

ASSOCIATION OF AN UNUSUAL MARINE MAMMAL MORTALITY EVENT WITH *PSEUDO-NITZSCHIA* SPP. BLOOMS ALONG THE SOUTHERN CALIFORNIA COASTLINE

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ABSTRACT: During 2002, 2,239 marine mammals stranded in southern California. This unusual marine mammal stranding event was clustered from April to June and consisted primarily of California sea lions (*Zalophus californianus*) and long-beaked common dolphins (*Delphinus capensis*) with severe neurologic signs. Intoxication with domoic acid (DA), a marine neurotoxin produced during seasonal blooms of *Pseudo-nitzschia* spp., was suspected. Definitively linking harmful algal blooms to large-scale marine mammal mortalities presents a substantial challenge, as does determining the geographic extent, species composition, and potential population impacts of marine mammal die-offs. For this reason, time series cross-correlation analysis was performed to test the temporal correlations of *Pseudo-nitzschia* blooms with strandings occurring along the southern California coastline. Temporal correlations were identified between strandings and blooms for California sea lions, long-beaked common dolphins, and short-beaked common dolphins (*Delphinus delphis*). Similar correlations were identified for bottlenose dolphins (*Tursiops truncatus*) and gray whales (*Eschrichtius robustus*), but small sample sizes for these species made associations more speculative. The timing of the blooms and strandings of marine mammals suggested that both inshore and offshore foraging species were affected and that marine biotoxin programs should include offshore monitoring sites. In addition, California sea lion-strandings appear to be a very sensitive indicator of DA in the marine environment, and their monitoring should be included in public health surveillance plans.

Key words: California sea lion, common dolphin, domoic acid toxicity, harmful algal blooms, marine mammal strandings, *Pseudo-nitzschia* spp.

INTRODUCTION

During the first 6 mo of 2002, marine mammal strandings appeared to significantly increase along the California coast. This increase was declared an “unusual mortality event” (UME) by the National Marine Fisheries Service (NMFS) and was initially believed to involve primarily California sea lions (*Zalophus californianus*) and long-beaked common dolphins (*Delphinus capensis*). Animals that were alive at the time of stranding exhibited clinical signs, such as seizures and head weaving, of severe neurologic disease. These observations were compatible with those previously reported for outbreaks of

domoic acid (DA) toxicity in California sea lions (Gulland et al., 2002); therefore, DA toxicity was the main suspected cause of death in this UME. Domoic acid toxicity varies among individuals, even within species, but clinical signs usually present acutely. Death does not always result, and time to death may also be prolonged; in humans and California sea lions, death may follow exposure by weeks to months (Teitelbaum et al., 1990; Goldstein et al., 2008). In 2002, stranded animals were in good body condition, suggesting an acute course of disease and the ability to forage until near the time of stranding.

Domoic acid is a naturally occurring marine neurotoxin produced during sea-

sonal blooms of nine species of the pennate diatom *Pseudo-nitzschia* (Bates, 2000; Hasle, 2002). *Pseudo-nitzschia* spp. can overgrow and produce DA in a wide range of environmental conditions. The quantity of toxin produced varies among strains of the same species of *Pseudo-nitzschia*, as well as from bloom to bloom (Bates et al., 1998; Mos, 2001). Domoic acid is a kainoid water-soluble amino acid that acts as an agonist of N-methyl-D-aspartate, α -amino-5-methyl-3-hydroxysoxazolone-4-propionate, and kainate glutamate receptors in the brain, resulting in massive depolarization of neurons, increase in intracellular calcium ions, energy depletion, cellular swelling, and cell death, primarily in the neurons of the hippocampus (Berman and Murray, 1997; Larm et al., 1997; Hampson and Manalo, 1998).

In the last two decades, DA toxicity has been linked to several outbreaks of neurologic disease, with morbidity and mortality occurring in people in Eastern Canada and in marine birds and California sea lions along the Pacific coast of California. Neurologic signs were observed in all events of intoxication and were commonly characterized by abnormal behavior, disorientation, ataxia, seizures, reproductive failure, coma, and death (Perl et al., 1990; Work et al., 1993; Sierra-Beltrán et al., 1997; Scholin et al., 2000; Gulland et al., 2002; Brodie et al., 2006). Domoic acid toxicity was also suspected during a UME of dolphin strandings in the Gulf of California during an identified *Pseudo-nitzschia australis* bloom; this has been the most frequently implicated DA-producing algal species associated with wildlife mortality (Ochoa et al., 1998; Bates, 2000). Mounting evidence strongly suggests that DA is transmitted through the marine food web (Work et al., 1993; Scholin et al., 2000). The main vectors for DA identified to date include several species of the planktivorous fish (e.g., sardines and anchovies), mollusks, and krill that are common prey items for many of the pinniped and

cetacean species that stranded in California during 2002. Domoic acid accumulates in these vector species after direct consumption of toxic *Pseudo-nitzschia* cells; therefore, the toxin may ultimately be consumed by several marine mammal species at high trophic levels (Pauly et al., 1998; Bargu et al., 2002; Lefebvre et al., 2002a; Lefebvre et al., 2002b).

Despite advances in DA identification in animal fluids and environmental samples, and the development of a well-trained and dedicated California Marine Mammal Stranding Network (CMMSN), definitively linking harmful algal blooms to large-scale marine mammal mortalities remains a challenge, as does determining the geographic extent, species composition, and potential population impacts. Obstacles to making these firm linkages include: variability in marine mammal monitoring and record keeping by the mostly volunteer CMMSN; funding limits for diagnostic testing; and the low-quality historic data for most marine mammal species, and for geographic regions, available for comparison. This investigation aimed to characterize epidemiologically the 2002 UME in pinnipeds and cetaceans and to evaluate the association between that UME and *Pseudo-nitzschia* spp. blooms in local waters. In order to better understand the species and demographic factors involved in that UME, a reference population was needed. In consultation with experts from the CMMSN and the US Working Group on Marine Mammal UMEs, data from marine mammal strandings in California in 1999 were considered for comparison. That data set was selected because: it occurred after 1998, when DA-related morbidity and mortality was recognized and diagnosed in marine mammals (Scholin et al., 2000); it documented a year when only rare DA-related strandings were observed in California sea lions (Gulland et al., 2002; Greig et al., 2005; Silvagni et al., 2005); and paper-based records on all observed strandings were available.

MATERIALS AND METHODS

Marine mammal stranding data collection

Marine mammal strandings in California are reported to the NMFS (US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Region, CMMSN database). The NMFS provided paper-based records of these "level A" stranding data for this investigation that consisted of species, sex, age class, and stranding date and location. Level A data are collected primarily by members of the CMMSN, the general public, and other agencies such as local police, fire, animal control departments, and city lifeguards. From live and dead stranded animals, additional information on clinical signs, and DA confirmatory test results from fecal, urine, stomach contents, and amniotic fluid samples using the receptor binding assay (Van Dolah et al., 1997), were provided sporadically by some of the rehabilitation centers participating in the CMMSN. Unfortunately, these tests were not consistently performed and were most commonly conducted only for the first few marine mammals stranding in a temporal cluster. Once DA was identified in fluids, feces, or stomach contents, the rest of the marine mammal strandings were usually considered to be due to DA toxicity, unless physical examinations provided evidence to suggest another cause of stranding. Extreme variability in carcass age and quality also limited the CMMSN's ability to make specific diagnoses for most of the stranded animals. For these reasons, our investigation became focused on epidemiologic and demographic analyses using reference data from 1999, when DA strandings were rare.

For all animals, sex of affected individuals was determined, when possible, by visual examination of genital morphology and dimorphic characteristics. Animals were categorized into age classes (pup-calf, yearling, subadult, and adult) according to species-specific growth curves based on sex, body length, weight, development of teeth, and color of skin or pelage, and other external characteristics (Geraci and Lounsbury, 1993). When available, latitude and longitude points for strandings were reported by members of the CMMSN. Descriptive information on location, and attributes of strandings such as county, city, and beach details, were used to estimate latitude and longitude points when these data were not provided.

Initially, strandings occurring throughout the entire California coastline were examined. Because the majority (80%) of 2002 strandings

occurred in southern California, episodic peaks in strandings were not noted in northern California, and because anecdotal reports of clinical signs and DA testing were consistent with DA toxicity in southern California, our analyses for the UME were focused on southern California. This study examined 2,965 records of strandings along the coastline in southern California (San Luis Obispo to San Diego counties; Fig. 1) occurring between 1 January and 31 December of 1999 and 2002. San Simeon (35°39'N, 121°11'W) was used as a geographic cutoff for the southern California area. To compare the expected level of marine mammal strandings with the level observed in 2002, data were also obtained for strandings in 1999, a year which was perceived to have had a background or expected level of strandings as determined by experts from the CMMSN and the US Working Group on Marine Mammal UMEs. This reference year was selected because: monitoring for DA toxicity and the related clinical syndrome, which began in 1998, revealed only rare cases in 1999 (Gulland et al., 2002; Greig et al., 2005; Silvagni et al., 2005); there were no large peaks in strandings throughout 1999 that would indicate an associated outbreak (Fig. 2); and high-quality data were available from NMFS as a result of maturation of the CMMSN. Data on southern sea otters (*Enhydra lutris nereis*) were excluded from our analyses because a detailed investigation into causes of strandings in this species was already underway (Kreuder et al., 2005), and there was very little range overlap with our southern California focus. Data were assembled into a relational database (Microsoft Access, Microsoft Corporation, Redmond, Washington, USA) to facilitate data analyses.

Pseudo-nitzschia spp. bloom data collection

The presence of *Pseudo-nitzschia* spp. blooms was used as an indicator for presence of DA. Data on phytoplankton distribution along the California coastline were collected by the California Department of Health Services (DHS) through its ongoing Marine Biotxin Monitoring Program (Technical Report No. 03-14), specifically by the volunteer phytoplankton monitoring component coordinated by DHS. Records containing data on sampling dates, sampling site descriptions, latitude, and longitude were used in the analysis. Records also contained data on the proportion of the total phytoplankton in the sample identified as *Pseudo-nitzschia* spp. based on: cell counts (percent composition), recognizing that not all species in this genus produce DA; *Pseudo-nitzschia* spp. productiv-

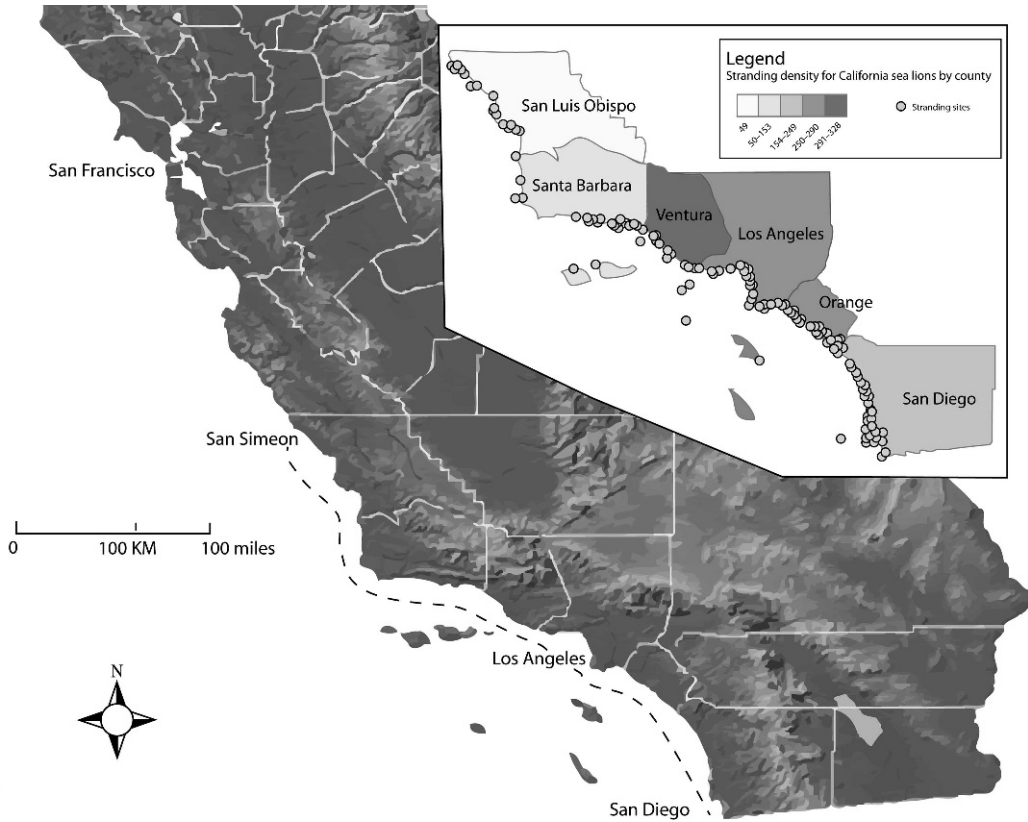


FIGURE 1. Map of central and southern California, USA, showing the focus area of study (dashed line) due to frequent strandings of marine mammals from San Luis Obispo to San Diego counties in 2002. Inset: stranding densities, by county, for California sea lions.

ity estimated as the volume of settled cell mass in each sample (settled volume); and sampling effort or total tow length, which was determined by the depth of tow and the number of vertical hauls.

Most plankton tows were conducted from piers or docks and involved several vertical hauls at a minimum depth of 3 m. Occasional samples were also collected by boat from various offshore locations at depths up to 9 m. Sampling frequency varied by location, ranging from once per week at key locations to once or twice per month at supplemental locations. Sampling frequency increased at different sites based on the observation of toxigenic species in the region, thereby improving the ability to track a potential toxic bloom (for programmatic and sampling details see: <http://www.dhs.ca.gov/ps/ddwem/environmental/Shellfish/>).

All phytoplankton samples received by DHS were examined qualitatively by light microscopy for the presence of toxigenic species. The occurrence of all phytoplankton genera iden-

tified in the sample was recorded, along with a semiquantitative estimate of the percent composition and settled volume. After examination of the sample, the settled volume estimate was adjusted by subtracting the volumetric contribution of inorganic matter (e.g., detritus, sand). A relative abundance index (RAI; Langlois, 2004) was derived for net tow samples for *Pseudo-nitzschia* spp. as a function of percent composition (PC), settled volume (SV ; ml), and sampling effort (SE ; ft):

$$RAI = \frac{(PC \times SV)}{SE}$$

The semiquantitative RAI standardizes the species composition data to account for differences in sampling effort and cell mass.

Statistical analyses

The chi-square (χ^2) test for independence was used to determine significant differences

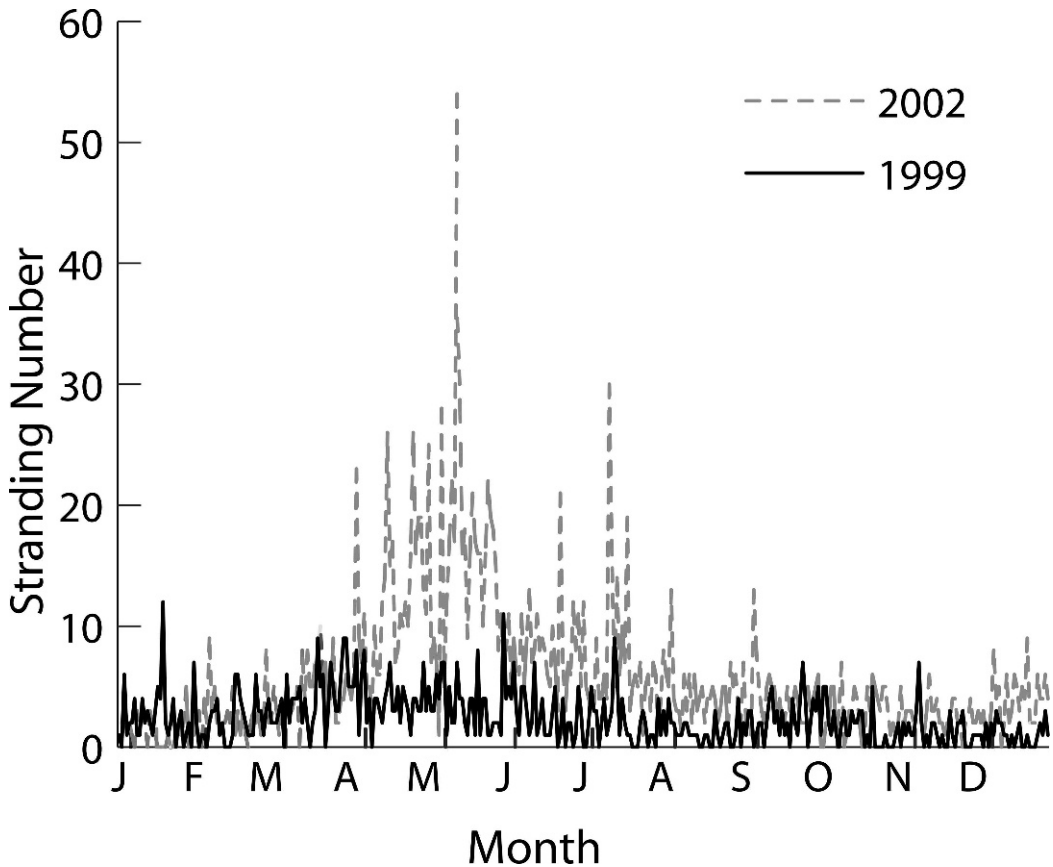


FIGURE 2. Number of daily marine mammal strandings in southern California during 1999 and 2002.

in the number of strandings between 1999 and 2002 for species in southern California that stranded during both years. For species with higher stranding numbers in 2002 vs. 1999, and with a high proportion of known sex and age classes, the distribution of sex and age classes were also compared using the chi-square test of independence or Fisher's exact test when expected cell frequencies were less than five. Differences were determined to be significant using a two-tailed test at $P < 0.05$ (MINITAB 14, Minitab Inc., State College, Pennsylvania, USA).

For marine mammal species having more than five strandings in southern California during 2002, temporal associations between date of stranding and *Pseudo-nitzschia* spp. RAI for the southern California coastline were evaluated using time series cross-correlation analysis (Box and Jenkins, 1976; Statistica 5.5, StatSoft Inc., Tulsa, Oklahoma, USA). Although strandings occurred on nearly a daily basis, the available data for *Pseudo-nitzschia* blooms were most consistent on biweekly time periods.

Therefore, the number of strandings for all species, and for each individual species, were summed for each 2-wk time period during 2002 across the southern California coastline. The median RAI of *Pseudo-nitzschia* was estimated for all sample locations in southern California for 2-wk time periods. All data from each sample site were included. Temporal associations were evaluated independently for each species by calculating cross-correlation coefficients (R_{xy}) for the number of strandings and the median *Pseudo-nitzschia* spp. RAI in each 2-wk interval. Cross-correlation coefficients were evaluated at eight forward and eight backward 2-wk time lags and were considered to be significant at $P < 0.05$.

RESULTS

A total of 1,237 marine mammal strandings (excluding southern sea otters) were observed along the entire California coastline in 1999, and 2,796 strandings were

observed in 2002, greater than double those strandings which occurred during 1999. The differences in southern California were even more marked, with 2,239 of the strandings (80%) in 2002 having occurred in the south, in comparison with only 726 (59%) in 1999 in the same area. California sea lions stranded more frequently than any other species (73%). In fact, southern California sea lion strandings (1,634) increased more than fourfold in 2002 compared to 1999 (358), but strandings increased only slightly for harbor seals (*Phoca vitulina*; 72 in 1999 compared to 97 in 2002) and actually decreased for northern elephant seals (*Mirounga angustirostris*; 112 in 1999 compared to 102 in 2002) and gray whales (*Eschrichtius robustus*; 22 in 1999 compared to 6 in 2002). The number of strandings in southern California increased over twofold for some other marine mammal species including long-beaked common dolphins (17 in 1999 compared to 49 in 2002), short-beaked common dolphins (*Delphinus delphis*; seven in 1999 compared to 16 in 2002), and bottlenose dolphins (*Tursiops truncatus*; three in 1999 compared to nine in 2002). Additionally, marine mammal species that are only very rarely observed on beaches also stranded in southern California in 2002, including five Cuvier's beaked whales (*Ziphius cavirostris*), four Risso's dolphins (*Grampus griseus*), and one humpback whale (*Megaptera novaeangliae*). As described above, DA testing was conducted for only a small subset of individual stranded animals. However, DA was confirmed (National Oceanic and Atmospheric Administration's Marine Biotoxins Program, Charleston, South Carolina, USA) by mass spectrometry with liquid chromatographic separation in stomach contents, feces, and urine from 11 out of 11 California sea lions and 23 of 26 common dolphins tested, as well as in one individual each of Risso's dolphin, Cuvier's beaked whale, gray whale, and humpback whale.

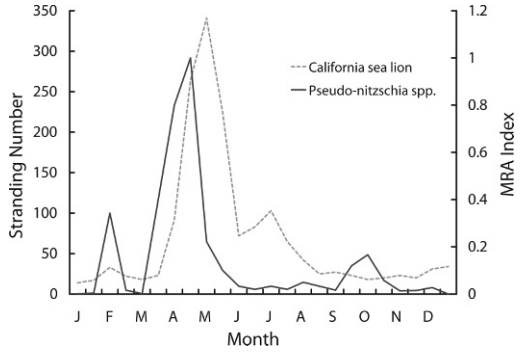


FIGURE 3. Number of monthly California sea lion (CSL) strandings and median *Pseudo-nitzschia* spp. bloom relative abundance (MRA) index in southern California in 2002.

Stranding frequencies for all pinniped and cetacean species in 2002 were significantly higher than in 1999 ($\chi^2=8.642$, $P<0.01$). Notable exceptions to this general trend were gray whales, harbor seals, and northern elephant seals. In 2002, most of the stranded California sea lions were female (78%), compared to the more-equal sex proportions observed in 1999 (51%; $\chi^2=61.90$, $P<0.01$). The proportion of adult California sea lions (60%) that stranded in 2002 was significantly higher than the proportion of adults (30%) that stranded in 1999 ($\chi^2=52.13$, $P<0.01$). In 2002, most common dolphins that stranded were male (83%), significantly higher than the proportion of males (48%) that stranded in 1999 ($\chi^2=11.98$, $P<0.01$). Pups were the most common age class represented for northern elephant seals (85%) and harbor seals (79%) in 2002. Age class information for other marine mammals was not sufficient to allow comparisons.

Figure 2 shows the daily distribution of stranding frequencies of marine mammals in southern California for 1999 and 2002. In 1999, there was very little monthly variation in contrast to 2002, when a substantial increase in strandings was detected from April to June. The highest number of strandings in southern California (721) occurred during May, approximately 12 times the average monthly

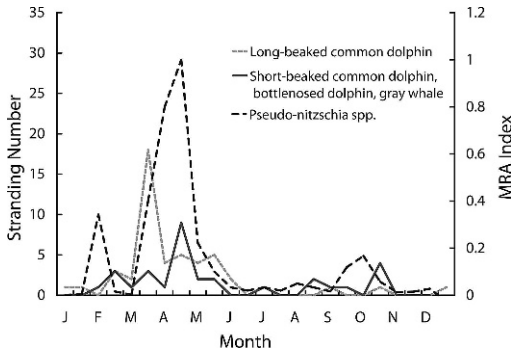


FIGURE 4. Number of monthly long-beaked common dolphin (LBCD), short-beaked common dolphin (SBCD), bottlenose dolphin (BD), and gray whale (GW) strandings and median *Pseudo-nitzschia* spp. bloom relative abundance (MRA) index in southern California in 2002.

stranding level in 1999. Figures 3 and 4 show that the temporal distribution of strandings in southern California for sea lions, long-beaked common dolphins, short-beaked common dolphins, bottlenose dolphins, and gray whales corresponds to the peak *Pseudo-nitzschia* spp. blooms.

A summary of the significant time series correlations found at different bloom time lags, for marine mammal species that stranded more frequently than expected in southern California compared with 1999 numbers, is presented in Table 1. Overall, the increase in marine mammal

strandings in southern California during 2002 was temporally correlated with an increase in *Pseudo-nitzschia* spp. blooms. In fact, marine mammal strandings were significantly correlated with high median *Pseudo-nitzschia* spp. RAI values in real time and for 6 wk following the peak in *Pseudo-nitzschia* relative abundance. California sea lions showed a similar correlation pattern with blooms to that for all strandings combined. All species that stranded in the area had significant correlations at multiple 2-wk time periods, except for gray whales and bottlenose dolphins, which showed significant correlations for only one 2-wk time period.

Despite small sample sizes for gray whales and bottlenose dolphins, all of the examined species-specific temporal stranding patterns were tightly grouped around the *Pseudo-nitzschia*-bloom peak, with the highest correlation values occurring between 2 wk prior and 2 wk after the highest levels of *Pseudonitzschia* spp. median RAI (Table 1). California sea lion and gray whale strandings were highly correlated with algal blooms for 2 wk after the peak in *Pseudonitzschia* spp. median RAI. Long-beaked common dolphin were most highly correlated at 4 wk prior to peak *Pseudo-nitzschia* spp. bloom activity in coastal waters. Short-beaked common dolphin and bottlenose dolphin strandings

TABLE 1. Significant 2-wk time periods of *Pseudo-nitzschia* spp. bloom values (stippled) and cross-correlation coefficients for the most significant bloom time lag (solid black) with marine mammal species that stranded in southern California during 2002.

Species	n	<i>Pseudo-nitzschia</i> blooms two-week time lag range						R _{xy}	P ≤
		backward time lags			forward time lags				
		-6	-4	-2	0	2	4		
All strandings	2239				■	■	■	0.879	0.01
California sea lion	1634				■	■	■	0.871	0.01
Long-beaked common dolphin	49	■	■	■	■			0.748	0.01
Short-beaked common dolphin	16	■		■	■	■		0.557	0.04
Gray whale	6					■		0.488	0.01
Bottlenose dolphin	9				■			0.359	0.04

had the most significant correlations at the time that the *Pseudo-nitzschia* spp. bloom activity was highest in coastal samples.

DISCUSSION

Marine mammal strandings in California increased considerably during 2002 compared to 1999. California sea lions were the primary species affected, and strandings increased in southern California in 2002 more than threefold above the expected baseline. The sudden increase in California sea lion strandings in 2002 was similar to increases in strandings of this species observed during 1998 and 2000 in San Luis Obispo County, strandings that have been attributed to DA toxicity (Greig et al., 2005). The significant temporal correlations between marine mammal strandings and *Pseudo-nitzschia* spp. blooms shown here are highly suggestive of DA toxicity having a role in the marine mammal UME in 2002. Confirmation of DA toxicity for each stranding requires histologic and toxicologic examination of animals in freshly dead condition; this was not logistically possible for the mostly volunteer CMMSN. Furthermore, DA is rapidly excreted in urine, and toxicity may not produce definitive histopathologic lesions; therefore, in most cases diagnostic confirmation of DA toxicity was not possible.

The large number of California sea lion strandings, and their spatial and temporal clustering, suggests that this very-abundant marine mammal species is highly susceptible to the acute effects of DA exposure (Gulland et al., 2002). Although long-beaked common dolphin, short-beaked common dolphin, and bottlenose dolphin strandings were less frequent, the number of strandings in 2002 was more than double the expected number estimated by strandings in 1999. Relatively few numbers of other cetacean species regularly strand in southern California, and these species stranded relatively infrequently in 2002. However, DA was

detected in one individual each of Cuvier's beaked whale, Risso's dolphin, gray whale, and humpback whale. Dying and dead individuals of these species appear to be less likely to become beachcast along the California coast, so from our limited stranding data, it is difficult to determine how susceptible these species are to DA toxicity. Sample sizes for these species were not large enough to examine demographic stranding patterns and temporal correlations with *Pseudo-nitzschia* spp. bloom activity, but these individuals stranded from January to March before the largest peak in *Pseudo-nitzschia* spp. was detected in nearshore waters. Detection of DA in these individuals may be an incidental finding, but these positive DA test results indicate that exposure to DA can occur in species that do not commonly inhabit and forage in the nearshore areas where blooms are most easily detected. Cuvier's beaked whales and Risso's dolphins have relatively similar natural histories; they forage in pelagic deep waters, mainly on cephalopods (Foster and Hare, 1990; Clarke, 1996; Wuertz et al., 1992; Pauly et al., 1998). In contrast, gray whales feed mostly on benthic invertebrates but will occasionally feed on krill and sardines (Pauly et al., 1998). Gray whales travel along the California coast on their migratory route between Mexico and the Bering Sea (from January to May); foraging along this migration is believed to be minimal (Bowen and Siniff, 1999). The migratory humpback whale feeds mainly on krill and occasionally on planktivorous fish (Pauly et al., 1998; Gendron, 1993). Although the humpback is thought not to feed during its migration, there are reports of feeding activity near the Baja California breeding grounds (Gendron, 1993). Little is known of the bioaccumulation of DA in many of these prey species, but *Pseudo-nitzschia australis* frustules have been detected in krill (Bargu et al., 2002) and in fecal samples from humpback whales that were observed feeding on DA-contaminated krill (Lefebvre et al., 2002a).

Evaluation of differences in stranding demographics allowed acute DA toxicity to be ruled out as a major cause of stranding for some species. Pups and yearlings were the predominant age classes observed in stranded phocid seals during 2002. This stranding pattern, and the numbers of individuals stranding, were consistent with those of previous years (Colegrove et al., 2005). The annual peak in harbor seal and northern elephant seal strandings occur after pupping (March to June) and weaning (February to March). Malnutrition has been identified as the most common cause of phocid seal pup strandings along the California coastline (Colegrove et al., 2005), and our data match the typical stranding data for these species. It is also unlikely that stranded preweaned pups were foraging during the peak *Pseudo-nitzschia* bloom activity; therefore, recent exposure to DA would have been unlikely.

California sea lion strandings in 2002 were mostly adult females; this is consistent with previous reports of DA toxicity in this species (Scholin et al., 2000; Gulland et al., 2002; Greig et al., 2005; Brodie et al., 2006) and contrasts with the demographics of sea lions stranding during epizootics of other types. Leptospirosis typically affects juvenile male sea lions in central and northern California during the fall (Gulland et al., 1996; Lloyd-Smith et al., 2007), and malnutrition associated with El Niño events typically affects yearlings (Greig et al., 2005). Species-specific ecologic and demographic factors may influence the distinct sex and age composition of stranded animals observed in association with DA toxicity. It is likely that more California sea lion females strand due to DA toxicity because males are not usually foraging in southern California during the *Pseudo-nitzschia* bloom season identified here, as it is the breeding season from May to July (Wells et al., 1999). In contrast, the majority of common dolphins that stranded with neurologic signs were adult males. The

common dolphin is a social and migratory species with demographically specific groups, including adult male bachelor pods, whose presence in the southern California region may also be seasonal. These bachelor pods may inhabit different areas than females; they may forage in higher-risk areas and preferably target known vector species as compared to females.

Strandings of California sea lions, short-beaked common dolphins, and bottlenose dolphins were highly correlated with *Pseudo-nitzschia* blooms and tended to occur at the same time, or soon after, the peak bloom detection. It is possible that a clustered outbreak due to another cause occurred at the exact same time as the peak bloom activity, resulting in a spurious association; however, no other viable hypothesis for this multispecies event has been identified. Strandings for these species persisted after the time of bloom occurrence; this is consistent with DA ecology, persistence in the food web, and trophic transfer. Sardines, anchovies, and other filter feeders that are known vectors of DA are among the California sea lion's preferred prey species near the Channel Islands (Lowry et al., 1990; Lowry et al., 1991). The relatively small number of individuals of bottlenose dolphins stranding during *Pseudo-nitzschia* bloom activity may reduce confidence that DA was involved, but may also give insight into the ecology of DA toxicity. Bottlenose dolphins tend to target less-efficient vector prey, feeding mainly on sciaenid, scombrid, and mugilid fish as well as cephalopods, and may therefore have less opportunity for exposure (Clarke, 1996; Walker et al., 1999; Gannon and Waples, 2004). Fewer short-beaked common dolphin than long-beaked common dolphin strandings were observed; their diets are thought to be similar and primarily consist of squid and anchovies, the latter being one of the better-known DA vector species (Evans, 1994). It is likely that relatively fewer short-beaked common

dolphins were observed due to their location at the time of greatest bloom activity. For species such as long-beaked common dolphins that occur slightly offshore, the strongest correlation of strandings with blooms actually preceded the time of peak bloom detection. Monitoring for bloom activity and DA presence in California at the time of this outbreak was conducted almost exclusively near the shore. It is possible that this species may have come into contact with offshore blooms before detection with the algal bloom monitoring programs was possible inshore. In response to this hypothesis, DHS' Marine Biotoxin Monitoring Program added offshore sampling sites in 2005 and have since detected blooms at these sites prior to detecting *Pseudo-nitzschia* at inshore sites (Langlois, 2005).

The highest numbers of strandings for California sea lions, long-beaked common dolphins, short-beaked common dolphins, and bottlenose dolphins along the southern California coast in 2002 were temporally associated with bloom activity that occurred mostly in the Ventura, Los Angeles, and Orange county areas (Fig. 1 inset). After the first detection of blooms in the southern California region, variably high concentrations of DA were also detected in shellfish sampling stations, specifically along the coast of Ventura and Los Angeles counties (Langlois, 2002). Domoic acid levels in shellfish increased during short periods in 2002; extremely high levels were detected briefly in January and, by late February, levels exceeded 100 ppm. The highest DA concentrations recorded to date were detected in April and persisted through June, when DA levels of up to 380 ppm were detected, 19 times greater than the federal alert level of 20 ppm (Langlois, 2002). It is unclear as to why *Pseudo-nitzschia* blooms may be more toxic or more frequent in this geographic area, but marine mammals may be at highest risk for DA toxicity near Ventura and Los Angeles counties—if blooms follow similar

geographic and temporal patterns in the future. Anthropogenic factors present in southern California such as coastal development, pollution by agricultural and industrial discharges, and overfishing have been linked to deterioration of coastal ecosystems by increasing the nutrient composition, thereby depleting these areas of species capable of top-down control of community structure, leading to eutrophication, hypoxia, and outbreaks of toxic algal blooms (Epstein and Rappaport, 1996; Jackson et al., 2001). However, the temporal patterns—most specifically the mortality of offshore foraging species just prior to the detection of blooms in the near shore environment—make these land-based causal theories less likely. Inclusion of offshore marine diatom and toxin monitoring sites should provide useful data for future evaluation of harmful algal bloom causes.

Inferences made from these results are limited by the surveillance capabilities for strandings and bloom activity in southern California during this UME, as stranding data originated from varied sources, each with different capabilities in surveillance and resources for diagnostics. Therefore, data quality varied greatly among regions. Some regions of the coastline are not intensely monitored because access to these beaches is extremely difficult. Additionally, it was not possible to perform more-detailed statistical analyses because confirmed diagnosis for the presence of DA in samples of blood and body excretions, using receptor binding assay, was not available for the majority of stranded animals, primarily due to economic and logistic constraints. In general, data generated by *Pseudo-nitzschia* spp. bloom surveillance are very patchy. Sampling effort capabilities for bloom detection varied by region and the amount of resources available. Blooms may differ in size, duration, and DA production. These factors are not measured with the current surveillance system, which is mostly qualitative. Due to economic constraints,

shellfish are monitored for presence of DA only after a meaningful increase in *Pseudo-nitzschia* spp. relative abundance is detected along the coastline. Therefore, it was not possible to use the data collected directly for DA levels in coastal waters or even prey species.

Multiple marine mammal species were reported to be involved in the UME during this investigation. It is possible that some of these species were affected by DA for the first time, but it is equally likely that surveillance has improved for both the causes of marine mammal mortalities and for harmful algal blooms. Time-series analysis proved to be a useful tool for evaluating linkages between marine mammal strandings and *Pseudo-nitzschia* blooms and should be included in a comprehensive outbreak response plan, along with systematic sampling of tissues and fluids for marine biotoxins and histopathology. In order to be most effective and efficient, plans for spatially and temporally specific data collection should be put into place prior to emergency events.

Our investigation has led us to conclude that California sea lion strandings, with clinical signs of DA toxicity, may be a sensitive indicator of the presence of DA-producing *Pseudo-nitzschia* spp., or of the toxin itself, in coastal California waters: They are a highly abundant species; they have demonstrated consistent acute clinical signs; and the likelihood of observing a California sea lion stranding is very high (Scholin et al., 2000; Gulland et al., 2002). This species is thus an important sentinel for ecosystem health assessments, and their strandings may be used as a trigger for more intensive public health monitoring for biotoxins. Constant DA surveillance, including California sea lion strandings, is recommended not only to address public health concerns but to evaluate options for coastal and marine resources management that might mitigate future impacts of harmful algal blooms on marine animal populations.

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