceed the known lethal dose reported for other mammal species. In terms of DA exposure in the minke whale in the present study, a single feeding bout involving ingestion of the 280 anchovies observed in the stomach at DA concentrations reported previously for anchovies would result in a dose of approximately 1.1 mg/kg, exceeding reported symptomatic oral

dose levels for mammals. This concentration, though not lethal in humans and monkeys, may nonetheless elicit symptoms of intoxication that may contribute to the whale's death when accounting for cumulative exposure across multiple feeding events.

DA exposure that would be sublethal in a terrestrial mammal may also have more pronounced toxic effects in a diving mammal due to various physiological adaptations to the marine environment (Geraci et al., 1989). For example, a large proportion of baleen whale body mass is metabolically inactive blubber that receives very little blood flow (Ash, 1957) and into which water-soluble toxins like DA would not likely partition. In addition, peripheral vasoconstriction induced by the "mammalian dive

response" (Berta & Sumich, 1990, p. 239) shunts blood away from detoxifying mechanisms in the liver and toward the heart and brain, which are particularly sensitive to the toxic effects of DA (Strain & Tasker, 1991; Silvagni et al., 2005). It has also been suggested that larger animals require proportionally less toxin exposure to achieve the same toxic effect as smaller animals (Casarett, 1975), indicating that any extrapolation of experimental DA exposure in small mammals may result in an underestimation of DA toxicity in larger mammals.

Although seasonal blooms of DA-producing *Pseudo-nitzschia* are a common occurrence in California coastal waters, marine animal mortalities that typically accompany such events generally include sea lions, sea birds, or common dolphins—species that are easily identified and recovered when stranding in large numbers. Large whales are undoubtedly affected by the harmful consequences of DA poisoning since they consume toxic prey similar to these more commonly affected species. The rarity of such strandings, however, has until now prevented a thorough assessment of DA poisoning impacts. This study demonstrates that baleen whales are, in fact, negatively impacted by DA through their diet, and it highlights the need for increased effort in understanding trophic transfer of algal toxins in all components of the marine food web. Since large cetaceans are conspicuous animals that can serve as important marine sentinels of ocean health, they can provide a critical component in estimating ecosystem-wide effects of harmful algal blooms in waters where marine mammals are found.

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