

Domoic acid exposure in pygmy and dwarf sperm whales (*Kogia* spp.) from southeastern and mid-Atlantic U.S. waters[☆]

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ABSTRACT

The neurotoxin domoic acid (DA) was detected in urine and fecal samples recovered from pygmy sperm whales (*Kogia breviceps*) and dwarf sperm whales (*Kogia sima*) stranding along the U.S. Atlantic coast from 1997 to 2008. Of the 41 animals analyzed from Virginia, North Carolina, South Carolina and Florida, 24 (59%) tested positive for DA at concentrations of 0.4–1.8 ng/mL in urine and 12–13,566 ng/g in feces as determined by liquid chromatography–tandem mass spectrometry (LC–MS/MS). Feces appeared to be the best indicator of DA exposure in *Kogia* spp., with 87% of all fecal samples analyzed testing positive for this toxin. Additional stranded animals ($n = 40$) representing 11 other cetacean species were recovered from the same region between 2006 and 2008 and analyzed by LC–MS/MS, however DA was not detected in any of these individuals. DA is produced naturally by diatoms in the genus *Pseudo-nitzschia*. Although blooms of DA-producing *Pseudo-nitzschia* have been associated with repeated large-scale marine mammal mortalities on the west coast of the U.S., there is no documented history of similar blooms on the southeast U.S. coast, and there were no observed *Pseudo-nitzschia* blooms in the region associated with any of these strandings. The feeding habits of *Kogia* spp. are poorly documented; thus, the vector(s) for DA exposure to these deep-diving species remains to be identified. Toxin accumulation in these pelagic whale species may be an indication of cryptic harmful algal bloom activity in offshore areas not currently being monitored. This study highlights the need for a better understanding of the role of toxigenic algae in marine mammal morbidity and mortality globally.

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1. Introduction

Marine mammals are among the most valuable marine sentinel species, organisms that act as indicators of current or potential negative impacts to oceans and human health (Wells et al., 2004; Bossart, 2006; Grosell and Walsh, 2006). Harmful algal blooms (HABs) and associated phycotoxins have been documented to negatively impact wild marine mammal populations in the United States, resulting in large-scale mortality events due to acute

toxicity (reviewed in Van Dolah, 2005), population-level impacts from reproductive failure (Brodie et al., 2006) and chronic detrimental health effects (Goldstein et al., 2008). During many of these mortality events, algal toxins were shown to accumulate in high concentrations in planktivorous vector organisms that graze on the toxic algae, and subsequently transfer large toxic burdens up the food web to apex predators (Anderson and White, 1989; Lefebvre et al., 1999; Flewelling et al., 2005). Prominent among the groups of harmful algae associated with severe wildlife mortality events are diatoms of the genus *Pseudo-nitzschia*, which includes at least 10 species known to produce the neurotoxin domoic acid (DA) (Fryxell and Hasle, 2003). During the last two decades, recurrent seasonal toxic *Pseudo-nitzschia* blooms occurring in U.S. coastal waters have been associated with multiple wildlife mortality events, with DA intoxication as the likely causative factor in marine mammal deaths estimated to number in the thousands (Work et al., 1993; Scholin et al., 2000; Heyning, 2003; Van Dolah, 2005; Schnetzer et al., 2007).

The majority of DA-producing *Pseudo-nitzschia* blooms in U.S. waters have been observed along the Pacific states (California to Washington), and to a lesser extent in the northeast (Bay of Fundy to the Chesapeake Bay) and in the northern Gulf of Mexico (Texas to Florida). Similarly, the majority of marine mammal strandings associated with DA accumulation and *Pseudo-nitzschia* blooms have been reported in the Pacific states, and in recent years, an emerging trend of DA detection in strandings has been observed for marine mammals in New England and Gulf of Mexico waters (NOAA Marine Biotoxins Program unpubl. data; TX, FL dolphins, Georges Bank humpback whales). Given the cosmopolitan distribution of toxin-producing species of *Pseudo-nitzschia* (Hasle, 2002), DA detection in marine mammal strandings from the majority of the above U.S. coastal states is not unexpected. However, the coastal states of the southeastern U.S. (SEUS) bordering the Atlantic Ocean (Virginia to Florida) have not historically observed blooms of DA-producing *Pseudo-nitzschia* until recently (S. Morton, T. Leighfield unpublished observation), and no data on DA body burdens from marine mammal strandings in the SEUS are recorded in the current literature.

The pygmy sperm whale, *Kogia breviceps*, and the dwarf sperm whale, *K. sima*, (hereafter jointly referred to as *Kogia*) are among the most poorly known pelagic cetacean (dolphins, porpoises and whales) species, yet are the second most common stranding cetacean in the SEUS (Scott et al., 2001; Odell et al., 2004). In September 2006, samples from four *Kogia* that mass-stranded in North Carolina's Outer Banks region were all found positive for DA exposure, marking the first detection of this toxin in marine mammals from the SEUS. This new finding highlighted the lack of data for DA in SEUS marine mammals and, in concert with recent detection of DA-producing blooms in SEUS waters (S. Morton, unpublished data), provided the impetus for a survey of DA accumulation in *Kogia* and other cetacean species from the SEUS. The primary objectives of this study were to determine the prevalence of DA in marine mammals stranding in a region with no prior history of DA-producing blooms, and to quantify and compare DA concentrations in various cetacean species recovered from this region across multiple years.

2. Methods

K. breviceps and *K. sima* that stranded in SEUS coastal waters between 1997 and 2008 were recovered and sampled by stranding organizations collaborating through the Marine Mammal Health and Stranding Response Program, under the direction of the National Marine Fisheries Service. The geographical range of the *Kogia* strandings included in this study covered Fisherman's Island, VA (37.093°N, 75.9382°W) at the northernmost point to Vero

Beach, FL (27.6462°N, 80.3532°W) at the southernmost point. Stranded animals representing 11 additional cetacean species were also recovered from SEUS coastal waters between 2006 and 2008, and stranding locations for these individuals comprised the same geographic range as the *Kogia*. Species investigated in this study (hereafter jointly referred to as "other cetaceans") included the fin whale (*Balaenoptera physalus*), common dolphin (*Delphinus delphis*), northern right whale (*Eubalaena glacialis*), pygmy killer whale (*Feresa attenuata*), Atlantic white-sided dolphin (*Lagenorhynchus acutus*), humpback whale (*Megaptera novaeangliae*), Blainville's beaked whale (*Mesoplodon densirostris*), melon-headed whale (*Peponocephala electra*), harbor porpoise (*Phocoena phocoena*), *Stenella* sp., bottlenose dolphin (*Tursiops truncatus*) and Cuvier's beaked whale (*Ziphius cavirostris*).

Animals in satisfactory carcass condition for suitable sample recovery (alive at initial stranding, fresh dead, or moderate decomposition) were necropsied and samples were collected for biotoxin analysis. Samples primarily consisted of feces, urine and gastric contents, although a limited number of other tissue and fluid types (liver, kidney, serum, brain, coelomic fluid, amniotic fluid, and milk) were also collected. Samples were placed in capped polypropylene tubes or sealed plastic bags, kept chilled until returning to the field station, and stored frozen (−20 °C) until shipped overnight on dry ice to the NOAA Marine Biotoxins Program, Charleston, SC. Samples were stored at −20 °C prior to analysis.

DA was extracted from feces and gastric samples by adding four volumes of extraction solvent (50% aqueous methanol) to the homogenized sample (typically 1–5 g), 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 3400 × 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 13,000 × g using 0.22 μm centrifugal filter devices (Nanosep MF; Pall Life Sciences) prior to analysis.

All animal samples 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 mm × 2 mm column. 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 16 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. To reduce instrument contamination, a diverter valve was used to divert the LC eluent to waste when not within the 6-min window bracketing the DA retention time. 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). The DA fragments monitored were *m/z* 266 (loss of HCOOH from DA), *m/z* 248 (loss of water from *m/z* 266), and *m/z* 193 (loss of C₂H₄O₂N from *m/z* 266).

Data for *Pseudo-nitzschia* spp. abundance throughout the SEUS region from 2001 to 2008 were provided by the Southeast Phytoplankton Monitoring Network (SEPMN) database. This program is based on volunteer field observations from various coastal sites along the SEUS (*n* = 143 sampling sites) and other U.S. states.

Table 1

DA concentration ranges in SEUS cetaceans relative to concentrations detected in large-scale marine mammal mortality events. Concentrations (median and range) are expressed in nanograms DA per gram of sample or milliliter of urine (ng/g or ng/mL).

Species	n	Urine	Feces	Gastric	Other*
<i>K. breviceps</i>	29	0.9 (0.4–1.8)	78 (12–13,566)	nd	nd
<i>K. sima</i>	12	1 (0.4–1.8)	65 (20–967)	nd	nd
<i>B. physalus</i>	1	nd	nd	nd	nd
<i>D. delphis</i>	4	nd	nd	nd	–
<i>E. glacialis</i>	1	–	–	nd	–
<i>F. attenuata</i>	3	nd	nd	–	–
<i>L. acutus</i>	1	nd	–	–	–
<i>M. densirostris</i>	2	nd	–	–	–
<i>M. novaeangliae</i>	4	nd	nd	nd	–
<i>P. electra</i>	3	nd	nd	nd	–
<i>P. phocoena</i>	1	nd	nd	nd	–
<i>Stenella sp.</i>	1	nd	nd	nd	–
<i>T. truncatus</i>	18	nd	nd	nd	nd
<i>Z. cavirostris</i>	1	–	nd	nd	–
California UMEs**	39	2160 (2–75,750)	5200 (8–324,000)		

* Other: liver, kidney, serum, brain, coelomic fluid, amniotic fluid, or milk.

** California UMEs: data from pinniped and cetacean unusual mortality events (UME) occurring in California (data from Gulland, 2000; Lefebvre et al., 1999; Scholin et al., 2000; NOAA unpublished data). nd: not detected, (–): no test made/no sample available.

When high abundances of known toxin-producing phytoplankton were observed, whole water samples were collected for subsequent cell enumeration and toxin determination.

Selected *Kogia* fecal samples ($n = 8$) 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). Samples were rinsed with distilled water and added to an equal volume of KMnO_4 for 24 h. After 24 h, an equal volume of HCl was added and the solution was heated until the sample color changed from purple to clear. 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 (10–100%) and a series of hexamethyldisilazane (HMDS) (25–100%) 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.

3. Results

A total of 41 *Kogia* and 40 other cetaceans were investigated in this study. Of the *Kogia* analyzed for this study, 59% (24 of 41) of the individuals sampled tested positive for DA by LC–MS/MS in at least one sample type (Table 1). DA was detected in 87% of fecal samples (20 of 23), and concentrations in DA-positive samples ranged from 12–13,566 ng/g (mean = 825 ng/g). The maximum value of 13,566 ng/g was an anomalously high DA concentration relative to the rest of the fecal samples (Table 1), and excluding this value, the concentration range for DA-positive samples was 12–967 ng/g (mean = 154 ng/g). DA was detected in 24% of urine samples (8 of 34). Quantifiable concentrations in DA-positive samples ranged from 0.4–1.8 ng/mL (mean = 1.4 ng/mL). DA was not detected in any of the gastric ($n = 5$) or other sample types analyzed ($n = 12$; liver, serum, kidney, brain, coelomic fluid, amniotic fluid, milk).

The proportion of DA-positive individuals for each of the two *Kogia* species was very similar, with DA being detected in 59% of *K. breviceps* (17 of 29) and in 58% of *K. sima* (7 of 12). When grouped

by stranding location (U.S. state), the proportion of DA-positive animals was highest for Florida (75%, 6 of 8), followed by North Carolina (63%, 10 of 16), Virginia (50%, 2 of 4) and South Carolina (46%, 6 of 13) (Fig. 1). Nearly all months in which *Kogia* were collected had at least one DA-positive individual, and all *Kogia* strandings occurring in spring (March–May) and mid-autumn/early winter (October–December) were positive for DA (Fig. 2).

LC–MS/MS analyses were also performed on samples recovered from the 11 other cetacean species described above. Stranding locations for other cetaceans closely approximated the geographical range of available *Kogia* sampling sites (Fig. 3). DA was not detected in any of the feces ($n = 20$), urine ($n = 28$), gastric ($n = 19$) or other sample types ($n = 7$) collected from other cetaceans ($n = 40$) included in this study (Table 1). The limit of detection for this method was generally 0.2 ng/mL for urine samples and 1.0 ng/g for feces, gastric and tissue samples.

Selected *Kogia* feces samples ($n = 8$) found to be DA-positive by LC–MS/MS analysis were subsequently analyzed by SEM to identify remains of *Pseudo-nitzschia*. No frustules or frustule fragments were detected in any of the eight samples available for analysis.

Cell concentration data collected by SEPMN confirmed the presence of four blooms of *Pseudo-nitzschia* spp. in the SEUS region between 2001 and 2008 (Fig. 1). These blooms were restricted to South Carolina coastal waters (Myrtle Beach, SC; 11/29/01) and the Outer Banks region of North Carolina (Duck, NC; 5/4/05, 11/1/06 and 9/5/07). The blooms were short-lived (<1 week) and very localized, as bloom concentrations were only found on the initial detection date during weekly sampling and were not detected at adjacent sampling sites. Total *Pseudo-nitzschia* abundance for these four blooms ranged from 3500 to 7300 cells/mL, and relative abundance of *Pseudo-nitzschia* for each bloom was >80% of total observed cells. Of the four blooms, only the 2006 and 2007 blooms had detectable concentrations of DA in associated seawater and/or shellfish. The species present during the non-toxic 2001 and 2005 blooms was identified as *P. pseudodelicatissima*, while the toxic blooms in 2006 and 2007 were composed of *P. multiseriata*, *P. pungens* and *P. pseudodelicatissima*. LC–MS/MS analysis of seawater samples collected during the 2006 and 2007 blooms detected particulate DA at concentrations of 0.9 and 0.01 $\mu\text{g/L}$, respectively. Blue mussels collected during the 2006 bloom were also positive for DA by LC–MS/MS, at a concentration of 9.6 ng/g.

4. Discussion

This study provides the first reported evidence of DA exposure in marine mammals from the SEUS. In addition, this study indicates *Kogia* as species of particular interest in their ability to accumulate DA when other cetacean species recovered from this region apparently do not. The majority (59%) of *Kogia* collected between Virginia and Florida tested positive for DA in urine and/or feces, whereas other cetaceans with the same available sample types and that stranded in the same geographical range had no detectable DA. Feces appeared to be the best indicator of DA exposure in the *Kogia* since it had much higher DA concentrations relative to urine samples, which had values approaching the detection limit for LC–MS/MS. This is consistent with experimental dosing studies in rodents which show that nearly the entire oral DA dose is cleared via feces (Iverson et al., 1989, 1990). Although 59% of *Kogia* collected for this study tested positive for DA in at least one sample type, this may be an underestimate of the actual proportion of animals exposed, since feces samples were not available from all *Kogia* collected. Including only *Kogia* individuals with available fecal samples, the proportion of DA-positive animals increases to 87%. Although DA was detected in the *Kogia* feces over a broad concentration range (12–13,566 ng/g; median = 65 ng/g), these

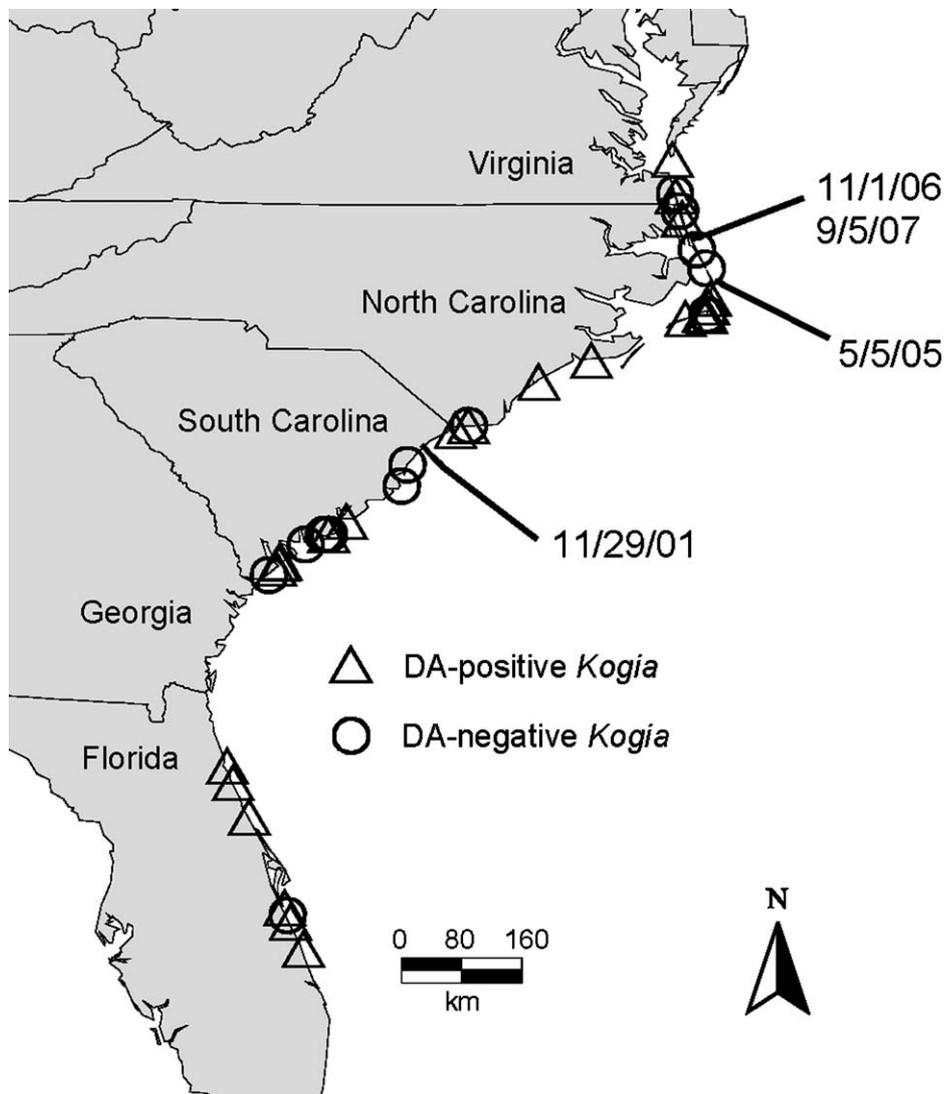


Fig. 1. *Kogia* stranding/sampling locations in the SEUS, between 1997 and 2008. Triangle markers: DA-positive animals, circles: DA-negative animals. Date markers indicate *Pseudo-nitzschia* blooms (>80% relative abundance) detected in the SEUS region between 2001 and 2008.

values were generally two orders of magnitude lower than those detected (8–324,000 ng/g; median = 5200 ng/g) during large-scale marine mammal epizootics associated with toxic *Pseudo-nitzschia* blooms (Gulland, 2000; Lefebvre et al., 1999; Scholin et al., 2000; NOAA Marine Biotoxins Program unpublished data) (Table 1). Using the DA levels from these epizootics as a point of reference, it

appears unlikely that the concentrations detected in *Kogia* feces correspond to acute DA exposure, however there is insufficient information to determine what associated health effects can be expected in these animals. Although most DA values in this study were much lower than those observed in west coast mortality events, one fecal sample (13,566 ng/g, 9/1/06) had DA levels that

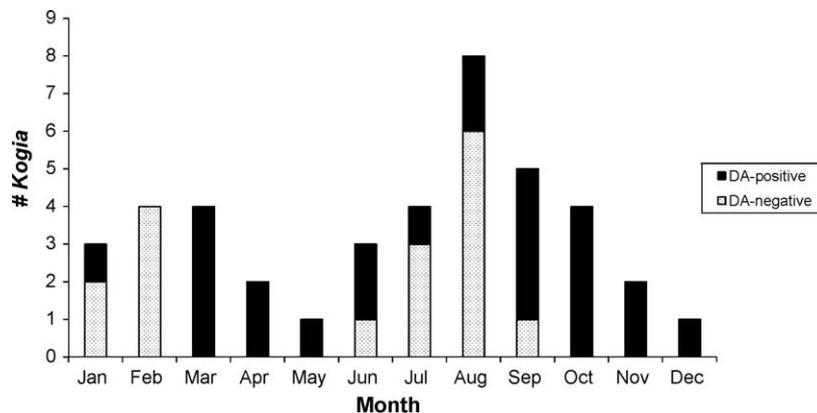


Fig. 2. Seasonal distribution of DA detected in sampled *Kogia*, grouped by month of stranding. Black bars: DA-positive animals, white bars: DA-negative animals.

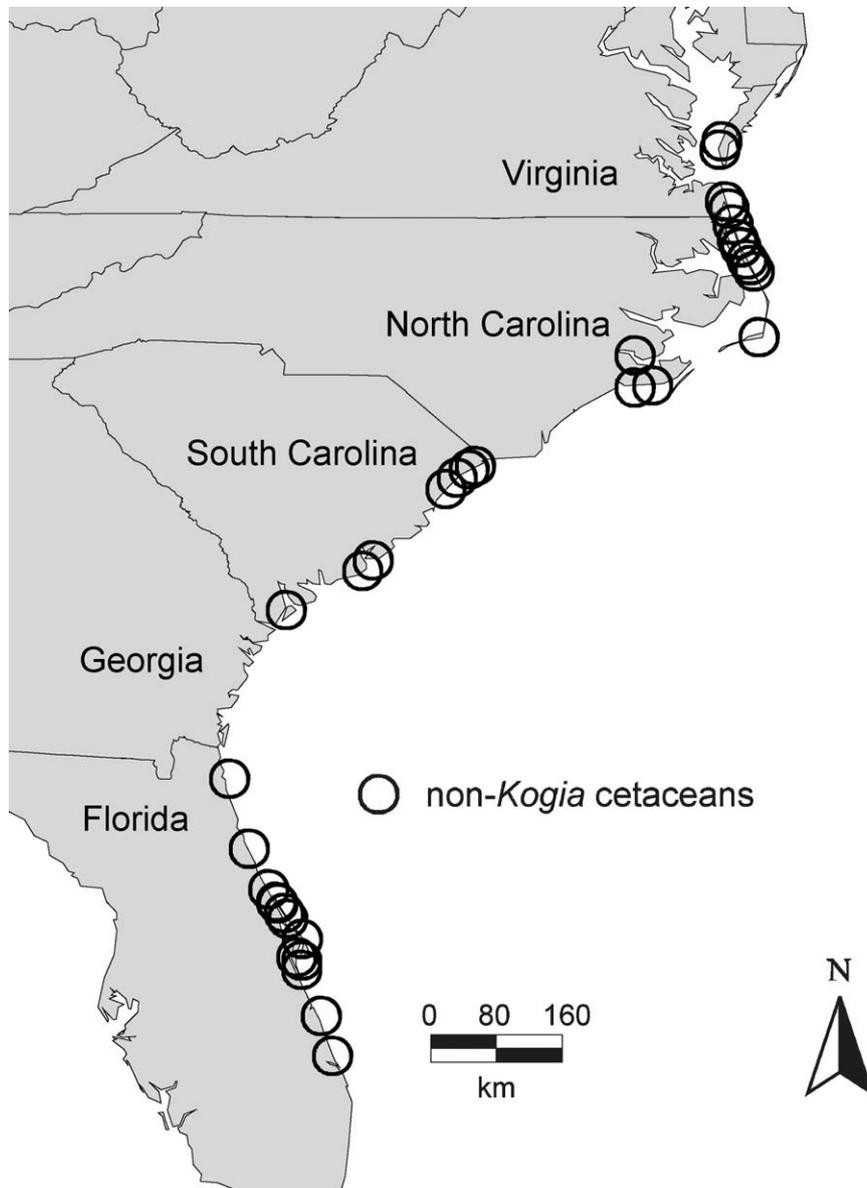


Fig. 3. Other cetacean stranding/sampling locations in the SEUS, between 2006 and 2008. Circles: DA-negative animals.

exceeded the median concentration reported for the mortality events cited above (Table 1), suggesting that DA can accumulate to potentially lethal levels in *Kogia*.

The prevalence of DA in these *Kogia* is an unexpected finding, since prior to November 2006 the SEUS had historically been a region free of DA-producing HABs. Although coastal *Pseudo-nitzschia* blooms were detected in the study region between 1997 and 2008, only the two blooms occurring in November 2006 and September 2007 had detectable levels of DA in associated seawater (up to 0.9 ng/mL) or shellfish samples (up to 9.6 ng/g) (T. Leighfield, unpublished). Though composed of the known DA producers *P. pungens*, *P. multiseriis* and *P. pseudodelicatissima* (Trainer et al., 1998; Douglas and Bates, 1992; Pan et al., 1996), the DA concentrations detected in the 2006 and 2007 blooms were very low in comparison to those detected in seawater and shellfish during toxic *Pseudo-nitzschia* blooms associated with cetacean mortality events (Anderson et al., 2006; Schnetzer et al., 2007; Langlois, 2007). However, regardless of the intensity of the detected blooms, neither the 2006 nor the 2007 DA-producing blooms in NC were spatially (within 100 miles) and temporally (i.e. within 1 month) associated with *Kogia* strandings in this study. The

only stranded animal (*T. truncatus*, 10/31/06) recovered in nearby NC waters during either bloom period was negative for DA by LC-MS/MS.

It is possible that the detection of DA in *Kogia* in the absence of observable blooms may be due to a lack of overlap of *Kogia* habitat with coastal phytoplankton monitoring sites. Although very little is known regarding the biology of *Kogia* in the SEUS, available literature indicates they are a pelagic species, with limited sighting data placing their main habitat offshore along the outer continental shelf and slope regions (Scott et al., 2001; Waring et al., 2006). No reported data exists for *Pseudo-nitzschia* abundance, distribution or species composition in these offshore regions, and until such data become available, a potential DA exposure source within the *Kogia* habitat remains unidentified. Gulf Stream-associated eddies have been demonstrated to stimulate highly productive, episodic upwelling events in offshore ocean waters such as the South Atlantic Bight (Bane et al., 2001; McGillicuddy et al., 2007), and if these phenomena occur within the pelagic *Kogia* habitat, they may provide a source of DA-producing blooms that affect the *Kogia* food web. However, many of the other cetacean individuals sampled in this study

(*F. attenuata*, *M. densirostris*, *P. electra*) are also poorly known species thought to occupy pelagic habitats (Waring et al., 2006), but were not positive for DA.

Another obstacle in explaining the presence of DA in these *Kogia* is the lack of an identifiable vector organism that facilitates trophic transfer of the toxin up the food web. In many of the frequent DA-related wildlife mortality events occurring where *Pseudo-nitzschia* blooms are common, DA has been shown to accumulate in very high concentrations in planktivorous organisms such as anchovies, sardines and krill, which then act as a toxin vector to apex predators (Scholin et al., 2000; Lefebvre et al., 2002a; Bargu et al., 2002). Analysis of *Kogia* prey items recovered from stranded animals suggest they feed mainly in the epi- and mesopelagic zones, consuming primarily mid- and deep-water squid species (McAlpine, 2002; Santos et al., 2006). Analysis of stomach contents indicates that 80% of squid species consumed by SEUS *Kogia* are associated with the productive areas of the deep continental shelf and/or slope (Candela, 1987). Although squid have been shown to accumulate DA in other regions (<0.5 µg/g in stomach and viscera; Bargu et al., 2008), these squid are not planktivorous and the DA concentrations reported are much lower than those from known DA vectors (>1800 µg/g in anchovy viscera, >40 µg/g in whole krill) (Lefebvre et al., 2002b; Bargu et al., 2002).

These *Kogia* feeding habits may also be a factor in the lack of detection of *Pseudo-nitzschia* frustules in feces samples collected for this study. SEM analysis of DA-positive cetaceans stranding during highly toxic *Pseudo-nitzschia* blooms frequently identifies frustules in feces from planktivorous baleen whales, while feces from small odontocetes stranding during the same bloom does not contain frustules (S. Morton, unpublished observation). Since *Kogia*, like many small odontocetes, occupy a trophic role of at least a tertiary consumer (Pauly et al., 1998), it seems unlikely that *Pseudo-nitzschia* frustules can survive across three trophic levels in a state identifiable by SEM.

Although DA exposure is associated with distinctive brain lesions and hippocampal atrophy in California sea lions (Gulland et al., 2002; Silvagni et al., 2005), it is unclear if these or similar lesions also occur in cetaceans due to morphological differences and small size of the hippocampus relative to other mammals (Manger, 2006). Due to the retrospective nature of the present study, brain tissue samples associated with these DA-positive *Kogia* were composed of tissue sets collected from various sites in the brain and examined by multiple pathologists, and as such may not be an appropriate diagnostic tool for DA exposure in these *Kogia* or other cetaceans (D. Rotstein, unpublished observations). A prospective study which specifically defines and standardizes criteria for DA-related lesions in cetaceans is necessary in order to determine if the DA levels in *Kogia* are associated with adverse health effects that manifest as identifiable lesions.

As a final note, it is interesting that *K. breviceps* and *K. sima* both have an enlarged section of ascending colon observed to contain a large volume (at least 12 L) of dark reddish fluid, the expulsion of which may be used as a behavioral defense mechanism similar to that of cephalopod ink (Caldwell and Caldwell, 1989; Scott and Cordaro, 1987). This enlarged colonic structure is unlike that found in any pelagic delphinid and may allow for sequestration of colonic contents for periods of up to 10 days (Manire et al., 2004; Marshall and Moss, 2006). These unique anatomical and behavioral adaptations may be a possible explanation for detection of DA in feces exclusively in *Kogia* along the SEUS, and warrants additional investigation.

Whatever the source of DA, exposure of *Kogia* to the toxin appears to be distributed across the entire geographical range of this study (Fig. 1), and across all seasons (Fig. 2). A more intensive sampling effort, both for *Kogia* and other cetacean strandings,

would be necessary to more accurately determine seasonal or spatial trends for DA exposure in these species. Nevertheless, the low DA concentrations detected in the majority of the *Kogia* throughout the 1997–2008 period suggests the potential for repeated or chronic DA exposure in areas not monitored for HAB activity. Data are insufficient to determine whether DA-producing blooms are new to the SEUS or if their detection is due to recent implementation of HAB monitoring networks. However, long-term, sublethal exposure to HAB toxins are a potential stressor to ecosystems and may have serious long-term implications for the health of marine mammal populations (Brodie et al., 2006; Leandro et al., unpublished).

5. Conclusions

This study demonstrates for the first time that marine mammals inhabiting SEUS waters are exposed to the harmful algal toxin domoic acid, at a frequency and magnitude indicative of year-round, chronic exposure. The detection of DA exclusively in the pygmy and dwarf sperm whale, *Kogia* spp., suggests a unique aspect of feeding behavior or habitat utilization as yet undiscovered, but important to the development of research on toxin trophic transfer in cetacean food webs. The significance of these findings in the context of an absence of associated *Pseudo-nitzschia* bloom activity suggests that DA exposure may take place in remote pelagic locations not monitored for HABs. Future research seeking to address food web dynamics of DA exposure to *Kogia* should focus on offshore sampling of phytoplankton species and abundance, as well as prey items consumed by *Kogia* in these regions. This knowledge will aid in establishing toxin exposure routes and evaluating potential impacts on pelagic cetacean populations.*****

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