

Complexity of domoic acid-related sea lion strandings in Monterey Bay, California: foraging patterns, climate events, and toxic blooms

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ABSTRACT: The neurotoxin domoic acid (DA) produced by the diatoms *Pseudo-nitzschia* has been responsible for deaths of marine mammals and birds in Monterey Bay, California, USA. In this study we examined links between DA-related strandings of the seasonally migratory California sea lion *Zalophus californianus* and regional occurrences of DA-producing diatoms using a decade-long time series. Results suggest a more complex pattern of stranding than anticipated, one not related simply to regional abundance of the toxin producers. Stranding patterns of sea lions exhibiting signs of acute DA toxicosis may be best explained by multiple causative factors including timing of toxic blooms with respect to the sea lion breeding cycle, adequacy of sea lion prey during the breeding season, as well as the geographic range of this pinniped outside of the breeding season. Three DA-related stranding events occurred in Monterey Bay in 1998, 2000 and 2007, when toxic DA blooms were present in the Bay and the California coast was experiencing El Niño/Southern Oscillation conditions. Foraging centers near the breeding site likely provided inadequate food for individuals, leading to northerly movement of these highly mobile predators to other geographic sites resulting in exposure to toxic DA-producing blooms. High toxic *Pseudo-nitzschia* and DA concentrations did not, however, result in DA-related stranding events in 2002, 2003 and 2004, when weaker than normal upwelling events were present in the Bay. Relative productivity of central versus southern California with respect to the breeding season thus appears to strongly influence the frequency of DA-related strandings in Monterey Bay, California.

KEY WORDS: California sea lion · Harmful algal blooms · Animal stranding · Foraging behavior · Domoic acid · *Pseudo-nitzschia* · ENSO · Monterey Bay

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INTRODUCTION

Domoic acid (DA) was first recognized on the west coast of the United States in 1991, when more than 200 brown pelicans *Pelicanus occidentalis* and Brandt's cormorants *Phalacrocorax penicillatus* were found dead on beaches in Monterey Bay, California, after consuming tainted planktivorous fish (Fritz et al. 1992,

Work et al. 1993). Subsequently, blooms of the planktonic diatom genus *Pseudo-nitzschia*, the DA source, were implicated as causal agents of deaths of marine mammals, especially California sea lions (CSL) *Zalophus californianus* along the central California coast (Scholin et al. 2000). More recently, toxic *Pseudo-nitzschia* blooms have been increasingly observed in southern California coastal waters, with strandings of

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sea lions reaching or exceeding numbers found earlier in Monterey Bay (Schnetzler et al. 2007).

DA is introduced via the food web to marine mammals and birds through a variety of intermediaries, including both pelagic and benthic organisms that feed on toxin-producing *Pseudo-nitzschia*. *P. australis* has been the primary DA source in California DA poisoning events. Schooling planktivorous fish, commonly sardines *Sardinops sagax* and anchovies *Engraulis mordax*, are the most likely vectors (Lefebvre et al. 2002a,b, Schnetzler et al. 2007). Other key toxin vectors in the Bay's coastal food web have also been identified, including several species of swarm-forming herbivorous krill (mainly *Euphausia pacifica* and *Thysanoessa spinifera*), market squid *Loligo opalescens*, and benthic invertebrates (Bargu et al. 2002, 2008, Kvittek et al. 2008). DA contamination of blue *Balaenoptera musculus* and humpback *Megaptera novaeangliae* whales in California was traced to krill feeding on toxic *Pseudo-nitzschia* at sites where these baleen whales were foraging (Lefebvre et al. 2002b).

Diatoms in the genus *Pseudo-nitzschia* have been common members of the phytoplankton community in coastal waters off central and southern California since the early 1900s, though the association of *Pseudo-nitzschia* blooms with animal deaths is relatively recent (Fryxell et al. 1997). Prior to the 1990s, the *Pseudo-nitzschia* species, including those now recognized as toxin producers, were often assigned to species in the genus *Nitzschia*, with some now known to include multiple species, not all toxic (Fryxell et al. 1997). In Monterey Bay, the blooms have typically occurred both in spring and fall, with peak abundances varying greatly inter-annually (Fryxell et al. 1997). High cell numbers of *Pseudo-nitzschia* ($>10^4$ cells l^{-1}) have often been associated with the end of upwelling events or weak upwelling events during warm years along with low concentrations of macronutrients (Fryxell et al. 1997, Kudela et al. 2004). Recent studies with access to offshore samples in the southern California region have shown that blooms can originate offshore on the continental margin and then move onto the coast (Anderson et al. 2006). As in central California, the southern blooms appear to be associated with transitions between upwelling and more stratified nutrient poor conditions (Fryxell et al. 1997).

Distribution of phytoplankton has been correlated with physical and chemical features of the water. Diatoms appear to have an inverse relationship with sea surface temperatures (Tont 1987), and short-term climate events such as the El Niño/Southern Oscillation (ENSO) have been shown to cause shifts in the phytoplankton community, promoting dinoflagellates and/or less abundant diatom groups over the typically more dominant larger diatoms (Balech 1960, Tont

1987, Reid & Stewart 1989). Changes in physical oceanographic conditions can also impact lower trophic levels, with changes in prey availability affecting the distribution of marine mammal foraging locations (Keiper et al. 2005, Weise & Harvey 2008). In both central and southern California, the occurrence of *Pseudo-nitzschia* blooms is also suggested to be tied to shorter term climate events such as ENSO and possibly also to phenomena on time scales of the Pacific decadal oscillation (PDO) (Fryxell et al. 1997).

Mobile marine mammals are considered to be good coastal sentinels for signaling remote or offshore biotoxin events (Bossart 2006, 2007, Goldstein et al. 2008). In the Gulf of Mexico, strandings of bottlenose dolphins *Tursiops truncatus* were observed coastally, likely resulting from offshore brevetoxin-producing *Karenia brevis* bloom events (Flewelling et al. 2005). Similarly, CSL are considered to be a sentinel marine mammal species for DA events off the California coast; during blooms of toxic *Pseudo-nitzschia* these mammals commonly strand on beaches due to DA intoxication, displaying distinctive neurological signs, including seizures (Gulland et al. 2002). However, in the last decade, the concentration of toxic *Pseudo-nitzschia* and/or particulate DA in the environment has not always been related to strandings of animals showing signs of acute DA intoxication (Goldstein et al. 2008). Hence, we questioned whether DA-related CSL strandings in the Bay were also linked to toxic events occurring elsewhere within the range of this mobile predator, whose movements vary seasonally.

Using a decade long data series, the goal of this study was to examine the potential links between CSL strandings, DA, toxic cell numbers, and overall primary production of Monterey Bay waters. These data include records of sea lion strandings on beaches within the Bay, concentration measurements of the 2 dominant toxin producing species of *Pseudo-nitzschia*, and the associated levels of particulate DA at multiple sites around the Bay.

MATERIALS AND METHODS

Phytoplankton sampling. Phytoplankton surveys in the central California region were designed to investigate the temporal and spatial patterns of abundance of the DA-producing *Pseudo-nitzschia* species that could lead to poisoning of marine mammals and seabirds. Between 1998 and 2007 the temporal patterns of the toxin-producing species of *Pseudo-nitzschia*, namely *P. australis* and *P. multiseriata*, at 2 sites within Monterey Bay, California were examined using a nearshore and an ~1000 m deep, more open water location. The 2 sites, the Santa Cruz municipal wharf and the Mon-

terey Bay Aquarium Research Institute Stn M1 ~20 km offshore, were sampled weekly (Fig. 1). Beginning in 1999, water from both sites was obtained using surface buckets and returned to the University of California, Santa Cruz, where a subsample was taken and the 2 toxic *Pseudo-nitzschia* species were counted, using species-specific molecular probe methods. A duplicate subsample provided water for the measurement of DA concentrations in particulate material, using the high performance liquid chromatography (HPLC) method described below. At the wharf site, an additional subsample of the water was used to measure chlorophyll *a* (chl *a*). An additional series of monthly water samples began in November 2002 and continued until the end of 2007, providing us with additional offshore stations in the Bay. These samples were collected as part of the NOAA-funded 'Wind to Whales' (WZW) project (Center for Integrated Marine Technologies, 2002–2007) and by CeNCOOS (2006–2007), part of the US Integrated Ocean Observing System (<http://cimt.ucsc.edu/index.htm>). These latter surveys consisted of 7 transect lines ~10 km in length from the 50 m isobath out to 122° 05' W, where 11 hydrographic stations were monitored during each survey (Fig. 1). Each hydrographic station included a phytoplankton net tow, a conductivity-temperature-depth (CTD) cast, and a sur-

face water sample for measuring toxic *Pseudo-nitzschia* abundance and particulate DA. Since our data collection did not start until 1999, we used published data for toxic cells and DA concentrations during the 1998 stranding event (Scholin et al. 2000).

Chl *a* analyses. Chl *a* concentrations in surface water, available only for the Santa Cruz wharf site, were used as the selected biomass indicator of phytoplankton. All aliquots were filtered onto 25 mm GF/F filters (Whatman), pigments in cells on the filters were then extracted for 24 h in 90% aqueous acetone at -20°C, and subsequently analyzed for chl *a* using a Turner fluorometer (Parsons et al. 1984).

Toxic *Pseudo-nitzschia* cell enumeration. Water column abundance of toxic *P. australis* and *P. multiseriis* were determined for each sample using whole cell hybridization with species-specific large subunit rRNA-targeted fluorescent probes (Miller & Scholin 1996). Aliquots of seawater ranging from 5 to 30 ml were filtered onto 1.2 µm isopore polycarbonate filters (Millipore) and preserved with a saline ethanol solution for at least 1 h. After rinsing with hybridization buffer, i.e. 5X SET (1/5 dilution of 3.75 M NaCl, 25 mM EDTA, 0.5 M Tris HCl, pH 7.8), 0.1% (v/v) IGEPAL-CA630 (Sigma), 25 µg ml⁻¹ polyadenylic acid (Sigma), each sample was incubated with species-specific

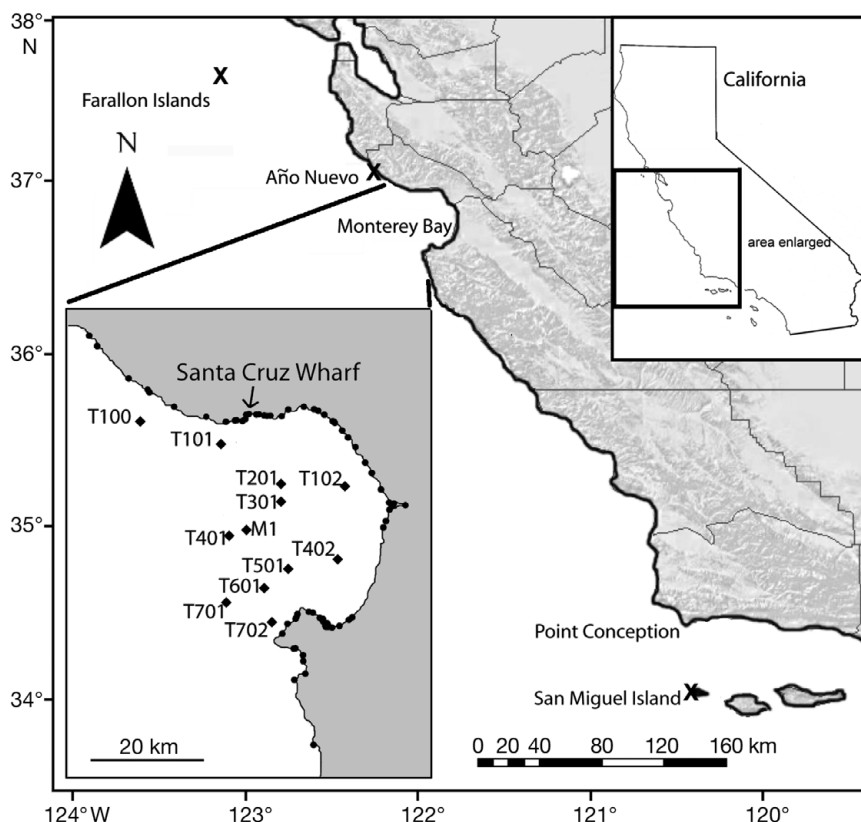


Fig. 1. California, USA, and central California sea lion breeding islands (X). Inset map on the left is Monterey Bay, showing the stranding locations for sea lions with signs of acute domoic acid (DA) poisoning (●), and sampling sites (♦) for toxic *Pseudo-nitzschia* cell counts and associated DA values, 1998 to 2007. Note that 'T' stations were visited monthly from 2002 to 2007 on the 'Wind to Whales' cruises (Center for Integrated Marine Technologies, 2002–2007), and the nearshore Santa Cruz municipal wharf and open water M1 stations were visited weekly from 1998 to 2007

probes for *P. australis*, *P. multiseriis*, a positive eukaryote control probe, and a negative control probe designed for *Alexandrium tamarense*. A third control consisted of a sample with no probe added. After 1 h, filters were rinsed and placed on microscope slides. Intact cells that retained the fluorescein-labeled probe were then counted on a Zeiss Standard 18 compound microscope, equipped with epifluorescence (microscope illuminator 100, Zeiss, with a high pressure mercury lamp, 50 W). The entire area of each filter was counted and used to generate results that were converted into cells l^{-1} .

Domoic acid detection in water samples. DA concentrations in particulate matter (i.e. cells) were determined using the FMOc (9-fluorenylmethylchloroformate)-HPLC method (Pocklington et al. 1990) in every sample taken between 1999 and 2005; subsequently, DA was measured only when toxic cells exceeded $10^4 l^{-1}$, reflecting levels at which clinical signs of DA intoxication or deaths of sea lions occurred in the Bay between 2006 and 2007 (Scholin et al. 2000). The method involves pre-column derivatization of DA in cell extracts with FMOc reagent to form the fluorescent FMOc derivative of DA. Prior to analysis, particulate samples collected on GF/F filters (Whatman) were extracted in 2.5 ml 10% aqueous MeOH. Filters were vortexed to homogenize cells, followed by sonication using a sonicator probe for 2 min. Extracts were centrifuged for 3 min at $1399 \times g$, the supernatant removed, and filtered through a $0.22 \mu m$ filter (Millipore). For derivatization, 200 μl of extract was vortexed with 50 μl 1 M borate buffer (pH 6.2), 10 μl dihydrokainic acid (DHKA), and an internal standard (100 $\mu g ml^{-1}$ DHKA in 10% aqueous MeOH) for 10 s, and then 250 μl FMOc-Cl reagent was added and vortexed again for 45 s. Three ethyl acetate washes were performed and the aqueous layer was removed for HPLC analysis. A 5 μl sample was injected into a Hewlett-Packard HP1090M HPLC equipped with an HP1046A fluorescence detector set for excitation at 264 nm and emission at 313 nm, with a mobile phase flow rate of $0.2 ml min^{-1}$ comprised of 40% aqueous MeCN, 0.1% trifluoroacetic acid (TFA). Isocratic separations were performed on a reverse phase C_{18} column (2.1 mm \times 25 mm, Vydac 201TP52; Separations Group) heated to $40^\circ C$. A calibration curve was generated using the certified domoic acid standard (DACS-1C) (National Research Council of Canada) of 5, 10, 25, 50, 100 and 250 $ng ml^{-1}$ in 10% aqueous MeOH ($r^2 = 0.99$).

CSL stranding data. Sea lions found live and debilitated along the Monterey Bay coast from 1998 to 2007 were transported to The Marine Mammal Center in Sausalito, California (TMMC) for clinical examination, treatment and rehabilitation for release back to the wild, when possible. Data from CSL that stranded with

signs of acute DA poisoning was collected including information on seizures, disorientation and death (as described by Gulland et al. 2002), sex, age class, and stranding date and location. Based on sex, body length, weight, development of teeth, and color of skin or pelage, and other external characteristics, the sea lions were then categorized by age classes according to growth curves: pup, 0 to 1 yr; yearling, 1 to 2 yr; juvenile male, 2 to 4 yr; subadult male, 4 to 8 yr; subadult female, 2 to 5 yr; adult male, 8+ yr; and adult female, 5+ yr (Greig et al. 2005).

DA detection in sea lion feces. DA concentrations were detected in available feces samples collected from stranded CSL suffering from acute effects of DA toxicity in Monterey Bay in 2007. DA extractions were conducted using the procedure described by Bargu et al. (2002). A 1:4 ratio of sea lion feces to 50% MeOH extraction solvent was vortexed for 30 s, homogenized for 2 min using a biohomogenizer with a 1.4 and 2.5 cm generator (Biospec), and sonicated for 2 min (30 to 40 W) in an ice bath with a Sonicator 3000 equipped with a microtip (Misonix). The resulting slurry was then centrifuged for 20 min at $1399 \times g$ and the supernatant passed through a $0.2 \mu m$ filter (Millipore). Consequently, 2 to 4 ml of filtrate were passed through a strong anion exchange solid phase extraction column (JT Baker™) which was preconditioned with 6 ml nanopure water, followed by 3 ml 100% methanol, and finally 3 ml 50% MeOH (Lefebvre et al. 1999). After washing the column with 5 ml of 10% MeCN, DA was eluted with 5 ml of 0.5 M NaCl in 10% MeCN at a rate of 1 drop s^{-1} (Hatfield et al. 1994). The column was not allowed to run dry at any time during solid phase extraction.

Fecal samples were analyzed for the presence of DA using an isocratic elution profile on a Hewlett-Packard 1090 HPLC equipped with a diode array detector set at 242 nm with a bandwidth of 10 nm. The reference signal was set at 450 nm with a bandwidth of 10 nm. A reverse phase Vydac C_{18} column (catalog #201TP52, 2.1 \times 25 mm, Separations Group) equipped with a Vydac guard column (particle size 5 μm) was used. The mobile phase (90/10/0.1, water/MeCN/TFA) was degassed with helium for 10 min prior to analysis. A calibration curve was generated using DACS-1C DA standards of 0.15, 0.3, 0.5, 1.0, 2.0, 4.0, 8.0, and 16 $\mu g ml^{-1}$ ($r = 0.99$). The instrument detection limit, which is equivalent to the concentration that corresponded to 3 times the standard deviation of the signal from the lowest detectable standard ($n = 3$), was 0.018 ppm. Injections were 20 μl with a flow rate of $0.3 ml min^{-1}$. Two DA-free CSL fecal samples were selected and spiked with 16 ppm DA (DACS-1C) to test the matrix interference for the instrument; spiking recoveries were 94.8 and 101.8%, respectively.

RESULTS

Toxic *Pseudo-nitzschia* were frequently detected in Monterey Bay during the 10 yr time period (Fig. 2A). Monthly average cell numbers of the 2 toxic *Pseudo-nitzschia* (*P. australis* and *P. multiseriata*) for all stations were combined for each month during the study interval (Fig. 2A). *P. australis* always was the more abundant of the 2 toxic species during the study interval. A seasonal pattern was observed, with cell numbers $\geq 10^4$ l⁻¹ found mostly in the spring and sometimes in the fall (Fig. 2A). Although toxic *Pseudo-nitzschia* were frequently present in both near and offshore regions of Monterey Bay, concentrations of toxic *Pseudo-nitzschia* and associated particulate DA levels between 2004 and 2007 were an order of magnitude lower compared to previous years (Fig. 2A,B). The prior cell densities, i.e. those before spring 2004, seem to have returned in the spring of 2007 (Fig. 2A). Chl *a* concentrations at the Santa Cruz site ranged from 0.1 to 110 $\mu\text{g l}^{-1}$ (Fig. 2D). Generally, toxic *Pseudo-nitzschia* were most abundant when chl *a* levels were low or moderate, demonstrating no correlation between the 2 indices ($r = 0.06$, $p > 0.05$, $n = 606$).

DA levels in Monterey Bay during the study period (Fig. 2B) were highly correlated with cell abundances of the 2 toxic *Pseudo-nitzschia* species ($r = 0.78$, $p < 0.001$, $n = 504$). The highest average DA concentrations for the Bay occurred in May 2007 and reached 16.4 $\mu\text{g l}^{-1}$, and correlated with high levels (6×10^4) of *Pseudo-nitzschia* cells l⁻¹ in the water.

CSL stranded with acute toxicity at different sites within Monterey Bay between 1998 and 2007 (Fig. 1). A total of 125 were admitted for care to TMMC and the number that stranded varied by year (Fig. 2C). An acute DA 'stranding event' was identified when a minimum of 5 sea lions were admitted into rehabilitation over a 48 h period, the animals were exhibiting signs of DA toxicosis, they were clustered spatially within 80 km of coastline of each other, and histopathological findings included hippocampal necrosis in at least one case (as defined by Goldstein et al. 2008). Such events occurred in 1998, 2000 and 2007, when numbers of the toxic species were moderate or high in the Bay ($> 10^4$ cells l⁻¹) (Fig. 2, shaded months). Overall, the number of sea lions admitted for rehabilitation each month with acute signs of DA intoxication was significantly correlated with average DA concentrations and the abundance of toxin-producing *Pseudo-nitzschia* in that

month ($r = 0.22$, $p = 0.04$, $n = 86$ and $r = 0.23$, $p = 0.02$, $n = 98$, respectively). However, high toxic *Pseudo-nitzschia* numbers and DA levels occurred in 2002, 2003 and 2004, which did not result in DA-related CSL stranding events (Fig. 2C).

Overall, the majority (48%) of sea lions stranding with acute DA toxicity were adult females ($n = 60$), followed by subadult males (18%, $n = 23$), adult males (14%, $n = 17$), juvenile males (8%, $n = 10$), subadult females (5%, $n = 6$), and even fewer younger individuals (4 yearling males, 2 yearling females, 2 male pups, 1 female pup) (Table 1). Among the adult animals, more females stranded than males in all years except in 2007; the number of subadult animal strandings appeared to increase over the 10 yr period (Table 1). DA was detected in 4 of 5 fecal samples collected in May 2007 from 5 sea lions that exhibited acute signs of DA poisoning at varying concentrations (1.4 to 96.8 $\mu\text{g DA g}^{-1}$ feces).

DISCUSSION

Results from Monterey Bay showed a complex relationship between DA concentrations, toxic *Pseudo-nitzschia* abundance in the water and related sea lion strandings. High DA concentrations in Monterey Bay did not always corresponded with an increase in DA-related CSL stranding events. The presence of CSL in the Bay varied seasonally in normal, non-ENSO years, as animals move to and from the breeding sites on the Channel Islands, but their seasonal movement pattern shifted during ENSO years.

CSL are one of the most abundant pinnipeds in the California Current System (Henkel & Harvey 2008),

Table 1. *Zalophus californianus*. Annual summary of age class and sex distribution of stranded California sea lions admitted for rehabilitation to The Marine Mammal Center with signs of acute domoic acid (DA) poisoning, 1998 to 2007. Age classes included pup (0–1 yr), yearling (1–2 yr), juvenile male (2–4 yr), subadult male (4–8 yr), subadult female (2–5 yr), adult male (8+ yr) and adult female (5+ yr)

Year	Adult		Subadult		Juvenile		Yearling		Pup		Total
	M	F	M	F	M	F	M	F	M	F	
1998	0	30	1	0	2	0	0	0	0	0	33
1999	0	0	0	0	0	0	0	0	0	0	0
2000	6	10	4	0	0	0	0	0	0	0	20
2001	0	5	0	0	0	0	0	0	0	0	5
2002	1	3	1	0	2	0	2	0	0	0	9
2003	1	6	0	3	2	0	1	0	0	1	14
2004	1	2	1	0	1	0	0	1	1	0	7
2005	1	2	1	1	1	0	0	1	0	0	7
2006	1	2	4	1	0	0	1	0	0	0	9
2007	6	0	11	1	2	0	0	0	1	0	21
Total	17	60	23	6	10	0	4	2	2	1	125

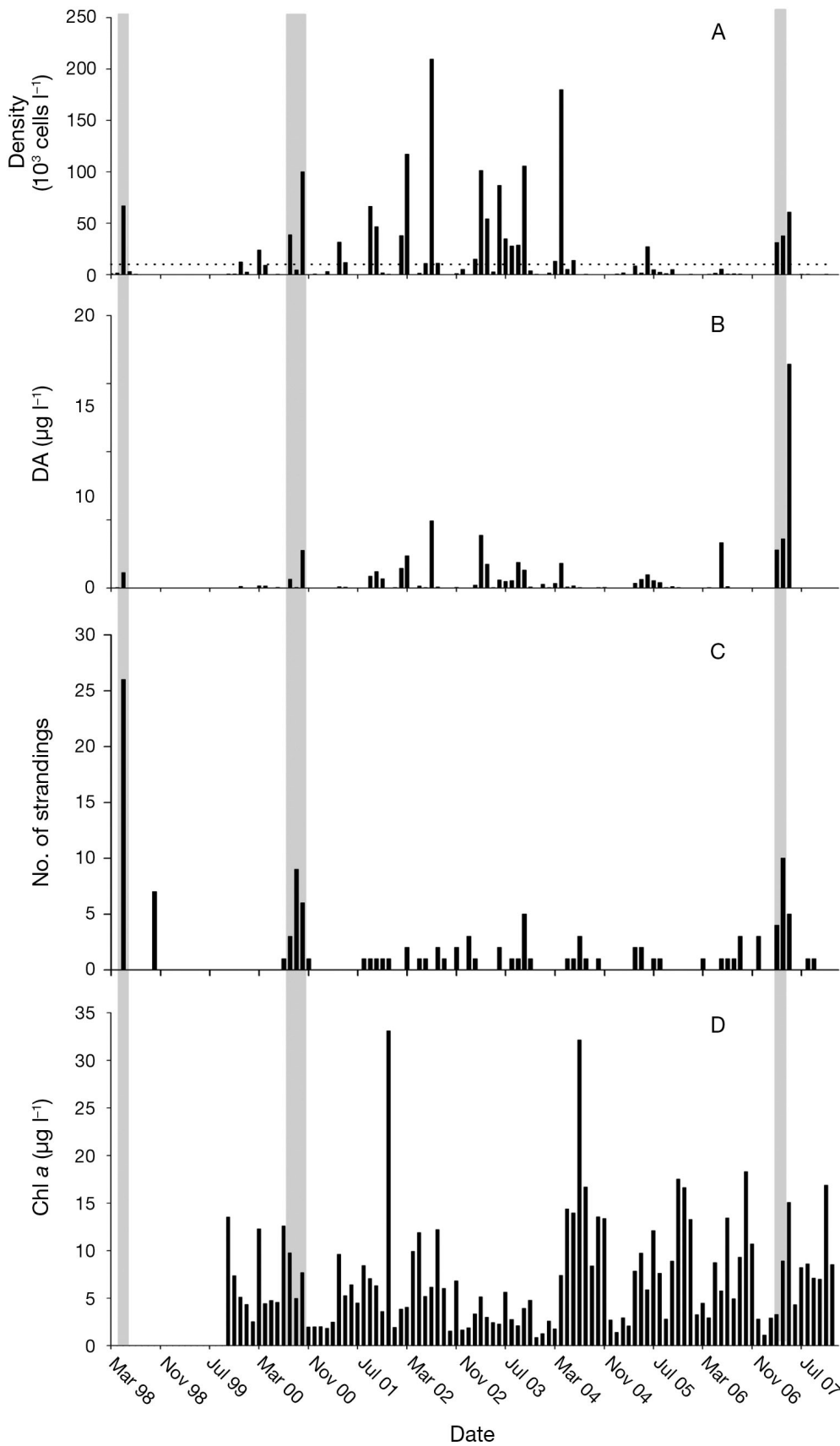


Fig. 2. Monterey Bay, California, USA, time-series data, 1998 to 2007, showing monthly averages of (A) toxic *Pseudo-nitzschia* (*P. australis* + *P. multiseriata*) cells l^{-1} for all Bay stations; dashed line represents 10^4 cells l^{-1} coincided with times when California sea lions (CSL) *Zalophus californianus* stranded in Monterey Bay with signs of acute domoic acid (DA) toxicity, (B) particulate DA in the water (average $\mu g l^{-1}$ for all Bay stations), (C) number of CSL stranding with signs of acute DA poisoning (total for the entire Bay), and (D) phytoplankton biomass (average chl a for Santa Cruz site only). Toxic *Pseudo-nitzschia* and particulate DA data for 1998 are averages from (Scholin et al. 2000). Shaded areas show months in 1998, 2000 and 2007 when large numbers of CSL stranded with signs of acute DA poisoning

with large annual breeding and foraging movements that differ by age and sex (Melin et al. 2000). The breeding season is between May and August, with peak pupping in mid-June (Melin et al. 2000). During the summer, most sea lions are on or near their breeding sites, on islands south of Point Conception, along the coasts of southern California, Baja California, and in the Gulf of California (Weise et al. 2006) (Fig. 1). Adult males usually do not leave during the breeding season, as they are defending territories to maintain access to females. Females alternate between nursing their pup and foraging offshore, spending up to 50% of their time over the continental shelf (Melin et al. 2000). Foraging locations of CSL vary by breeding site, and sea lions from San Miguel Island, the most northwestern site and the closest to Monterey Bay (330 km south), primarily foraging north of Point Conception along the coast of central California (Melin et al. 2000) (Fig. 1). At the end of the summer, most adult and subadult males leave the breeding islands and migrate northward to feeding areas on the shelf off central and northern California, Oregon, and Washington, returning in late spring (Weise et al. 2006). Although females primarily feed off the shelf during the breeding season, they shift to slope and offshore habitats later in the year (Melin et al. 2000). Juveniles and non-lactating females disperse along the California coast (Melin et al. 2000).

CSL feed on various pelagic and benthic prey, primarily northern anchovy, sardines, herring, and squid (Lowry et al. 1991). Sea lion prey distribution and abundance depends on the level of primary productivity, and changes in productivity may alter foraging locations (Weise & Harvey 2008). Ecosystem productivity may be reduced with climate-related events such as ENSO and, on a longer time scale, climate cycles such as the PDO. Such climatic changes in the marine environment affect the distribution and abundance of prey resources, which in turn influences the distribution and behavior of top marine predators (DeLong et al. 1991, Brodeur et al. 2006).

In the California Current, shorter-term climate events such as ENSO alter production and geographic distribution of many important fish stocks by reducing upwelling favorable winds and vertical stratification, thereby reducing nutrient input to surface waters, resulting in lower primary and secondary production (Kahru & Mitchell 2000, Chavez et al. 2002, Weise & Harvey 2008). Anchovy and sardines, important prey for CSL, are known to shift in their location and abundance, with sardines being more common in warm years and anchovies more abundant in colder years, as these planktivores are also dependent on prey abundance (Barth et al. 2007, Weise & Harvey 2008). CSL are able to respond to climatic variability by changing

their diet, foraging areas, and time at sea (DeLong et al. 1991, Keiper et al. 2005). Additionally, female CSL leave their pups for longer periods to forage if local food resources are sparse (Melin et al. 2000). Increased female and pup strandings have been documented throughout the pupping season during ENSO years (DeLong et al. 1991).

Sea lion mortalities due to acute DA poisoning in central California appear to be associated with the presence of DA-producing blooms at primary foraging sites. If productivity is low in southern California but higher in central and northern California, sea lions may move northward from the breeding ground to find prey, and, at that time, intoxication may occur if DA producers are abundant at these sites, such as in Monterey Bay. During our 1998 to 2007 study period, several ENSO warming events occurred along the California coast. In Monterey Bay, toxic *Pseudo-nitzschia* blooms, which included DA-producing *P. australis* and *P. multiseriis* blooms, coincided with the unusual warming events, especially at times when productivity was low in southern California, but normal or higher in central California. The first event in 1998 (Scholin et al. 2000) occurred when California coastal waters were affected by very strong ENSO conditions beginning late in the summer of 1997 and continuing into the summer of 1998 (Lynn et al. 1998), with associated decreased upwelling, increased water temperatures, and increased mixed layer depth (Kahru & Mitchell 2000). When a large proportion of adult sea lions stranded in May ($n = 26$) in Monterey Bay, productivity was lower than in normal, non-ENSO years, but still higher than outside of the Bay (Chavez et al. 2002). Sardines also were reported to be more abundant in the nearshore Bay during this period (Keiper et al. 2005, Weise & Harvey 2008), along with moderate to high levels of toxic *Pseudo-nitzschia* (Scholin et al. 2000). During this time of the year, the majority of the CSL population would have been expected to be near breeding sites to the south. Instead, increased CSL strandings were reported for the central California region, suggesting a possible delayed return to breeding areas in response to reduction in prey availability there (Lowry & Forney 2005). Most of the stranded sea lions were adult females ($n = 24$), which was unusual, and they may have been foraging in the Bay in response to reduced prey availability further south. Additionally, the increase in adult females in central California in 1998 resulted in an increase in the number of pups born at Año Nuevo and the South Farallon Islands north of Monterey Bay (Fig. 1) (106 pups in 1998 vs. 23 in 1997), and below normal birth rates at rookeries in southern California (Lowry & Forney 2005).

The other 2 DA-related sea lion stranding events occurred in the late summer/fall of 2000 and spring of

2007, and also coincided with moderate to high numbers of toxic *Pseudo-nitzschia* and associated high levels of DA in Monterey Bay (Fig. 2). Similarly, more sea lions were observed during these time periods in the Bay than in other years (Goldstein et al. 2008, Henkel & Harvey 2008). In 2000, coastal waters off the southern California region experienced weaker than normal upwelling and lower productivity, whereas production remained near normal in central and northern California (Durazo et al. 2001, Henkel & Harvey 2008). Adult females ($n = 10$) stranded in larger than usual numbers in Monterey Bay. Since most of the strandings in 2000 were observed in late August and throughout September and October, i.e. after the breeding season, adult and subadult males were expected to be near the Bay, foraging during their northward migration. However, most of the female sea lion population would have been expected to remain near the Channel Islands nursing their pups. With lowered productivity and decreased prey abundance further south, females were likely traveling northwards in greater numbers to forage (Henkel & Harvey 2008), resulting in exposure to high levels of DA and increased numbers of related strandings.

In contrast, during 2007, higher numbers of males (mostly adult and subadult, $n = 20$) stranded than females ($n = 1$). Strandings in 2007 mainly occurred in March and April, shortly before the beginning of the breeding season, and coincided with a highly toxic (maximum of $7.4 \mu\text{g DA l}^{-1}$) *Pseudo-nitzschia* bloom (up to 6×10^5 cells l^{-1}) in Monterey Bay. Southern California upwelling in the spring of 2007 began slightly early, whereas strong and persistent upwelling-favorable winds occurred in Monterey Bay throughout the year (Goericke et al. 2007, McClatchie et al. 2008). Thus, it is likely that most males were already migrating southward to the breeding site and foraging as they transited through Monterey Bay, where a toxic *Pseudo-nitzschia* bloom was present.

The presence of moderate to high numbers ($>10^4$ cells l^{-1}) of toxic *Pseudo-nitzschia* associated with high DA levels in the Bay, coincided with the CSL stranding events in 1998, 2000 and 2007, but did not result in DA-related stranding events in other years. During the spring and summer of 2002 and 2003, and spring of 2004, monthly averaged toxic cells in the Bay reached 2×10^5 cells l^{-1} , with toxicities exceeding $4.5 \mu\text{g DA l}^{-1}$, but with no occurrence in DA related sea lion stranding events here. It is possible that during some of the DA increases in the Bay the sea lions may have had access to a less contaminated prey source and thus DA-related stranding events were lower. However, the ability of sea lions to detect and avoid DA-tainted prey if a less contaminated alternative exists is not known. Between 2002 and 2004, weaker-

than-normal northwesterly winds affected the central coast and resulted in weak, delayed upwelling in the Bay area. In contrast, waters were more productive and intense blooms of toxic *Pseudo-nitzschia* were occurring off southern California (Goericke et al. 2004, 2005, Langlois 2004, 2005, 2006, 2007, Peterson et al. 2006, Schnetzer et al. 2007). More than 1000 sea lions were stranded in the south due to DA-related toxicity (Schnetzer et al. 2007, de la Riva et al. 2009), where toxic *Pseudo-nitzschia* blooms occurred during this period. Between summer 2004 and 2007 toxic *Pseudo-nitzschia* populations appeared to be suppressed along the central coast, and therefore DA-related CSL stranding events were unlikely and, indeed, occurred very infrequently (Table 1). Cell densities and DA levels noted earlier in the decade appeared to have returned to Monterey Bay by spring 2007.

High DA levels in 2 important prey species (anchovies and sardines, $>128 \mu\text{g DA g}^{-1}$ viscera) (Lefebvre et al. 1999, 2002a,b, Scholin et al. 2000) and in feces of stranded sea lions ($>96 \mu\text{g DA g}^{-1}$) (Lefebvre et al. 1999, Scholin et al. 2000, present study) were reported for 1998, 2000 and 2007 during the DA blooms in Monterey Bay, showing the food chain link between the DA vectors and affected sea lions. Although the dose needed and time from exposure to the presence of clinical signs and/or stranding is not known in CSL, neurological signs likely occur within 48 h (based on estimates in other species, Teitelbaum et al. 1990, Tryphonas et al. 1990). Thus, given the broad extent of Monterey Bay (~49 km wide, NNE to SSW, and about 14 km from its seaward entrance to most western beach), and the average daily movements of sea lions (31 km d^{-1} , Thomas et al. 2009), it is most likely that DA-intoxicated sea lions consumed contaminated prey within the Bay.

This study suggests that DA-related strandings of sea lions may be a more complex phenomenon than previously expected. In a given site within the migratory range of this marine predator, the presence of toxic DA blooms is a necessary but not sufficient condition to predict local DA-related stranding events. Instead, the interaction of large-scale weather events such as ENSO, which can differentially affect productivity along the coastline, together with complex, seasonally related activities of migratory populations that center around fixed geographical locations combine to produce these regional DA-associated stranding events.

Thus, combined information on regional primary production, locations of foraging sea lions, phytoplankton composition (toxic/non-toxic), and DA concentrations rather than DA concentrations alone can be used to better predict DA-related sea lion strandings, an increasing phenomenon along the California coast.

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