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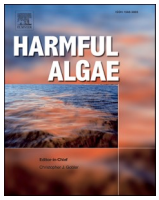

Original Article

Marine algal toxins and their vectors in southern California cetaceans

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ABSTRACT

Published baseline data on biotoxin exposure in cetaceans is sparse but critical for interpreting mortality events as harmful algal blooms increase in frequency and duration. We present the first synthesis of domoic acid (DA), saxitoxin (STX), okadaic acid (OA), and microcystin detections in the feces and urine of stranded and bycaught southern California cetaceans, over an 18 year period (2001–2018), along with corresponding stomach content data. DA was detected in 13 out of 19 cetacean species, most often in harbor porpoise (*Phocoena phocoena*) (81.8%, $n = 22$) and long-beaked common dolphins (*Delphinus delphis bairdii*) (74%, $n = 231$). Maximum DA concentrations of 324,000 ng/g in feces and 271, 967 ng/ml in urine were observed in *D. d. bairdii*. DA was detected more frequently and at higher concentrations in male vs. female *D. d. bairdii*. Higher fecal DA concentrations in *D. d. bairdii* were associated with a greater proportion of northern anchovy (*Engraulis mordax*) in the diet, indicating it may be a primary vector of DA. Fecal DA concentrations for *D. d. bairdii* off Point Conception were greater than those from animals sampled off Los Angeles and San Diego counties, reflecting greater primary productivity and higher *Pseudo-nitzschia* spp. abundance in that region and a greater abundance of *E. mordax* in the diet. STX was detected at low levels (fecal max = 7.5 ng/g, urine max = 17 ng/ml) in 3.6% ($n = 165$) of individuals from 3 out of 11 species. The occurrence of *E. mordax* in 100% of the 3 examined stomachs suggests this species could be a primary vector of the detected STX. OA was detected in 2.4% of tested individuals ($n = 85$) at a maximum fecal concentration of 422.8 ng/g. Microcystin was detected in 14.3% ($n = 7$) of tested individuals with a maximum liver concentration of 96.8 ppb.

1. Introduction

Harmful algal blooms and their associated biotoxins impact marine and human life in coastal waters globally and are increasing in frequency due to increased anthropogenic nutrient inputs and oceanographic changes associated with ocean-warming (Cosper et al., 1989; Hallegraeff, 2004). Blooms are episodic and variable in duration, with associated die-offs of marine life documented across a wide range of species and negative impacts to marine fisheries (Cavole et al., 2016; Cosper et al., 1989; Hoagland and Scatasta, 2006; Landsberg, 2002; Moore et al., 2019). Domoic acid (DA) and saxitoxin (STX) are the primary algal toxin threats along the Pacific coast of North America, while others such as okadaic acid (OA) and microcystins are less common but potentially emerging threats. Algal toxins can bioaccumulate in invertebrates and fish, which are then consumed by higher trophic level animals such as marine mammals (Broadwater et al., 2018; Lewitus et al., 2012).

DA is an excitotoxin produced by diatoms of the genus *Pseudo-nitzschia*, which are consumed by filter feeders such as bivalves, crustaceans, and planktivorous fish. Production of DA by diatoms can be limited by biotic and abiotic factors and not all *Pseudo-nitzschia* spp. produce DA (Bates et al., 2018; Fryxell et al., 1997). DA toxicity in

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California sea lions (*Zalophus californianus*) results in abnormal behavior, ataxia, seizures, reproductive failure, and death (Brodie et al., 2006; Gulland et al., 2002; Scholin et al., 2000) and has been linked to consumption of DA-contaminated planktivorous fish (Lefebvre et al., 1999). Although the impacts of DA on the physiology and ecology of *Z. californianus* have been studied extensively, similar work on cetaceans has been limited to single case studies, exposure data, or indirect links to mortality (D'Agostino et al., 2017; Fire et al., 2010; Lefebvre et al., 2016; Ochoa et al., 1998; Torres de la Riva et al., 2009).

Dinoflagellates of the genera *Alexandrium* are the major producers of STX along the U.S. West Coast (Lewitus et al., 2012), which is among the most toxic biotoxins (Krock et al., 2018). STX presence has historically occurred in central and northern California but may be increasing in the Southern California Bight (SCB) (Lewitus et al., 2012). STX blocks neurotransmission and can cause neurological symptoms and paralysis (Jeffery et al., 2004; Levin, 1991). The health impacts of STX on marine mammals have not been well studied. However, several studies have provided circumstantial evidence for STX impacts on marine mammals: a sea otter die off in Alaska (DeGange and Vacca, 1989), a mass mortality event of sei whales (*Balaenoptera borealis*) in southern Chile (Häussermann et al., 2017), a humpback whale mortality event off the East Coast of the U.S. (Geraci et al., 1989), (Hernandez et al., 1998), potential sublethal impacts to North Atlantic right whales (*Eubalaena glacialis*) (Doucette et al., 2006; Durbin et al., 2002), and Mediterranean monk seal (*Monachus monachus*) deaths in northwest Africa (Hernandez et al., 1998). High concentrations of STX were detected in marine mammal carcasses during a 2008 multi-taxa mortality event in the Saint Lawrence Estuary, Canada (Starr et al., 2017), and more recently low baseline STX concentrations were reported in Alaskan marine mammals (Lefebvre et al., 2016).

Along the U.S. West Coast, OA is produced by dinoflagellates of the genera *Dinophysis* (Lewitus et al., 2012) and can cause gastrointestinal illness, dysfunction at the cellular and molecular levels, and promote tumors (Valdiglesias et al., 2013). Exposure to OA has been reported in Gulf of Mexico bottlenose dolphins (*Tursiops truncatus*), Peruvian fur seals (*Arctocephalus australis*), South American sea lions (*Otaria byronia*), and manatees (*Trichecus manatus latirostris*)(Fire et al., 2017, 2011). However, the impacts of this toxin on marine mammals are unknown.

Microcystins are toxins produced by freshwater cyanobacteria that can enter the marine environment via freshwater outflows. They can cause liver damage and promote tumors (Carmichael et al., 2001; Humpage and Falconer, 1999). Microcystins have been detected at low levels in Florida *T. truncatus* (Brown et al., 2018) and implicated in the death of southern sea otters (*Enyhdra lutris*) feeding on marine bivalves in contaminated freshwater plumes (Miller et al., 2010).

Published data on algal toxins in West Coast cetaceans is sparse and this study provides baseline data on the presence and concentration of DA, STX, OA, and microcystin in southern California cetaceans. This is

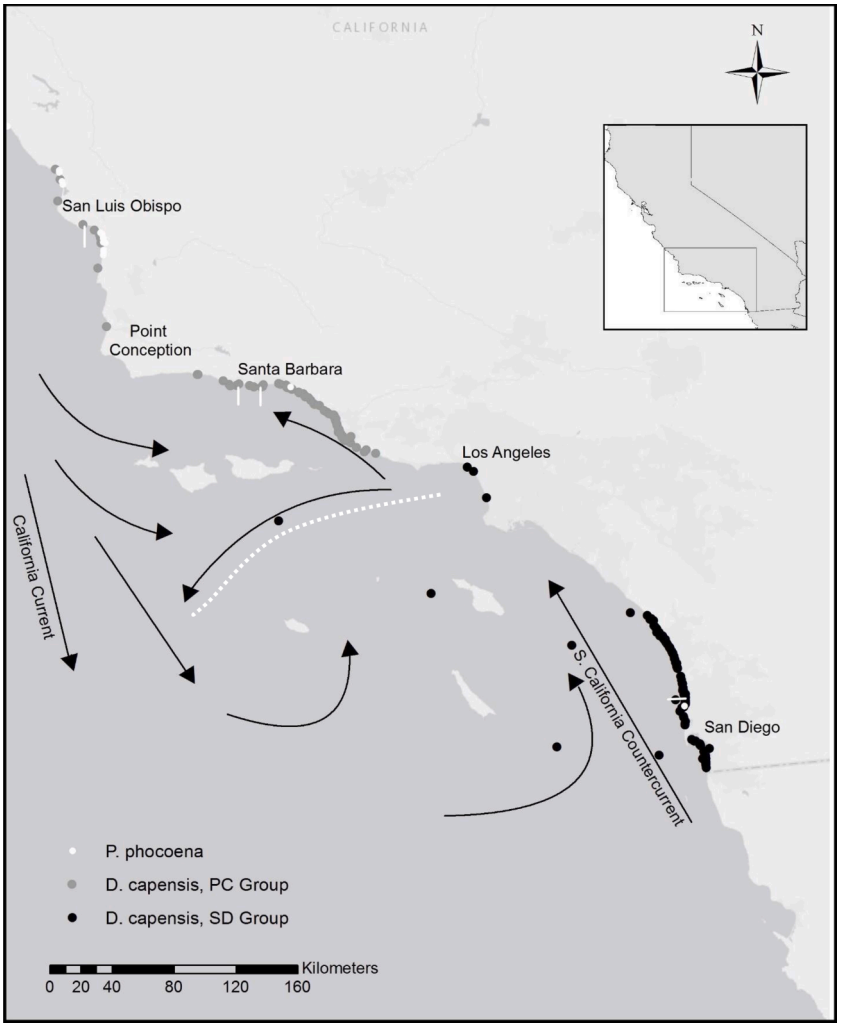

Fig. 1. Southern California Bight (SCB) study area, showing ocean circulation pattern (Hickey, 2005) and *D. d. bairdii* and *P. phocoena* sample locations. Solid white lines indicate pier sampling locations, north to south: Cal Poly, Goleta, Stearns Wharf, and Scripps. The hashed line delineates the two sampling regions into Northern (Point Conception (PC)) and Southern (San Diego (SD)).

also the first study to investigate diet in relation to the prevalence and concentration of biotoxins in marine mammals. Risk factors for *D. d. bairdii* were assessed by comparing diet, sex, age class, and location relative to toxin exposure.

2. Methods

2.1. Study area

The SCB study area is a region in southern California where the coastline curves eastward with a complex topography that includes islands, basins, and ridges that influence regional water circulation patterns (Fig. 1). The northern area of the SCB, around Point Conception, is more productive than the southern region (McClatchie, 2014). This, in combination with preliminary data indicating high DA concentrations in dolphin specimens in this northern region, led us to compare biotoxins, plankton, and dolphin diet between the northern and southern regions of the SCB, designated as Point Conception (PC) and San Diego (SD) spatial groups. SD and PC spatial groups were separated based on circulation patterns of the SCB (Fig. 1).

2.2. Sample collection

A total of 397 cetacean carcasses were obtained from strandings (n = 367) or incidental mortalities in commercial fisheries (n = 30) in the SCB between the years 2001 and 2018. These carcasses comprised 19 cetacean species, but the majority of cetaceans sampled were *D. d. bairdii* (Tables 1–3). Feces (collected from the colon or large intestine), urine, and/or gastric contents were collected from each carcass and frozen at −20°C until analysis. Each carcass was tested for the presence of one or more of the following biotoxins, dependent on sample volume available: DA (n = 381), STX (n = 165), OA (n = 85), and microcystin (n = 7).

Stranded dolphins largely encompass individuals that are sick prior to stranding, while some die from more acute causes, such as trauma. Thus, it is important to note that reported biotoxin detections largely represent a non-random portion of the population. *D. d bairdii* that were determined to have died from trauma (Moore et al., 2013) (n = 33), were used to investigate DA exposure in presumed healthy dolphins.

2.3. Biotoxin analyses

Biotoxin detections were determined using available feces, urine, and gastric content data; positive animals were defined as those with biotoxin concentrations greater than the detection limit of the test method in any submitted sample.

2.3.1. Domoic acid

A total of 320 fecal, 162 urine, and 9 gastric samples were analyzed to determine DA concentrations from 381 cetaceans (Table 1). Samples collected between the years 2001 and 2008 were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the National Centers for Coastal Ocean Science Charleston Laboratory. Specimens collected between 2008 and 2017 were largely analyzed at the Northwest Fisheries Science Center with a subset (n = 13) analyzed at the University of California at Santa Cruz, both using a DA specific enzyme-linked immunosorbent assay (ELISA). DA ELISA results are generated by comparing sample extracts to a supplier provided standard curve in each plate to minimize variability between assays. LCMS results are generated by comparing sample extracts to a standard curve prepared using certified reference material across the observed sample

Table 1
Summary of cetaceans tested for domoic acid (DA) with corresponding concentrations (ng/g or ng/ml) by matrix. Means were calculated using DA positive samples only. F = feces, U = Urine, G = gastric contents.

Common Name	Scientific Name	Number of samples tested F/U/G	Individuals positive/ Individuals tested	% positive	FecesMean ±SE	Feces Max.	Urine Mean±SE	UrineMax.
Blue whale	*Balaenoptera musculus*	0/1/0	0/1	0.0				
Bottlenose dolphin	*Tursiops truncatus*	25/14/0	7/27	25.9	68 ± 59 (n = 4)	245	102 ± 100 (n = 5)	500
Bryde's whale	*Balaenoptera brydei*	1/0/0	0/1	0.0				
Cuvier's beaked whale	*Ziphius cavirostris*	1/0/0	1/1	100.0		8		
Dall's porpoise	*Phocoena dalli*	4/2/0	2/5	40		N/A		0.6
Fin whale	*Balaenoptera physalus*	5/0/0	4/5	80.0	4457 ± 4431 (n = 4)	17,750		
Gray whale	*Eschrichtius robustus*	5/2/0	2/5	40.0	11 ± 2 (n = 2)	13		
Harbor porpoise	*Phocoena phocoena*	19/13/0	18/22	81.8	824 ± 613 (n = 13)	7915	56 ± 25 (n = 10)	257
Humpback whale	*Megaptera novaengliae*	4/0/0	1/4	25		55		
Long-beaked common dolphin	*Delphinus delphis bairdii*	189/104/4	171/231	74	11,703 ± 3634 (n = 128)	324,000	12,564±4464 (n = 82)	271,967
Mesoplodon sp.	*Mesoplodon* sp.	1/0/0	0/1	0.0				
Minke whale	*Balaenoptera acutorostrata*	1/1/0	1/1	100.0		258,665		2931
Northern right whale dolphin	*Lissodelphis borealis*	14/9/0	9/16	56.3	13 ± 3 (n = 8)	23	7 ± 4 (n = 7)	23
Pacific white-sided dolphin	*Lagenorhyncus obliquidens*	10/4/1	2/11	18.2	1008 ± 992 (n = 2)	2000		
Pygmy sperm whale	*Kogia breviceps*	1/0/0	0/1	0.0				
Risso's dolphin	*Grampus griseus*	6/2/0	3/7	42.9	671 ± 664 (n = 3)	2000		
Short-beaked common dolphin	*Delphinus delphis*	32/10/4	7/40	17.5	143 ± 109 (n = 3)	518	143 ± 188 (n = 3)	360
Sperm whale	*Physeter macrocephalus*	1/0/0	0/1	0.0				
Striped dolphin	*Stenella coeruleoalba*	1/0/0	0/1	0.0				
Total		320/162/9	228/381	60.0				

Table 2
Summary of cetaceans in the San Diego (SD) (n = 151) and Point Conception (PC) (n = 14) groups tested for saxitoxin (STX) between the years 2003 and 2017. Maximum STX concentrations are presented as ng/g STX equivalents for feces and urine. F = feces, U = urine, G = gastric contents.

Common name	Scientific Name	Number of samples tested F/U/G	Individuals positive/ Individuals tested	% Positive	Max [STX] in feces (ng/g)	Max [STX] in urine (ng/ml)
Bottlenose dolphin	*Tursiops truncatus*	13/6/0	0/15	0		
Fin whale	*Balaenoptera physalus*	2/0/0	1/2	50	7.4	
Gray whale	*Eschrictius robustus*	1/0/0	0/1	0		
Harbor porpoise	*Phocoena phocoena*	1/2/0	0/6	0		
Humpback whale	*Megaptera novaengliae*	1/0/0	0/1	0		
Long-beaked common dolphin	*Delphinus delphis bairdii*	86/26/1	4/101	4	7.5	17
Northern right whale dolphin	*Lissodelphis borealis*	7/3/0	1/8	12.5	4.2	
Pacific white-sided dolphin	*Lagenorhyncus obliquidens*	4/3/0	0/6	0		
Pygmy sperm whale	*Kogia breviceps*	1/0/0	0/1	0		
Risso's dolphin	*Grampus griseus*	2/0/0	0/2	0		
Short-beaked common dolphin	*Delphinus delphis*	20/1/0	0/22	0		
Total		138/41/1	6/165	3.6		

Table 3
Summary of cetaceans tested between 2008 and 2018 for okadaic acid (OA) in the San Diego (SD) group. F = feces, U = urine, G = gastric contents.

Common name	Scientific name	Number of samples tested F/U/G	Individuals positive/ Individuals tested	Max [OA] in feces (ng/g)
Bottlenose dolphin	*Tursiops truncatus*	8/2/0	0/8	
Bryde's whale	*Balaenoptera brydei*	1/0/0	0/1	
Fin whale	*Balaenoptera physalus*	1/0/0	0/1	
Humpback whale	*Megaptera novaengliae*	2/1/0	1/2	332
Long-beaked common dolphin	*Delphinus delphis bairdii*	60/4/1	0/61	
Northern right whale dolphin	*Lissodelphis borealis*	3/1/0	0/3	
Pacific white-sided dolphin	*Lagenorhyncus obliquidens*	1/0/0	0/1	
Risso's dolphin	*Grampus griseus*	1/0/0	0/1	
Short-beaked common dolphin	*Delphinus delphis*	7/0/0	1/7	423
Total		84/8/1	2/85	

concentration range. Results from both methods are comparable although the ELISA method has a slightly lower limit of detection for urine than the LC-MS/MS method (0.4 ng/ml vs 1 ng/ml) (Frame and Lefebvre, 2013), which could result in slightly more detections for the 2008 - 2017 data (11 samples contained DA < 1 ng/ml).

For LC-MS/MS analyses, filtered urine samples and methanolic extracts of feces were analyzed for the presence of DA as described in Fire et al. (2009). This method utilized reversed phase chromatography (Phenomenex Luna C18(2)) with gradient elution using water and acetonitrile both containing 0.1% formic acid, using an Agilent 1100 HPLC coupled to an ABI-SCIEX API-4000 triple quadrupole mass spectrometer in ESI+ mode. Monitored domoic acid fragments mass/charge ratios were m/z 266, 248, and 193. The retention time of domoic acid in samples was determined based on the retention time of a certified domoic acid reference standard from the NRC Canada (Halifax, NS). The limit of detection for DA was 1 ng/mL urine or 4 ng/g feces.

For ELISA, DA was extracted from samples as described in Lefebvre et al. (2016). DA was quantified in filtered extracts using a commercially available competitive ELISA kit, following the instruction protocol supplied by the manufacturer (Biosense® Laboratories, Bergen, Norway and Eurofins Abraxis, Warminster, PA) with minor modifications described in Lefebvre et al. (2016). The limits of detection for DA in sample material were 4 ng/g for feces, 2 ng/g for gastric contents, and 0.4 ng/ml urine.

2.3.2. Saxitoxin

STX was extracted from urine (n = 41), feces (n = 138), and gastric samples (n = 1) collected between 2008 and 2017 (Table 2), as described in detail in Lefebvre et al. (2016). Toxin levels were quantified as STX equivalents using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for STX (Eurofins Abraxis STX ELISA) following the instruction protocol supplied by the manufacturer with slight modifications based on sample matrix. For STX ELISA, a 1:50 dilution of the 1:4 80% ethanol extracts was sufficient to eliminate matrix effects in all sample types. The ELISA kit primarily measures STX (with limited cross-reactivity to several other Paralytic Shellfish Poisoning (PSP) congeners, as listed in the product documents). All PSP levels are listed as STX equivalents and may underestimate the total PSP toxicity. The detection limit for STX in all sample matrices was 3 ng/g or ml.

2.3.3. Okadaic acid

Urine (n = 8), feces (n = 84), and gastric samples (n = 1) collected in the SD group between 2008 and 2018 (Table 3) were extracted following methods outlined in Fire et al. (2011) and purified using 500 mg C18 solid-phase extraction (SPE) cartridges. SPE-cleaned samples were analyzed for OA and related congeners using an OA specific, commercially available enzyme-linked immunosorbent assay (ELISA), following procedures outlined by the manufacturer (Eurofins Abraxis, Warminster, PA). Extracts were pre-diluted to eliminate false positive results due to matrix effects and run in tandem with negative controls. The limit of detection for this assay was ~164 ng/g OA for feces and 41 ng/mL OA for urine.

2.3.4. Microcystins

Coastal *T. truncatus* were selected as a sentinel cetacean species to monitor for microcystin because they generally stay within 500 m of shore (Hanson and Defran, 1993) and therefore could travel through and feed in areas of freshwater outflows. In addition, since microcystin is known to cause necrotizing hepatitis, livers from 2 *D. d. bairdii* with this condition were tested for this toxin. In total, six fecal and two liver samples (~1 g wet wt.), collected between 2011 and 2018, were extracted using methods outlined in Mekebri et al. (2009) with

modifications. Samples were homogenized for ~1 min in 10 mL of 90% MeOH/0.1% TFA, followed by 1 h of sonication and 30 min of centrifugation at 3400 *x g*. Extracts were filtered (0.45 μm) and further purified by SPE cleanup using Strata-X 30 mg polymeric columns. SPE-cleaned samples were analyzed for microcystins, nodularins and related toxin congeners using an MC/NOD ADDA ELISA (Eurofin Abraxis, Warminster, PA) kit, following procedures outlined by the manufacturer. Extracts pre-diluted to eliminate false positive results due to matrix effects were run in tandem with negative controls. The limit of detection for this assay was ~107 ng/g MC for feces and 11 ng/g for liver.

2.4. Cell counts

Cell counts for *Alexandrium* spp., *Dinophysis* spp., and *Pseudo-nitzschia* spp. were provided by the Southern California Coastal Ocean Observing System (SCCOOS) for the years 2008 - 2018. The occurrence of these species were defined as dates with non-zero cell counts. The *P. seriata* designation for SCCOOS refers to the larger size class of *Pseudo-nitzschia* spp. that are generally toxigenic rather than the actual species, whereas the *P. delicatissima* designation refers to the smaller size class that is generally non-toxigenic. Thus, they are referred to as "*P. delicatissima* group" and "*P. seriata* group" since they are not true species identifications. *Pseudo-nitzschia* spp. cell counts at Scripps Pier, prior to 2008, were enumerated to the lowest taxonomic group rather than the species level of more recent data. *Pseudo-nitzschia* spp. cell counts for Scripps Pier in February 2004 were extracted from Busse et al. (2006), whereas raw data, collected according to methods outlined in Curtiss et al. (2008) were analyzed for the time period October 2004 through April 2007.

Cell count data was used as a proxy for the presence and abundance of phytoplankton that may produce toxins. Biases inherent in combining cell count data from different sources, with different methods, and varying taxonomic level resolution may exist, but represents the best data available. Cell count data from Scripps Pier were used for SD Group comparisons while cell count data from Stearns Wharf, Goleta Pier, and Cal Poly Pier were used for PC Group comparisons.

2.5. Diet

The stomachs of 111 *D. d. bairdii* and 11 *P. phocoena* that had corresponding biotoxin data were examined (Table 4) to determine prey items present, via the examination of otoliths and cephalopod beaks. Biotoxin presence was not determined for the prey in the examined stomachs because soft tissues were unavailable in these archived samples. Although the cetacean stomach has three compartments, only prey from the forestomach were identified because they represent the most recent feeding and therefore the most undigested remains (Harrison et al., 1967; Robertson and Chivers, 1997). However, retention and degradation rates can vary between hard and soft parts as well as by species, introducing biases associated with the method used (Pierce and Boyle, 1991). For example, beaks may be retained longer than otoliths or flesh, potentially overestimating the importance of squid in the diet (Bigg and Fawcett, 1985).

Stomach contents were rinsed and sorted using a series of mesh screen sieves (9.5 mm, 1.4 mm, 500 μm). Otoliths and cephalopod beaks were separated into left and right, and upper and lower, respectively, and were used to estimate number of prey. The standard Relative Index of Precision was not calculated as prey weight was unavailable. Instead, the relative abundance (%A) and frequency of occurrence (%O) were calculated for each species of prey, as follows:

$$\%A = \left(N_i \div T_p\right) \times 100$$

$$\%O = \left(N_{si} \div T_s\right) \times 100$$

where N_i is the number of prey item i and T_p is the total number of prey items; N_{si} is the number of stomachs with prey item i and T_s is the total number of stomachs examined.

2.6. Analyses

Analyses focused on examining differences in 1) biotoxin presence/absence proportions between sexes and geographic region and 2) biotoxin concentrations and prey species abundance between age and sex classes. A two-tailed Fisher Exact test ($\alpha = 0.05$) was used to assess differences in categorical biotoxin presence/absence data. To assess sex differences in biotoxin concentrations and prey abundance, which are not normally-distributed, we used a nonparametric Wilcoxon rank sum test ($\alpha = 0.05$). Only DA or STX positive specimens were included in calculations of means and medians, and concentration comparisons. Age classes for *D. d. bairdii* were defined using standard total length as defined in Preti (2020): juveniles TL < 150 cm; young subadults 150≤TL<190 cm; presumed adults TL ≥ 190 cm. Sample size in the juvenile category was insufficient for analysis and thus excluded from age class analyses. All statistical tests were done in the R-programming language (R Core Team, 2018).

3. Results

3.1. Domoic acid

For individuals with both urine and feces tested for the presence of DA ($n = 127$), 82.7% had the same result for both matrices. Occasions where urine and feces from the same individual had conflicting presence/absence results were associated with low DA concentrations (feces: mean = 43.1 ng/g, S.E. = 18.2 ng/g; urine: mean = 7.8 ng/ml, S.E. = 6.1 ng/ml). In these instances, 57.1% of urine samples were positive when fecal samples were negative. DA concentration was higher in feces than in urine ($p = 0.04012$, $W = 2863$) for individuals with both matrices tested.

DA was detected in 68.4% of cetacean species tested, with a maximum DA concentration of 324,000 ng/g, observed in the feces of a *D. d. bairdii*. Maximum DA concentrations known to cause acute toxicity in pinnipeds (1000 ng/g (feces) or 10 ng/ml (urine); (Goldstein et al., 2008)) were recorded in the following species: minke whale (*Balaenoptera acutorostrata*), fin whale (*Balaenoptera physalus*), *D. d. bairdii*, short-beaked common dolphin (*D. d. delphis*), Risso's dolphin (*Grampus griseus*), Pacific white-sided dolphin (*Lagenoryhncus obliquidens*), northern right whale dolphin (*Lissodelphis borealis*), harbor porpoise (*Phocoena phocoena*), and bottlenose dolphin (*Tursiops truncatus*) (Table 1).

Prevalence of DA exposure and associated mean DA concentrations are compared for species with 20 or more individuals tested, while those with smaller sample sizes are simply tabulated to document DA exposure (Table 1). Overall, DA was predominant in *P. phocoena* (81.8%),

Table 4
Number of stomach and prey samples examined in the study with corresponding domoic acid detection results of dolphin urine or feces (PC=Point Conception; SD=San Diego; DA+ = domoic acid positive; *D. d. bairdii* =long-beaked common dolphin; *P. phocoena* =harbor porpoise).

	D. d. bairdii, DA +			*D. d. bairdii*, DA, below detection limit			*P. phocoena*, DA +			Total
	PC	SD	Total	PC	SD	Total	PC	SD	Total	
Total No. of Prey	4291	3766	8057	391	1010	1401	507	0	507	9965
No. of Stomachs	55	37	92	3	16	19	11	0	11	122

followed by *D. d. bairdii* (74%), *T. truncatus* (25.9%), and *D. d. delphis* (17.5%). Mean fecal concentration was relatively high for *P. phocoena* (824 ng/g, S.E = 613 ng/g) and *D. d. bairdii* (11,703 ng/g, S.E. = 3634 ng/g) and relatively low for *T. truncatus* (68 ng/g, S.E. = 59 ng/g) and *D. d. delphis* (143 ng/g, S.E. = 109 ng/g). In presumed healthy *D. d. bairdii* that died from trauma ($n = 33$), 54.6% tested positive for DA with mean concentrations of 18.7 ng/g and 7.6 ng/ml in feces and urine, respectively.

Although DA was detected in cetacean feces during all seasons, the majority of detections occurred in the spring and summer seasons alongside peaks in *Pseudo-nitzschia* spp. cell counts. DA detections in PC cetaceans occurred during spring and summer seasons during most years of this study, except 2004, 2006, and 2014–2016 in which little to no data is available due to low stranding rates (Fig. 2). Spring data for 2015 is currently unavailable due to samples being withheld in litigation related to the Refugio oil spill (California Department of Fish and Wildlife, 2016). Fecal DA concentrations that are indicative of acute toxicity in *Z. californianus* (above 1000 ng/g)(Goldstein et al., 2008) were routinely observed in the PC area during spring and summer seasons from 2002 to 2013.

The majority of positive SD cetacean DA detections occurred in 2007, 2010–2012, and 2017; these years were associated with *Pseudo-nitzschia* spp. blooms, except 2007 when cell count data is unavailable (Fig. 3). Fecal DA detections in 2011 were unusual in that they were present winter through summer, during periods of lower cell counts. However, these cell counts (30,000 – 63,000 cells/L) were still well above the bloom threshold of 10,000 cells per liter (Lefebvre et al., 2002a). Highest fecal DA concentrations occurred in 2007 and 2017, indicating significant toxic bloom events during those years, with 2017 associated with a *P. seriata* group bloom.

3.1.1. Domoic acid prevalence by sex, geographic region, and diet

The proportion of *D. d. bairdii* males testing positive for DA (79%, $n = 146$) was higher relative to females (64%, $n = 83$; $p = 0.012$), and DA concentrations were higher in males (Table 5; $p = 0.021$, $W = 1385.5$). *D. d. bairdii* fecal DA concentrations varied spatially (Table 6), with concentrations higher in the PC group than the SD group ($p < 0.001$, $W = 3002.5$). Since there was a disparity in female sample sizes between regions (PC $n = 15$, SD $n = 30$), male fecal DA concentrations were compared between regions since they had relatively equal sample sizes (PC $n = 48$, SD $n = 34$) and were also found to be higher in the PC group than the SD group ($W = 419$, p-value = 0.00019). Accordingly, *Pseudo-nitzschia* spp. were detected more frequently in the PC area compared to the SD area (*P. delicatissima* group: $p < 0.001$; *P. seriata* group: $p = 0.030$). *P. delicatissima* group cell concentrations did not vary by region ($p = 0.9121$, $W = 141,550$), whereas *P. seriata* cell group concentrations were higher in the PC area compared to the SD area ($p < 0.001$, $W = 46,630$).

For the entire study area, the dominant prey for DA positive *D. d. bairdii* was northern anchovy (*Engraulis mordax*) (Fig. 4; Table 7). The relative abundance of *E. mordax* identified in stomach contents was not different between male and female *D. d. bairdii* ($p = 0.388$, $W = 470$). However, it was higher in *D. d. bairdii* adults compared to sub-adults ($p = 0.01762$, $W = 523$) and prey differences were observed between the northern and southern spatial groups (Fig. 1). The dominant prey of the PC group was *E. mordax* (63.5%) while the prey of the SD group comprised roughly equal proportions of *E. mordax* (31.7%) and market squid (*Doryteuthis opalescens*) (34.9%; Fig. 4). *E. mordax* was also the dominant prey for PC *D. d. bairdii* that were below the detection limit for DA, although the sample size was small ($n = 3$ stomachs). However, the abundance of *E. mordax* and *D. opalescens* in the diet of SD *D. d. bairdii* was lower in those that were below the detection limit for DA compared to those that were positive for DA (Table 7 and 8). Prey diversity was lower overall in the PC group vs. the SD group, regardless of whether DA was detected (Tables 7 and 8).

The primary prey consumed by DA-positive *P. phocoena* ($n = 10$) were *E. mordax* and *D. opalescens* (Table 9). Stomach contents for one *P. phocoena* that was below the detection limit for DA contained only *D. opalescens*.

3.2. Saxitoxin

STX was detected in 3 of 11 cetacean species tested: *D. d. bairdii, L. borealis* and *B. physalus*. Only 3.6% of individuals tested ($n = 165$) were positive and the maximum STX concentration of 17 ng/ml was observed in urine (Table 2). All animals that were STX positive were also positive for DA. STX detections for *D. d. bairdii* were associated with higher coastal *Alexandrium* spp. cell counts off San Diego in April 2011, while two SD STX detections were coincident with higher *Alexandrium* spp. cell counts in PC (Fig. 5).

Overall, *Alexandrium* spp. were more often present ($p < 0.001$) in the PC area, and when present, had higher cell counts in the PC area compared to the SD area ($p < 0.001$, $W = 609.0$) (Fig. 5). A comparison of STX detections between spatial areas was not possible due to skewed sample availability (PC, $n = 11$; SD, $n = 155$) and low detection rates.

Three stomachs from STX positive *D. d. bairdii* were examined. *E. mordax* was the most frequently occurring prey in these stomachs, whereas Pacific sardine (*Sardinops sagax*) was the most abundant (Table 10).

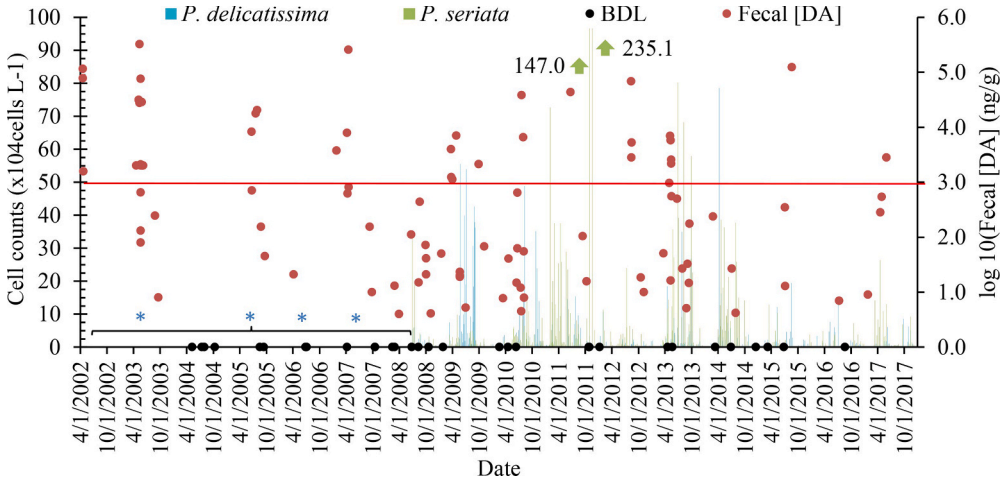

Fig. 2. *Pseudo-nitzschia* spp. cell counts at Goleta, Stearns Wharf, and San Luis Obispo Piers and domoic acid (DA) concentrations quantified in fecal samples in cetaceans from the Point Conception Group. Date indicates the day that either plankton was collected or the animal stranded. "BDL" = fecal specimens below DA detection limit (black dots). Red dots represent DA positive fecal samples. Black bracket indicates period where pier data was unavailable. Blue asterisks indicate the presence of *Pseudo-nitzschia* spp. blooms (2003 = 0.4×10^5 to 2×10^6 cells L^{-1}; 2005 - 2006 > 5.0×10^4 L^{-1}) detected via boat-based or shellfish surveys (Anderson et al., 2006, 2009; Smith et al., 2018). Red line indicates concentration at which acute toxicity documented in *Z. californianus* (1000 ng/g) (Goldstein et al., 2008).

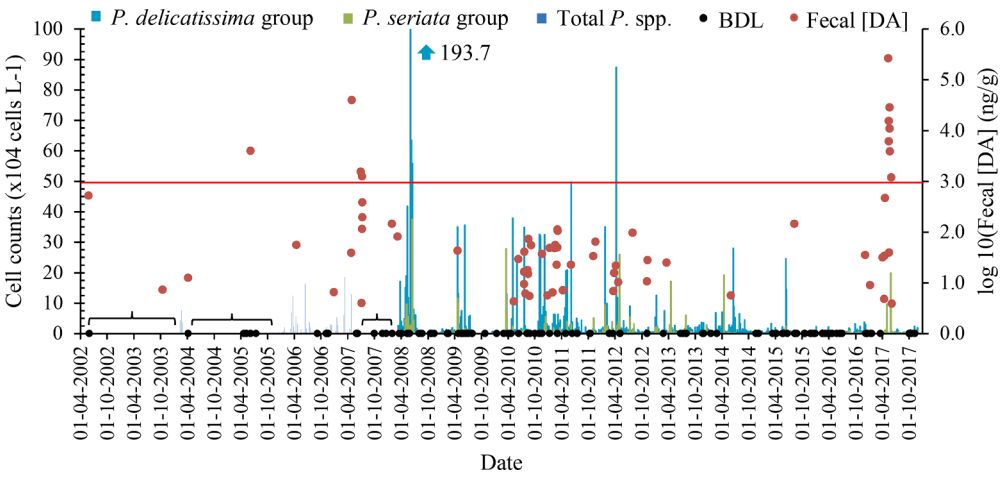

Fig. 3. *Pseudo-nitzschia* spp. cell counts at Scripps Pier and domoic acid detections in cetaceans from the SD Group. Date indicates the day that either plankton was collected or the animal stranded. "BDL" = fecal specimens below DA detection limit (black dots). Red dots represent DA positive fecal samples. Red line indicates concentration at which acute toxicity has been documented in *Z. californianus* (1000 ng/g) (Goldstein et al., 2008). Black brackets indicate periods without cell counts available.

Table 5
Domoic acid (DA) concentration (ng/g) in *D. d. bairdii* feces by sex.

Sex	n	Mean	Median	Min	Max
Female	45	11,461	46	4	263,623
Male	82	11,965	205	4	324,000

Table 6
Domoic acid (DA) concentration (ng/g) in *D. d. bairdii* feces by location.

Group	n	Mean	Median	Min	Max
Point Conception	64	16,679	889	4.5	324,000
San Diego	64	6727	41	4	263,623

3.3. Okadaic acid

Out of 85 individuals and 9 cetacean species tested for okadaic acid (Table 3), only the feces of one humpback whale (*Megaptera novaengliae*) that stranded on October 10, 2014 and one *D. d. delphis* that stranded on July 28, 2018 were positive, both in the SD region, at concentrations of 331.6 ng/g and 422.8 ng/g, respectively. *Dinophysis* spp. were present in the SD region during these time periods (Fig. 6). Neither of these OA-positive individuals tested positive for domoic acid or saxitoxin. *Dinophysis* spp. occurred more frequently in the PC area than the SD area ($p < 0.001$), but cell concentrations did not differ between regions ($p = 0.850$, $W = 25{,}754$).

3.4. Microcystin

The feces of one female neonate and five male *T. truncatus* individuals (3 calves, 1 juvenile, and 1 adult) were tested for microcystin and all were below the detection limit. The livers of two *D. d. bairdii* whose pathological review indicated necrotizing hepatitis were tested for microcystin; one adult male (KXD0163) collected on April 22, 2009 at Camp Pendleton, San Diego, was positive, at a concentration of 96.8 ng/g.

4. Discussion

DA, STX, OA, and microcystin were all detected in SCB cetaceans. However, DA was regularly detected in several species and observed at concentrations that are similar to those previously reported in known marine mammal DA toxicosis cases (Goldstein et al., 2008). This paper establishes a baseline for future monitoring of biotoxin levels in U.S. West Coast marine mammals and facilitates comparisons with future HAB events that are expected to continue with increased variability and intensity, along with changes in habitat boundaries and prey diversity and distribution (Santora et al., 2017, 2020; Trainer et al., 2020). The baseline data provided in this study will help managers document and interpret potential changes in exposure and mortality related to these ecosystem shifts.

4.1. Domoic acid

DA was reliably detected in both feces and urine. The slightly higher

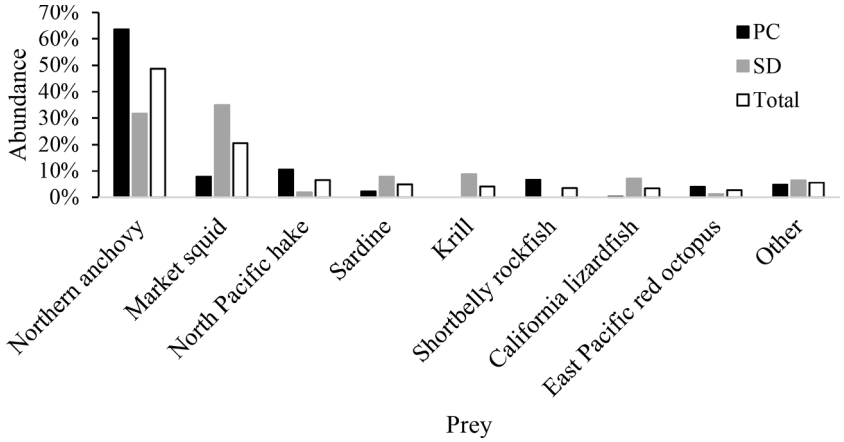

Fig. 4. Prey abundance of domoic acid (DA) positive *D. d. bairdii* by region (PC = Point Conception, SD = San Diego, Total = combined regions).

Table 7
Spatial differences in overall prey abundance (%A) and prey occurrence (%O) of *D. d. bairdii* in the Point Conception (PC) ($n = 55$) and San Diego (SD) ($n = 37$) groups whose feces or urine were positive for domoic acid (DA).

		Point Conception			San Diego			Entire Study Area		
Common Name	Scientific Name	No. of Prey	%A	%O	No. of Prey	%A	%O	No. of Prey	%A	%O
Northern anchovy	*Engraulis mordax*	2726	63.5%	80.0%	1194	31.7%	64.9%	3920	48.7%	73.9%
Market squid	*Doryteuthis opalescens*	338	7.9%	32.7%	1312	34.8%	56.8%	1650	20.5%	42.4%
Pacific hake	*Merluccius productus*	453	10.6%	27.3%	75	2.0%	10.8%	528	6.6%	20.7%
Pacific sardine	*Sardinops sagax*	100	2.3%	20.0%	294	7.8%	35.1%	394	4.9%	26.1%
Eucarida	*Eucarida*	0	0.0%	0.0%	329	8.7%	10.8%	329	4.1%	4.3%
Shortbelly rockfish	*Sebastes jordani*	287	6.7%	5.5%	0	0.0%	0.0%	287	3.6%	3.3%
California lizardfish	*Synodus lucioceps*	10	0.2%	5.5%	268	7.1%	32.4%	278	3.5%	16.3%
East Pacific red octopus	*Octopus rubescens*	172	4.0%	27.3%	49	1.3%	21.6%	221	2.7%	25.0%
Plainfin midshipman	*Porichthys notatus*	21	0.5%	9.1%	48	1.3%	8.1%	69	0.9%	8.7%
White croaker	*Genyonemus lineatus*	44	1.0%	16.4%	8	0.2%	10.8%	52	0.6%	14.1%
Pacific sanddab	*Citharichthys sordidus*	33	0.8%	21.8%	1	0.0%	2.7%	34	0.4%	14.1%
"ten-footed" crustacean	*Decapoda*	0	0.0%	0.0%	32	0.8%	2.7%	32	0.4%	1.1%
Rockfish	*Sebastes sp.*	20	0.5%	3.6%	12	0.3%	10.8%	32	0.4%	6.5%
Queenfish	*Seriphus politus*	0	0.0%	0.0%	32	0.8%	18.9%	32	0.4%	7.6%
Pacific butterfish	*Peprilus simillimus*	22	0.5%	14.5%	0	0.0%	0.0%	22	0.3%	8.7%
California smoothtongue	*Leuroglossus stilbius*	0	0.0%	0.0%	17	0.5%	2.7%	17	0.2%	1.1%
Jack mackerel	*Trachurus symmetricus*	3	0.1%	1.8%	14	0.4%	8.1%	17	0.2%	4.3%
Armhook squid	*Gonatus sp.*	1	0.0%	1.8%	13	0.3%	13.5%	14	0.2%	6.5%
Verrill's two-spot octopus	*Octopus bimaculatus*	0	0.0%	0.0%	10	0.3%	2.7%	10	0.1%	1.1%
Shiner perch	*Cymatogaster aggregata*	9	0.2%	7.3%	0	0.0%	0.0%	9	0.1%	4.3%
Northern lampfish	*Stenobrachius leucopsarus*	3	0.1%	3.6%	6	0.2%	2.7%	9	0.1%	3.3%
Spotted cusk-eel	*Chilara taylori*	3	0.1%	3.6%	4	0.1%	5.4%	7	0.1%	4.3%
Croaker	*Sciaenidae*	7	0.2%	5.5%	0	0.0%	0.0%	7	0.1%	3.3%
Butterfish	*Peprilus sp.*	0	0.0%	0.0%	6	0.2%	8.1%	6	0.1%	3.3%
California headlight fish	*Diaphus theta*	4	0.1%	1.8%	1	0.0%	2.7%	5	0.1%	2.2%
Boreopacific armhook squid	*Gonatopsis borealis*	3	0.1%	1.8%	2	0.1%	2.7%	5	0.1%	2.2%
Sculpin	*Icelinus sp.*	5	0.1%	1.8%	0	0.0%	0.0%	5	0.1%	1.1%
Pacific dover sole	*Microstomus pacificus*	5	0.1%	3.6%	0	0.0%	0.0%	5	0.1%	2.2%
Octopus squid	*Octopoteuthis sp.*	0	0.0%	0.0%	5	0.1%	5.4%	5	0.1%	2.2%
Topsmelts	*Atherinops affinis*	4	0.1%	5.5%	0	0.0%	0.0%	4	0.0%	3.3%
Shortbelly rockfish	*Sebastes jordani*	4	0.1%	1.8%	0	0.0%	0.0%	4	0.0%	1.1%
Bigfin lampfish	*Symbolophorus californiensis*	0	0.0%	0.0%	4	0.1%	5.4%	4	0.0%	2.2%
Enoploteuthid squid	*Abraliopsis sp.*	0	0.0%	0.0%	3	0.1%	5.4%	3	0.0%	2.2%
Pacific argentine	*Argentina sialis*	3	0.1%	3.6%	0	0.0%	0.0%	3	0.0%	2.2%
Krill	*Euphausiidae*	0	0.0%	0.0%	3	0.1%	2.7%	3	0.0%	1.1%
Black-eye squid	*Gonatus onyx*	3	0.1%	3.6%	0	0.0%	0.0%	3	0.0%	2.2%
Pacific mackerel	*Scomber japonicus*	1	0.0%	1.8%	2	0.1%	2.7%	3	0.0%	2.2%
Mexican lampfish	*Triphoturus mexicanus*	0	0.0%	0.0%	3	0.1%	8.1%	3	0.0%	3.3%
Shortspine combfish	*Zaniolepsis frenata*	1	0.0%	1.8%	2	0.1%	2.7%	3	0.0%	2.2%
Dogtooth lampfish	*Ceratoscopelus townsendi*	0	0.0%	0.0%	2	0.1%	5.4%	2	0.0%	2.2%
Jumbo squid	*Dosidicus gigas*	0	0.0%	0.0%	2	0.1%	2.7%	2	0.0%	1.1%
Octopus	*Octopus sp.*	0	0.0%	0.0%	2	0.1%	2.7%	2	0.0%	1.1%
Boreal clubhook squid	*Onychoteuthis borealijaponica*	0	0.0%	0.0%	2	0.1%	2.7%	2	0.0%	1.1%
Silversides	*Atherinidae*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Chiroteuthid squid	*Chiroteuthis sp.*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Speckled sanddab	*Citharichthys stigmaeus*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Glass squid	*Cranchia scabra*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Berry armhook squid	*Gonatus berryi*	1	0.0%	1.8%	0	0.0%	0.0%	1	0.0%	1.1%
Broadfin lampfish	*Lampanyctus ritteri*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Slender sole	*Lyopsetta exilis*	1	0.0%	1.8%	0	0.0%	0.0%	1	0.0%	1.1%
Stout argentine	*Nansenia crassa*	1	0.0%	1.8%	0	0.0%	0.0%	1	0.0%	1.1%
Pelagic tuberculate octopus	*Ocythoe tuberculata*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Basketweave cusk-eel	*Otophidium scrippsi*	1	0.0%	1.8%	0	0.0%	0.0%	1	0.0%	1.1%
Righteye flounders	*Pleuronectidae*	1	0.0%	1.8%	0	0.0%	0.0%	1	0.0%	1.1%
Blue lanternfish	*Tarletonbenia crenularis*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Unidentified squid	*Teuthoidea*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Pink surfperch	*Zalembius rosaceus*	0	0.0%	0.0%	1	0.0%	2.7%	1	0.0%	1.1%
Combfish	*Zaniolepis sp.*	1	0.0%	1.8%	0	0.0%	0.0%	1	0.0%	1.1%
	Total Prey	**4291**			**3766**			**8057**		
	Total Species	**34**			**42**			**58**		

detection rate in urine (56%) may be due to the lower detection limit of urine (0.4 ng/g) compared to feces (4 ng/g). The higher concentrations of DA observed in feces compared to urine reflects the pathway of DA through the body. DA exposures to marine mammals are through oral consumption and the dose is dependent on the vector species consumed (Bejarano et al., 2007). After consumption, DA containing prey is digested, absorbed then transported throughout the organism in blood. The fraction of DA absorbed is predicted to be low, as described both *in vitro* (Kimura et al., 2011) and *in vivo* (Iverson et al., 1990; Truelove et al., 1997). As such, feces is expected to contain higher concentrations of DA than urine. For DA exposures to wildlife, urine best represents the absorbed dose, and feces represents the oral exposure dose. The toxicokinetic elimination profile from oral or intravenous exposure to DA in monkeys has been described in detail by Jing et al. (2018), and showed a low bioavailability of DA, with renal clearance as a significant route of elimination of the absorbed dose.

DA concentrations that could result in immediate impairment and/or death (Goldstein et al., 2008) were observed in nine cetacean species

Table 8
Overall prey abundance (%A) and prey occurrence (%O) of *D. d. bairdii* in the Point Conception (PC) ($n = 3$) and San Diego (SD) ($n = 16$) groups whose urine or feces were below the detection limit for domoic acid (DA).

		Point Conception			San Diego		
Common name	Scientific Name	No. of Prey	%A	%O	No. of Prey	%A	%O
Cusk-eels	*Ophididae*	0	0.0%	0.0%	233	23.1%	6.3%
Northern anchovy	*Engraulis mordax*	362	92.6%	66.7%	210	20.8%	31.3%
Market squid	*Doryteuthis opalescens*	9	2.3%	33.3%	171	16.9%	37.5%
Plainfin midshipman	*Porichthys notatus*	0	0.0%	0.0%	128	12.7%	12.5%
Shortbelly rockfish	*Sebastes sp. cf S. jordani*	0	0.0%	0.0%	51	5.0%	6.3%
East Pacific red octopus	*Octopus rubescens*	0	0.0%	0.0%	44	4.4%	6.3%
Pacific hake	*Merluccius productus*	0	0.0%	0.0%	33	3.3%	18.8%
Pacific sardine	*Sardinops sagax*	1	0.3%	33.3%	25	2.5%	18.8%
California lizardfish	*Synodus lucioceps*	0	0.0%	0.0%	20	2.0%	25.0%
California tonguefish	*Symphurus atricauda*	0	0.0%	0.0%	16	1.6%	6.3%
Jacksmelt	*Atherinopsis californiensis*	0	0.0%	0.0%	15	1.5%	12.5%
Jack mackerel	*Trachurus symmetricus*	0	0.0%	0.0%	14	1.4%	18.8%
Pacific mackerel	*Scomber japonicus*	0	0.0%	0.0%	10	1.0%	18.8%
Queenfish	*Seriphus politus*	0	0.0%	0.0%	6	0.6%	12.5%
Fiery armhook squid	*Gonatus pyros*	0	0.0%	0.0%	5	0.5%	6.3%
Armhook squid	*Gonatus sp.*	0	0.0%	0.0%	5	0.5%	18.8%
Silversides	*Atherinidae*	0	0.0%	0.0%	4	0.4%	12.5%
White croaker	*Genyonemus lineatus*	0	0.0%	0.0%	4	0.4%	12.5%
Berry armhook squid	*Gonatus berryi*	0	0.0%	0.0%	3	0.3%	6.3%
Topsmelt	*Atherinops affinis*	1	0.3%	33.3%	3	0.3%	6.3%
Lingcod	*Ophiodon elongatus*	0	0.0%	0.0%	2	0.2%	6.3%
Longfin sanddab	*Citharichthys xanthostigma*	0	0.0%	0.0%	1	0.1%	6.3%
Anchovies	*Engraulidae*	0	0.0%	0.0%	1	0.1%	6.3%
Black-eyed squid	*Gonatus onyx*	0	0.0%	0.0%	1	0.1%	6.3%
Octopus	*Octopus sp*	0	0.0%	0.0%	1	0.1%	6.3%
Tuberculate pelagic octopus	*Ocythoe tuberculata*	0	0.0%	0.0%	1	0.1%	6.3%
Blackeye goby	*Rhinogobiops nicholsii*	0	0.0%	0.0%	1	0.1%	6.3%
Croakers	*Sciaenidae*	0	0.0%	0.0%	1	0.1%	6.3%
Halfbanded rockfish	*Sebastes semicinctus*	0	0.0%	0.0%	1	0.1%	6.3%
Pacific sandab	*Citharichthys sordidus*	1	0.3%	33.3%	0	0.0%	0.0%
Pacific butterfish	*Peprilis simillimus*	1	0.3%	33.3%	0	0.0%	0.0%
Teleosts	Teleostei	16	4.1%	33.3%	0	0.0%	0.0%
	Total Prey	**391**			**1010**		
	Total Species	**7**			**29**		

Table 9
Summary of prey abundance ($n = 507$) of domoic acid (DA) positive *P. phocoena*, collected between the years 2007 and 2011, in the Point Conception (PC) ($n = 9$) and San Diego (SD) ($n = 1$) groups.

Common Name	Scientific Name	Abundance
Northern anchovy	*Engraulis mordax*	53.45%
Market squid	*Doryteuthis opalescens*	43.39%
White croaker	*Genyonemus lineatus*	0.39%
Pacific red octopus	*Octopus rubescens*	0.39%
Pacific sardine	*Sardinops sagax*	1.18%
Rockfish	*Sebastes spp.*	0.59%
Chub mackerel	*Scomber japonica*	0.59%

that includes baleen whales, dolphins, and porpoise (Table 1), indicating a serious health risk to these individuals. Prey items for these species range from zooplankton to fish and squid. Total sample size for some of these species was small, which limits interpretation of prevalence and extension to population level impacts. The maximum fecal DA concentration (324,000 ng/g) in a SCB *D. d. bairdii* was orders of magnitude higher than previously observed in Alaskan marine mammals (Lefebvre et al., 2016), but similar, albeit higher, than maximum concentrations reported in central California marine mammals (134,178 ng/g, *Z. californianus* (Rust et al., 2014); 207,000 ng/g, blue whale (*Balaenoptera musculus)* (Lefebvre et al., 2002b).

Low DA concentrations in presumed healthy *D. d. bairdii* that died from trauma indicate the potential for chronic DA exposure at low concentrations. One hypothesis is that DA intoxication caused these dolphins to be more prone to trauma and thus were not healthy. However, the low DA concentrations in this subset of dolphins does not support this argument. Chronic low level exposure is a concern because it has been shown to increase sensitivity in subsequent exposures in vertebrate models (Lefebvre et al., 2012) and cause spatial learning impairment in mammalian models that is reversible through cessation of exposure (Lefebvre et al., 2017). This effect on cognitive function could impact foraging behavior and navigation during bloom periods.

Temporal patterns in fecal DA detections in southern California cetaceans indicates that they are seasonally (spring/summer) exposed to DA, concomitant with seasonal peaks in *Pseudo-nitzschia* spp. counts. If seasonal chronic exposure occurs, they could recover during the winter and fall seasons, as cognitive effects in mammalian models were reversible after nine weeks with no toxin exposure (Lefebvre et al., 2017). Overall, positive DA detections were associated with *Pseudo-nitzschia* spp. blooms. However, some blooms did occur without corresponding cetacean DA detections (Figs. 2 and 3). In some cases this reflects a lack of cetacean sample availability and in others it may indicate that not all *Pseudo-nitzschia* spp. blooms are toxic (Smith et al., 2018), captured by pier data, potent vectors (i.e. *E. mordax*) were not being consumed by cetaceans at those times, or a time-lag exists due to the trophic transfer of the toxin.

4.1.1. Domoic acid exposure by sex, geographic region, and diet

Higher DA detection rates and fecal DA concentrations in male *D. d. bairdii* indicates that they may be more at risk for DA exposure and toxicity. This finding is supported by Torres de la Riva et al. (2009), who found increased stranding rates of southern California male *D. d. bairdii* during a *Pseudo-nitzschia* spp. bloom. Male susceptibility to DA does not appear to be related to differences in diet, since abundance of *E. mordax* in the diet did not vary between sexes. This is further supported by two studies that reported similar diets between male and female *D. d. bairdii* in our study area (Osnes-Erie, 1999; Preti, 2020). However, it could be related to foraging location since it is possible that sex, age, or

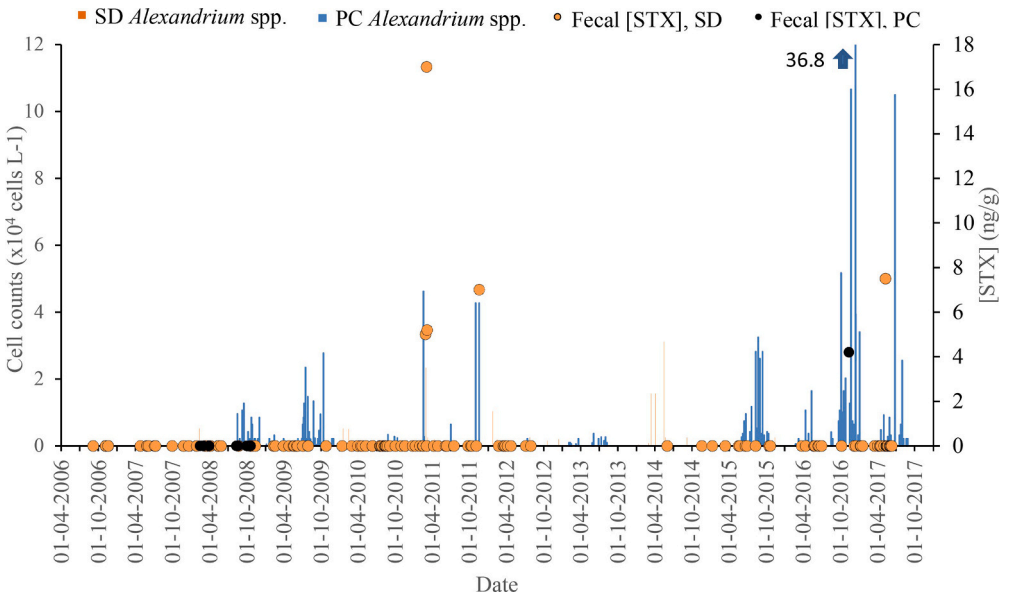

Fig. 5. Saxitoxin (STX) detections in urine and feces of southern California cetaceans and *Alexandrium* spp. cell counts in the Southern San Diego (SD) and Northern Point Conception (PC) groups. Date indicates the day that either plankton was collected or the animal stranded.

Table 10
Overall prey abundance (%A) and prey occurrence (%O) of 3 *D. d. bairdii* in the San Diego (SD) group whose urine or feces were saxitoxin (STX) positive.

Common Name	Scientific Name	No. of Prey	%A	%O
Northern anchovy	*Engraulis mordax*	79	39.1	100
Pacific sardine	*Sardinops sagax*	120	59.4	66.7
Market squid	*Doryteuthis opalescens*	2	0.99	33.3
Tuberculate pelagic octopus	*Ocythoe tuberculata*	1	0.50	33.3

reproductive class segregation in *Delphinus* spp. exists (Danil et al., 2010; Ferrero and Walker, 1995) as in other small delphinids (Miyazaki and Nishiwaki, 1978). It is possible that males could school separately and forage in more productive bloom areas. Similarly, differing DA exposure between sexes has been documented in *Z. californianus* and attributed to differences in foraging location (Bargu et al., 2010). Alternatively, physiological differences between sexes may make males more susceptible to neurotoxicity, as was observed in rats (Baron et al., 2013).

The increased DA exposure risk in Point Conception *D. d. bairdii* is likely driven by greater productivity (McClatchie, 2014) and consequently a more frequent occurrence of *Pseudo-nitzschia* spp., and specifically higher cell counts of the more toxigenic species (*P. seriata* group) in this region. In addition, the lower species diversity in the diet (primarily *E. mordax*) of PC *D. d. bairdii* predisposes them to DA exposure because *E. mordax* appears to be a more potent vector than other species (Bargu et al., 2008; Bejarano et al., 2007; Lefebvre et al., 2002a). A more diverse diet, as seen in SD *D. d. bairdii* (Tables 7 and 8), likely dilutes their exposure to DA, which was reflected in their lower fecal DA concentrations. This may also explain the lower fecal DA concentrations found in *P. phocoena* (Table 1, Table 9). The occurrence of *E. mordax* in

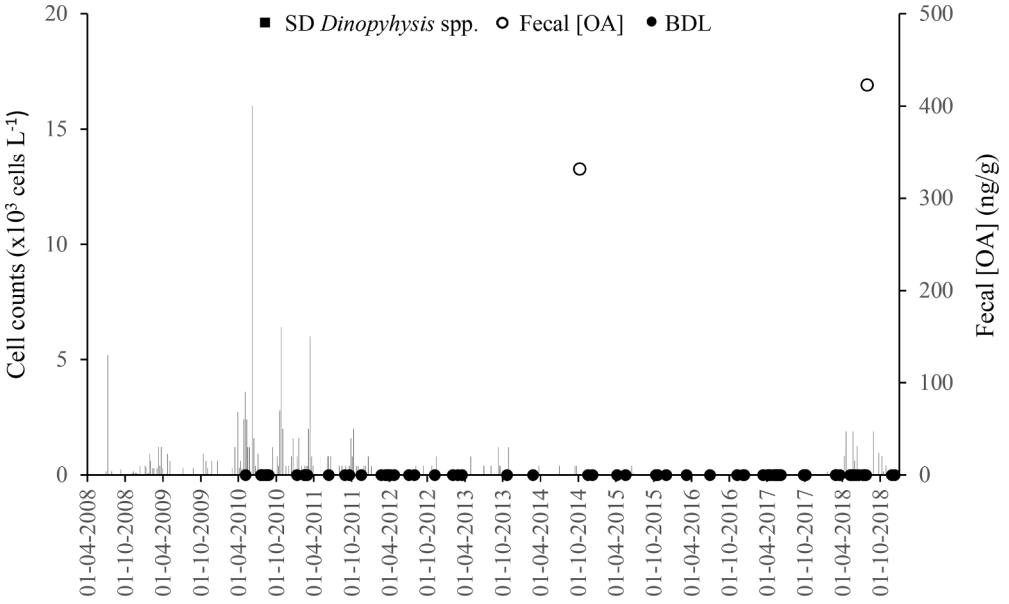

Fig. 6. Cetacean fecal okadaic acid (OA) detections and *Dinophysis* spp. cell counts in the San Diego (SD) group. Date indicates the day that either plankton was collected or the animal stranded.

the diet of DA positive *D. d bairdii* in the present study was higher than that reported previously for bycaught *D. d. bairdii* specimens in the study region (i.e., 73.9%O vs. 37.5%O; (Preti, 2020)). The diet of those bycaught specimens was more similar to that found in *D. d bairdii* below the detection limit for DA (31.3%O) and the most important prey item for *D. d bairdii* in that study was *D. opalescens*. This difference likely reflects the normal diet of the population vs. the unique diet of DA-exposed animals.

The increased abundance of *E. mordax* in the diet of adult *D. d bairdii* was also noted in another study of this species in the region (Preti 2020) and suggests that larger, older, sexually mature animals could be predisposed to DA exposure via this vector compared to their younger counterparts. It is plausible that this difference in diet between age classes exists due to varying nutritional needs of different life history stages. Sexually mature *D. d bairdii* likely benefit from a more nutrient-rich prey, such as *E. mordax*, compared to *D. opalescens* (Costa et al., 1991), that can provide more energy during periods of time with increased demands such as during reproduction.

The high detection rate of DA in southern California cetaceans suggests that it may be widespread in the ecosystem, which is further supported by its detection in numerous prey taxa along the West Coast of the U.S.: filter feeding bivalves, both benthic and pelagic fish, market squid (*D. opalescens*), and krill (Bargu et al., 2002, 2008; Lefebvre et al., 2002b; Vigilant and Silver, 2007). DA was detected more often and at higher mean concentrations in some species of cetaceans (i.e., *P. phocoena* and *D. d. bairdii)* than others (i.e. *T. truncatus* and *D. d. delphis*) and this may be related to diet. Both *D. d. bairdii* and *P. phocoena* have a higher prevalence of *E. mordax*, a known vector of DA, in their diet than *D. d. delphis* and *T. truncatus* (Preti, 2020; Toperoff, 2002; Walker, 1981).

4.2. Saxitoxin

Saxitoxin was not common in southern California cetaceans, but when detected it was concurrent with *Alexandrium* spp. blooms in the SCB. Two freshly dead SD cetaceans were positive for STX, but the strandings did not co-occur with a documented SD *Alexandrium* spp. bloom, suggesting offshore blooms may have occurred that were not detected by pier sampling or because prey can accumulate STX over a period of weeks and therefore not match a specific bloom period (Jester et al., 2009). STX concentrations were well below the maximum concentrations reported in marine mammals along western North America (Lefebvre et al., 2016; Starr et al., 2017). However, data on STX toxicity in marine mammals is limited, making interpretation difficult. A die-off of multiple taxa in Canada was associated with an *Alexandrium tamarense* bloom and the maximum marine mammal fecal concentration of STX for this event was 570 ng/g (Starr et al., 2017). Fire et al. (2020) suggested a 5-fold baseline threshold to indicate the severity of a STX event, which illustrates the importance of obtaining baseline data.

Concurrent exposure of both STX and DA in six cetaceans was observed in this study, which has also been reported in other marine mammals (Doucette et al., 2012; Lefebvre et al., 2016) and with other algal toxins (Fire et al., 2011; Twiner et al., 2011). Although the impact of this synergy is unknown, *in vitro* experiments of OA and gymnodimine indicate that co-occurrence of toxins may increase toxicity (Dragunow et al., 2005).

In California, STX has been found to accumulate in filter feeding bivalves, the common sand crab (*Emerita analoga*), three species of planktivorous fish, and two species of rock crab (Bretz et al., 2002; Jester et al., 2009). It is likely that the vector of STX in the dolphins of this study were planktivorous fish, specifically *E. mordax*, as this was common to all three dolphins (Table 10). Similarly, Atlantic mackerel (*Scombrus scomber*), a partially planktivorous fish, was identified as a possible vector of STX in *M. novaengliae* from the Atlantic ocean (Geraci et al., 1989).

4.3. Okadaic acid

The OA detection (422.8 ng/g) in the *D. d. delphis* is the highest reported concentration for marine mammals to date, with previous reports in *O. byronia, T. truncatus, T. m. latirostris, and A. australis* feces up to 9 ng/g, 10 ng/g, 16 ng/g, and 36 ng/g, respectively (Capper et al., 2013; Fire et al., 2017, 2011). Since this toxin is lipophilic, the biological residence time is long and may take up to 4 weeks to be excreted from the intestine (Ito et al., 2002). This makes it difficult to determine when the vector was consumed. In the case of the *M. novaengliae*, the vector of OA had been consumed one week or more before it stranded as it had been sighted alive, injured and immobile, unable to feed, for that period of time before death. *Dinophysis* spp. were present in the SD area where these individuals stranded, during these periods. However, higher cell counts of *Dinophysis* spp. have occurred without concurrent or time-lagged (consistent with a four week excretion time) fecal OA detections (Fig. 6).

4.4. Microcystin

Microcystin was not detected in the *T. truncatus* samples. This could be due to small sample size, an inappropriate matrix (feces), missed low level ELISA microcystin detections (Brown et al., 2018), or reflect a low prevalence of this toxin in their prey type or in the SCB. An examination of nine stomachs by Walker (1981) indicated that coastal California *T. truncatus* prey mainly upon croakers and perches, which in turn prey upon worms, crustaceans, and small fish (Love, 2011). This contrasts the diet of *D. d. bairdii* for whom there was one positive microcystin detection; *D. opalescens* and *E. mordax* are considered their most important prey items (Preti, 2020). Zooplankton are a known vector of microcystin in freshwater fish (Sotton et al., 2014) and so it is possible that the consumption of planktivorous fish (*E. mordax*) by *D. d bairdii* could make them more prone to microcystin exposure than *T. truncatus*, which do not largely prey on planktivorous fish. Although *D. d. bairdii,* who typically live within 30 km of shore, may not be exposed as often to fresh water plumes as coastal *T. truncatus*, it is plausible that their migrating prey are exposed. *E. mordax* can be found from the surf zone to 300 miles off the coast (Love, 2011).

The microcystin detection in one of two tested *D. d. bairdii* individuals was at a concentration (96.8 ppb) similar to those that were linked to sea otter deaths (1.4–104.5 ppb) (Miller et al., 2010). The prey ingested by this individual are unknown as its stomach was empty. Although false positive results have occurred in ELISA analyzed dolphin livers (Brown et al., 2018), we are confident in our results because we tested several samples of the same type collected from managed-care (aquarium) animals as negative controls and found acceptable levels of non-specific binding of antibodies to the sample matrix. *D. d. bairdii* warrants further testing and could potentially serve as a sentinel species for this toxin.

The positive microcystin detection confirms the transfer of this toxin from inland waters (Fetscher et al., 2015; Howard et al., 2017) to the SCB marine ecosystem as observed by Tatters et al. (2017) who detected microcystin at 50% of San Diego coastal sampling sites during the year 2015. A decision to monitor microcystin was made retrospectively and thus the availability of archived samples for this study was low, particularly due to low stranding rates of *T. truncatus* in the region. Increasing the sample size for microcystin testing in the future should help clarify the role of this toxin in coastal SCB marine mammals.

5. Conclusions

Observed spatial and temporal trends in cell counts and biotoxin detections and concentrations provide a reference for interpreting future harmful algal bloom and marine mammal mortality events. Overall, DA was frequently detected in SCB cetaceans, identified in the majority of cetacean species, and at concentrations indicative of acute toxicity

(Goldstein et al., 2008) in many of these species. The risk of DA exposure and toxicity was greater for *D. d. bairdii* inhabiting the northern range of the SCB during spring and summer, *D. d. bairdii* males, adult *D. d bairdii*, and those ingesting a diet rich in *E. mordax*. Further studies on additional prey vectors, offshore DA monitoring, and acute toxicity in cetaceans would help fill some knowledge gaps. STX, OA, and microcystin were detected less frequently in SCB cetaceans and in few cetacean species. However, the single microcystin detection was at a concentration known to cause death in sea otters (Miller et al., 2010) and the concentrations of okadaic acid were the highest recorded in marine mammals to date (Capper et al., 2013; Fire et al., 2017, 2011), suggesting that these biotoxins could impact the health of individual SCB cetaceans. Overall, DA is the biotoxin of greatest concern for *D. d. bairdii,* due to the prevalence and concentrations observed. All four biotoxins warrant future monitoring to better understand their role across multiple cetacean species and to detect shifting baselines.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The scientific results and conclusions, as well as any views or opinions expressed herein, are those of the authors and do not necessarily reflect the views of NOAA or the Department of Commerce. Funding support for toxin analyses was provided in part by NOAA John H. Prescott Marine Mammal Rescue Assistance Grant Program #NA17NMF4390082.

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