BioOne COMPLETE

THE ROLE OF DOMOIC ACID IN ABORTION AND PREMATURE PARTURITION OF CALIFORNIA SEA LIONS (ZALOPHUS CALIFORNIANUS) ON SAN MIGUEL ISLAND, CALIFORNIA

Authors: Goldstein, Tracey, Zabka, Tanja S., DeLong, Robert L., Wheeler, Elizabeth A., Ylitalo, Gina, et al.

Source: Journal of Wildlife Diseases, 45(1) : 91-108

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-45.1.91

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

Journal of Wildlife Diseases, 45(1), 2009, pp. 91–108
© Wildlife Disease Association 2009

THE ROLE OF DOMOIC ACID IN ABORTION AND PREMATURE PARTURITION OF CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*) ON SAN MIGUEL ISLAND, CALIFORNIA

Tracey Goldstein,[1,8,10] Tanja S. Zabka,[1] Robert L. DeLong,[2] Elizabeth A. Wheeler,[1] Gina Ylitalo,[3] Sibel Bargu,[4,9] Mary Silver,[4] Tod Leighfield,[5] Frances Van Dolah,[5] Gregg Langlois,[6] Inga Sidor,[7] J. Lawrence Dunn,[7] and Frances M. D. Gulland[1]

[1] The Marine Mammal Center, 1065 Fort Cronkhite, Marin Headlands, Sausalito, California 94965, USA
[2] National Marine Mammal Laboratory, Alaska Fisheries Science Center, 7600 Sand Point Way, Seattle, Washington 98115, USA
[3] US Department of Commerce, NOAA Fisheries, Northwest Fisheries Science Center, 2725 Montlake Blvd. East, Seattle, Washington 98112, USA
[4] Department of Ocean Sciences, Earth and Marine Sciences, Building A-312, University of California, Santa Cruz, California 95064, USA
[5] National Ocean Services Laboratory, Center for Coastal Environmental Health and Biomolecular Research, 219 Fort Johnson Road, Charleston, South Carolina 29412, USA
[6] California Department of Public Health, 850 Marina Bay Parkway, Richmond, California 94804, USA
[7] Mystic Aquarium and Institute for Exploration, 55 Coogan Blvd., Mystic, Connecticut 06355, USA
[8] Current address: Wildlife Health Center, University of California, TB 128 Old Davis Road, Davis, California 95616, USA
[9] Current address: Department of Oceanography and Coastal Sciences, School of the Coast and Environment, Louisiana State University, Baton Rouge, Louisiana 70803, USA
[10] Corresponding author (email: tgoldstein@ucdavis.edu)

ABSTRACT: Domoic acid is a glutaminergic neurotoxin produced by marine algae such as *Pseudo-nitzschia australis*. California sea lions (*Zalophus californianus*) ingest the toxin when foraging on planktivorous fish. Adult females comprise 60% of stranded animals admitted for rehabilitation due to acute domoic acid toxicosis and commonly suffer from reproductive failure, including abortions and premature live births. Domoic acid has been shown to cross the placenta exposing the fetus to the toxin. To determine whether domoic acid was playing a role in reproductive failure in sea lion rookeries, 67 aborted and live-born premature pups were sampled on San Miguel Island in 2005 and 2006 to investigate the causes for reproductive failure. Analyses included domoic acid, contaminant and infectious disease testing, and histologic examination. *Pseudo-nitzschia* spp. were present both in the environment and in sea lion feces, and domoic acid was detected in the sea lion feces and in 17% of pup samples tested. Histopathologic findings included systemic and localized inflammation and bacterial infections of amniotic origin, placental abruption, and brain edema. The primary lesion in five animals with measurable domoic acid concentrations was brain edema, a common finding and, in some cases, the only lesion observed in aborted premature pups born to domoic acid–intoxicated females in rehabilitation. Blubber organochlorine concentrations were lower than those measured previously in premature sea lion pups collected in the 1970s. While the etiology of abortion and premature parturition was varied in this study, these results suggest that domoic acid contributes to reproductive failure on California sea lion rookeries.

Key words: Abortion, California sea lion, contaminants, domoic acid, infectious disease, premature births, reproduction.

INTRODUCTION

California sea lions (*Zalophus californianus*) range along the Pacific coast of North America and breed on islands off southern California, Baja California, Mexico, and in the Gulf of California (Lluch, 1969; Mate, 1977). One of the primary breeding rookeries is on San Miguel Island, one of California's Channel Islands (Odell, 1971; Fig. 1). In the late 1970s, a high proportion (20%) of California sea lion pups born on the island were reported to die following premature parturition (Gilmartin et al., 1976). Higher concentrations of the organochlorine contaminants dichloro-diphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCB) were found in tissues from premature parturient dams and their pups than those measured in full-term dam-pup pairs (DeLong et al., 1973). High levels of contaminants, particularly PCBs and DDTs, were discharged into the Southern

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

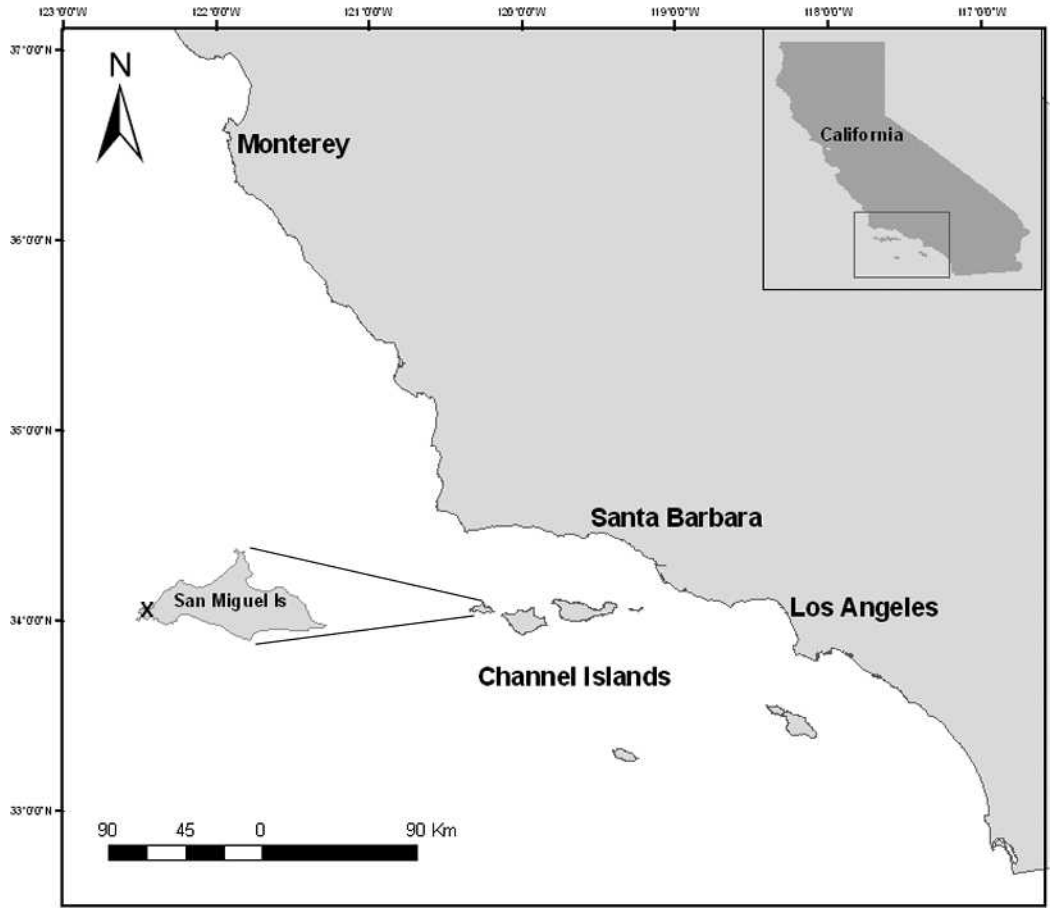


FIGURE 1. Map of California showing the locations of San Miguel Island and the sea lion rookery (x).

California Bight prior to passage of the Clean Water Act in 1977; however, some areas of the Bight are beginning to show environmental recovery (Kennish, 1998). Nonetheless, organochlorines such as PCBs and DDTs persist in this region, high levels are still observed in sea lions (Ylitalo et al., 2005; Greig et al., 2007), and experimental exposure studies have shown these chemicals to affect both immune function and reproductive success of pinnipeds (Reijnders, 1986; Ross et al., 1996). These chemicals have also caused reproductive failure in both experimental models and other wildlife species (Hart et al., 1971; Bruckner et al., 1973; Fry, 1995). Sea lions may accumulate these contaminants via prey since high levels of contaminants have been measured in demersal fish species (Zeng and Tran, 2002), as well as through transplacental transfer and lactation (Greig et al., 2007).

A calicivirus, San Miguel sea lion virus, and *Leptospira interrogans* serovar *pomona* have also been isolated from premature parturient dams and their pups. Both *Leptospira* spp. and caliciviruses have been associated with reproductive failure in domestic species, and antibody prevalence is widespread to both pathogens in California sea lions (Smith et al., 1979; Smith, 2000; Levett, 2001; Colagross-Schouten et al., 2002). Further studies therefore concluded that the high prevalence of reproductive failure was likely due to a combination of exposure to high organochlorine concentrations and infectious disease, rather than due to

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

contaminants alone (O'Shea and Brownell, 1998).

Reproductive failure has since been reported in California sea lions admitted to rehabilitation facilities while suffering from acute intoxication with domoic acid (Brodie et al., 2006). Domoic acid is a neurotoxin that binds to glutamate receptors in the brain, resulting in seizures (Jeffery et al., 2004). It is produced by algae, especially diatoms of the genus *Pseudo-nitzschia*. In 1998, domoic acid neurotoxic effects were documented in stranded California sea lions through exposure from contaminated prey such as northern anchovies (*Engraulis mordax*) and Pacific sardines (*Sardinops sagax*) (Scholin et al., 2000). Primary lesions observed upon histopathologic examination of the sea lions implicated limbic system seizure injury consistent with excitotoxin exposure and included hippocampal necrosis and/or atrophy (Silvagni et al., 2005). Almost one third of all affected adult females suffered from reproductive failure due to abortions or premature live births, and domoic acid was shown to cross the placenta, as the toxin was detected in both maternal urine and fetal fluids (Brodie et al., 2006). Domoic acid has also been shown to readily cross the placenta in experimentally exposed rat models (Dakshinamurti et al., 1993; Levin et al., 2005). It has been hypothesized that the nonkeratinized skin of the fetus facilitates transfer of the toxin by providing a poor barrier to the toxin after absorption into the amniotic fluid (Maucher and Ramsdell, 2007).

Since 1998, blooms of domoic acid–producing *Pseudo-nitzschia* spp. have been regularly documented in the Southern California Bight, especially between February and June, and they have been associated with marine mammal mortality events in the region (Anderson et al., 2006; Schnetzer et al., 2007; Goldstein et al., 2008). Normal pupping of California sea lions on San Miguel Island occurs in late May and June; aborted and premature live pups have been observed as early as February, increasing in frequency until mid-May, when pups may become viable (DeLong et al., 1973). The simultaneous occurrence of these toxin-producing spring algal blooms with premature pupping of California sea lions on rookeries, coupled with evidence of reproductive failure in domoic acid–intoxicated sea lions in rehabilitation, suggests that domoic acid toxicosis may play a role in reproductive failure in this species. The purpose of this study was to investigate the current causes of premature parturition in California sea lions on San Miguel Island, and to evaluate whether domoic acid contributed to this reproductive failure.

MATERIALS AND METHODS

Animals and sample collection

Sixty-seven aborted or live-born premature sea lions pups were collected on San Miguel Island (34.02°N, 120.26°W) from beaches on the western and northwestern points (Northwest Point to Adams Cove) in 2005 and 2006. Sampling was conducted during three periods in the third weeks of March (2006) and April (2005 and 2006). Therefore, these pups were considered to be premature since they were born prior to May 15, when pups become viable (DeLong et al., 1973). Six pups were found alive but agonal and were euthanized with intravenous Beuthanasia-D Solution (Schering-Plough Animal Health Corporation, Kenilworth, New Jersey, USA). Fetuses were weighed and measured for standard length (tip of snout to tip of tail), axillary girth, umbilical girth, ventral blubber thickness (mid-sternum), and complete sampling (as described next) was performed on 59 that were fresh and intact. Partial sampling was performed on 18 fetuses because they were either decomposing and/or partially scavenged.

Tissue samples from the visceral cavity (tongue, tonsil, salivary gland, thyroid, thymus, esophagus, trachea, lung, heart, tracheobronchial and mesenteric lymph nodes, stomach, intestines, liver, gall bladder, spleen, kidney, adrenal, urinary tract, reproductive tract), brain, eye, muscle, skin, umbilicus, and placenta (when available) were preserved in 10% buffered formalin for histologic examination. Swabs of amniotic fluid, placenta, liver, spleen, and stomach contents were collected into Amies transport media without charcoal

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

(BD Diagnostics, Franklin Lakes, New Jersey, USA) for aerobic and *Brucella* spp. culture, and swabs of stomach contents were also collected into Cary-Blair transport medium (BD Diagnostics) for culture of *Campylobacter* spp. Swabs were held at 4 C for up to 7 days until processing. Samples of placenta, brain, aqueous humor, tonsil, tracheobronchial lymph node, lung, heart, stomach contents, umbilicus, liver, spleen, adrenal, kidney, and skeletal muscle were frozen in liquid nitrogen then stored at −80 C until analysis. Fetal fluids (amniotic fluid, feces, urine, stomach contents, serum) were frozen in liquid nitrogen in the field and then stored at −80 C until analysis. Blubber and liver were collected into Teflon sheets, and bile was collected into glass vials, and samples were frozen in liquid nitrogen, and then stored at −20 C (blubber, liver) and −80 C (bile) until analysis.

Environmental sampling was conducted for *Pseudo-nitzschia* spp. and domoic acid during the aforementioned three sampling periods. Forty scat samples were collected randomly off the rookery and frozen at −20 C. Five vertical plankton tows off the coast of San Miguel Island were conducted using plankton nets with a 20-μm-mesh-size Nytex net (Research Nets, Bothell, Washington, USA) to obtain water-column samples. Plankton tows varied from a depth of 3 m (onshore) to 9 m (collected by boat offshore). A minimum volume of 100 ml of the net tow sample was placed in a 125-ml Nalgene® bottle (Nalge Nunc International Corporation, Rochester, New York, USA), preserved in 1% formalin, and stored at room temperature for phytoplankton identification.

Tissue samples were also collected, as described already, from fetuses aborted from 74 California sea lions in rehabilitation following domoic acid toxicosis (Gulland et al., 2002; Brodie et al., 2006), after which they were fixed in formalin and examined by light microscopy.

Sample analysis

Formalin-fixed tissues were processed by routine methods for paraffin wax embedding, sectioned at 5-μm intervals, and stained with hematoxylin and eosin at the Histology Laboratory, University of California, Davis, Veterinary Medical Teaching Hospital. Tissue sections were examined histologically by a single pathologist, and special review of select cases was performed by Dr. T. Spraker, Colorado State University Veterinary Teaching Hospital, Fort Collins, Colorado, USA.

Swabs in transport media were submitted to University of California Davis Veterinary Microbiology Laboratory for *Campylobacter* spp. culture and to the California Animal Health and Food Safety (CAHFS) Laboratory for aerobic and *Brucella* spp. culture. Aerobic cultures were performed on 5% sheep blood agar and MacConkey agar and incubated at 35–37 C for 48 hr. The *Brucella* cultures were performed on *Brucella* agar with and without ethyl violet and incubated at 35–37 C for 10 days. Identification was performed by conventional microbiology methods or by using the API system (Biomerieux Inc., Marcy l'Etoile, France; O'Hara et al., 2003). Swabs in Cary-Blair transport medium were streaked on a *Brucella* agar base with 5% sheep blood and vancomycin, amphotericin B, and cefoperazone (Campy-CVA agar; Hardy Diagnostics, Santa Maria, California, USA) and incubated at 37 C for 48 to 96 hr in 5.5-l AnaeroPack containers (Mitsubishi Gas Chemical America, Inc., New York, New York, USA) using CampyGen sachets (Oxoid, Inc., Ogdensburg, New York, USA) to produce a microaerophilic environment for culture of *Campylobacter* spp. (Stoddard et al., 2007).

Polymerase chain reaction (PCR) analysis was performed by the Colorado Veterinary Diagnostic Laboratories, Fort Collins, Colorado, USA, on kidney and aqueous humor samples for the detection of *Leptospira* spp. and on lung for *Chlamydia* DNA. Assays targeting the *ompA* gene, which codes for the major outer membrane protein, and the 16 s RNA gene were used to detect chlamydial and leptospiral DNA, respectively (Hewinson et al., 1997; Harkin et al., 2003). Marine *Brucella* testing was conducted at the Canadian Food Inspection Agency, Ottawa, Ontario, Canada, with primers targeting the *omp*2 locus by infrequent restriction site–PCR(IRS-PCR), taking into account the higher number of IS*711* elements in their genome to identify terrestrial mammal and marine *Brucella* species genes (Cloeckaert et al., 2003). Placental tissues were also examined for *Brucella* species by culture and real-time PCR targeting the *Brucella* genus-specific outer membrane protein *bcsp31* at the Mystic Aquarium Laboratory, Mystic, Connecticut, USA. Liver tissues were submitted to the University of Florida, College of Veterinary Medicine, Gainesville, Florida, USA, for Otarine herpesvirus-1 testing. Primers targeted the *DNA polymerase* gene as described by Buckles et al. (2006), with one modification (annealing temperature was increased from 60 C to 63 C). Umbilical tissues were tested for caliciviral RNA by the Institute of Animal Health, Pirbirght Laboratory, UK. A real-time RT-PCR assay targeting

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

conserved nucleotide sequences in the *RNA-dependent RNA polymerase* (3D) region of the genome was designed to specifically amplify marine caliciviral RNA (Reid et al., 2007). Brain tissues were tested for *Toxoplasma gondii* and *Sarcocystis neurona* DNA at the Lucy Whittier Molecular and Diagnostic Core Facility at the University of California, Davis, California, USA; real-time TaqMan® PCR was used with primers that targeted a conserved region of the 16 s RNA gene and an internal fluorescent-labeled TaqMan probe (5′ end, reported dye FAM [6-carboxyflourescein], 3′ end, quencher dye TAMRA [6-carboxytetramethylrhodamine]).

Special stains and immunohistochemistry were performed on tissues from selected cases based on histopathologic findings. Tests included the Grocott's silver stain (GMS) for fungi, Brown and Brenn (B&B) gram stain and Steiner stain for bacteria, and polyclonal antibodies for *Toxoplasma*, *Neospora*, and *Sarcocystis* spp. as described by Miller et al. (2001).

Fetal fluids were submitted to the National Oceans Services Laboratory Marine Biotoxin Laboratory, Charleston, South Carolina, USA, for domoic acid analysis using a direct competitive ELISA (Biosense Laboratories, Bergen, Norway; Maucher and Ramsdell, 2005). Stomach contents were diluted 1/100, urine was diluted 1/200, and amniotic fluid was diluted 1/200 prior to analysis. The detection limits of this assay were 0.5 ng/ml for stomach fluid, 15 ng/ml for urine, and 3.0 ng/ml for amniotic fluid. These dilutions were chosen based on the highest amount of matrix that could be added to the domoic acid ELISA without causing a false positive result. The sample extract from positive samples was then analyzed to confirm the presence of domoic acid using tandem mass spectrometry coupled with liquid chromatographic separation (LC-MS/MS) as described by Van Dolah et al. (1997). Rookery scat samples were subsampled and submitted to the University of California, Santa Cruz (Dr. M. Silver's laboratory), for analysis. Scanning electron microscopy (SEM) was used to identify *Pseudo-nitzschia* spp. Domoic acid was extracted from rookery scat samples as described by Bargu et al. (2002), and the extracts were analyzed for the presence of domoic acid using high-performance liquid chromatography (HPLC) equipped with a diode array detector (DAD) according to methods by Lefebvre et al. (2002).

Following subsampling for domoic acid analysis, the rest of the scat samples were prepared for prey identification at the National Marine Mammal Laboratory, Seattle, Washington, USA. Samples were thawed and rinsed in nested sieves (1.0 mm, 0.71 mm, 0.5 mm), fish structures were dried and stored in glass vials, and cephalopod remains were stored in vials with 70% isopropyl alcohol. Prey were identified to the lowest possible taxon by using sagittal otoliths, skeletal and cartilaginous remains from fish, and beaks and statoliths from cephalopods according to methods by Orr et al. (2004). The abundance of prey taxa in sea lion scat was described by using the minimum number of individuals (MNI) and percent frequency of occurrence (%FO).

Blubber and bile from a subset of pups were submitted to the Northwest Fisheries Science Center, Environmental Assessment Program, Seattle, Washington, USA, for contaminant analysis. Blubber samples were analyzed for concentrations of PCBs (congeners 17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101, 105, 110, 118, 128, 138, 149, 151, 153, 156, 158, 170, 171, 180, 183, 187, 191, 194, 199, 205, 206, 208); DDTs (metabolites *o,p*-dichlorodiphenyldichloroethane [DDD], *p,p*-DDD, *p,p*-dichlorodiphenyl-dichloroethylene, [DDE], *o,p*-DDT, *p,p*-DDT, and hexachlorobenzene); polybrominated diphenal esters (PBDE) flame retardants (congeners 28, 47, 49, 66, 85, 99, 100, 153, 154, 183); hexachlorobenzene; hexachlorocyclohexanes (α-HCH, β-HCH); chlordanes (lindane, *alpha*-chlordane, *cis*-nonachlor, gamma-chlordane, heptachlor, heptachlor expoxide, nonachlor III, oxychlordane, *trans* nonachlor); dieldrin; aldrin; mirex; and endosulfan sulfate by a gas chromatography/mass spectrometry method (Sloan et al., 2005). Summed contaminant concentrations were calculated by adding the concentrations of the individual congeners or metabolites as appropriate. Bile samples were analyzed for the metabolites of polycyclic aromatic hydrocarbons (PAHs) using high-performance liquid chromatography with fluorescence detection (HPLC/uvf) as described in Krahn et al. (1986). The concentrations of fluorescent PAHs in bile were determined using phenanthrene (PHN) and benzo[a]pyrene (BaP) as external standards and converting the fluorescence response of bile to phenanthrene (ng PHN equivalents/g bile) and benzo[a]pyrene (ng BaP equivalents/g bile) equivalents. Bile metabolites fluorescing at phenanthrene wavelengths were considered to be an indicator of exposure to low-molecular-weight PAHs, while metabolites fluorescing at benzo[a]pyrene (BaP) wavelengths were considered to be an indicator of exposure to high-molecular-weight PAHs.

Water samples were submitted to the

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

California Department of Public Health (CDPH), Richmond, California, USA, for examination by light microscopy for the presence of toxigenic genera of phytoplankton. The samples were collected as a part of CDPH's volunteer-based Marine Biotoxin Monitoring Program, which monitors for the occurrence of toxic algal blooms along the California coast. The percent composition of all phytoplankton genera identified in the sample and the settled volume were estimated, and then a relative abundance index (RAI) was derived for *Pseudo-nitzschia* spp. The RAI was based on an estimate of cell mass using estimates of settled cell volume (a), the percent composition of each species (b), and the sampling effort as determined by the total tow length (c): RAI = (a×b)/c, for each sample collected. When possible, phytoplankton were identified to the species level.

RESULTS

Environmental sampling

Pseudo-nitzschia species were identified in water samples collected during all three sampling periods and comprised up to 5% and 10% of the phytoplankton composition in 2005 and 2006, respectively. The phytoplankton community included a mix of toxic (*Pseudo-nitzschia australis* and *Pseudo-nitzschia seriata*) and nontoxic (*Pseudo-nitzschia delicatissima*) species and was for the most part dominated by the toxic species. Widespread elevated domoic acid concentrations were found in shellfish throughout southern California during the sampling periods, and phytoplankton samples tested for domoic acid during March 2006 at Cal Poly Pier off San Luis Obispo County tested as a part of the California Program for Regional Enhanced Monitoring for PhycoToxins (Cal-PReEMPT) were positive (G. Langlois, pers. obs.; http://ww2.cdph.ca.gov/HealthInfo/environhealth/water/Documents/Shellfish/MonthlyandQuarterlyReports/2006/Biotoxin_Monthly_0306.pdf). Seventy percent (26/37, three of the 40 scat samples could not be cleaned sufficiently to be processed) of the rookery scat samples contained *Pseudo-nitzschia* frustules observed by SEM and domoic acid detected by HPLC. Concentrations of domoic acid ranged from 10 to 13,300 ng/ml.

Fish otoliths and other skeletal structures were found in all of the scat samples. Eleven different prey types were identified in samples that tested positive for domoic acid (Fig. 2); northern anchovies (85%) had the highest percent frequency of occurrence, followed by Pacific sardines (48%) and Pacific hake (*Merluccius productus*, 44%). Of the other prey identified, only Pacific hake occurred alone in a scat sample that contained domoic acid, while the other fish occurred with other species.

Animal sampling

Most of the pups collected were in the early third trimester, since they were sampled in April (n=64), whereas fewer pups were in the late second trimester, sampled in March (n=13). Overall, the pups sampled in April were larger (Table 1). Dam condition was recorded for 22 of the premature pups: 21 appeared bright, alert, and in good nutritional status, and one was observed seizuring and ataxic but in good nutritional status.

Samples for domoic acid testing were not obtained from nine premature pups, and more than one sample type was tested from 12 pups, resulting in 79 samples from 68 premature pups submitted for testing. Domoic acid was detected in 17% (12/68) of fetuses and was measured in multiple fluids types, including stomach contents, urine, and amniotic fluid (Table 2). Concentrations of the biotoxin ranged from 0.5 to 18 ng/ml. Although the pup collected from the dam exhibiting signs of acute domoic acid toxicosis had detectable domoic acid in both samples submitted for testing (stomach contents measured 0.7 ng/ml, amniotic fluid measured 9.3 ng/ml), higher concentrations of domoic acid were measured in other pups.

A range of histologic lesions was observed in the premature sea lion pups (Table 3). An inflammatory condition occurred in 50 of 59 (80%) cases, of which 40% had at least one component of simple

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

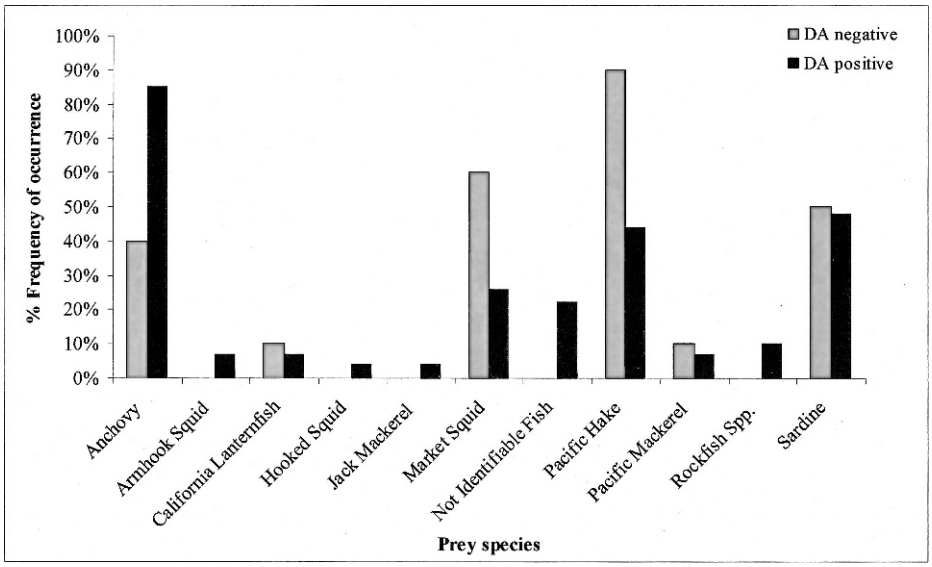


FIGURE 2. Frequency of occurrence of different prey types identified in California sea lion scat samples collected off the San Miguel Island rookery and tested for the presence of domoic acid.

bacterial origin. Most bacterial infections were systemic (22/25, bacteremia or endotoxemia) and affected the liver, with variable involvement of other tissues, including lung, placenta (umbilical cord epithelium, nonzonary chorion-fetal side or amnion), conjunctiva, and spleen, and these were often accompanied by inflammatory and squamous cell debris and/or bacteria in the stomach lumen. Lesions were characterized by mild to severe multifocal acute necrosis and inflammation and, less often, infarction.

An inflammatory response of unknown etiology was observed in lung and/or brain of 46% (27/59) of cases. Although this response sometimes occurred concurrently with inflammatory lesions of simple bacterial origin, it was distinct, as the inflammation was nonsuppurative to granulomatous, and was variably accompanied by multinucleated giant cells (or syncytial cells in the lung), eosinophils, and fewer neutrophils. This inflammatory response most often affected the lung (Fig. 3) and brain to spinal cord, with less frequent involvement of the lymph nodes, spleen, heart, and liver. Inflammation in the brain affected the meninges and/or white and gray matter, sometimes associated with central liquefactive necrosis and sometimes limited to meningitis and perivascu-

TABLE 1. Mean, standard deviation (SD), ranges, and sample size (n) of fetal life history variables of fresh premature pups for which a complete necropsy examination was obtained on San Miguel Island in 2005 to 2006.

Variable	2nd trimester fetuses					3rd trimester fetuses				
	Mean	SD	Min	Max	n	Mean	SD	Min	Max	n
Length (cm)	55	5	49	66	9	63	4	55	75	50
Axillary girth (cm)	28	2	26	33	9	35	3	30	42	50
Mass (kg)	3	0.8	3	5	9	5	1	3	9	49
Blubber thickness (mm)	4	1	2	5	9	6	2	2	13	49
Blubber % lipid	25	0.05	18	35	14	–	–	–	–	–

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

TABLE 2. Domoic acid concentrations measured in fluids collected from premature pups from San Miguel Island.

Sample type	No. positive/No samples tested	Domoic acid concentration (ng/ml)
Stomach contents	9/62	0.3–44.0
Urine	4/7	2.0–17.6
Amniotic fluid	2/6	3.0–9.3
Serum	0/3	<dl[a]
Feces	0/1	<dl[a]
Total	15/79	

[a] dl = detection limit.

lar cuffing. In two cases, necrotic foreign material with some semblance to a metazoan larva was within a granulomatous focus in the brain or lung (Fig. 4). Further elucidation of the foreign material was not possible. Brain edema occurred in approximately one third of the cases ($n=16$), sometimes not accompanied by any other lesions. The brain edema was mild to severe, perivascular to interstitial, and concentrated in the cerebrum to diencephalon. Brain edema did not occur exclusively in cases with known bacterial septicemia but did occur in two thirds of the cases (6/9) with detectable domoic acid but without other significant lesions. The zonary placenta was available for histologic examination in five cases (tissue blocks from an additional 15 cases could not be sectioned due to the presence of sand), three of which had evidence of placental abruption. Of these three cases, one had detectable domoic acid and brain edema without other evidence of disease, the second had detectable domoic acid and a mild septicemia, while the third had brain edema and a severe bacterial funisitis with mild septicemia (domoic acid not detected). Moderate to severe aspiration of squamous, epithelial, and other cellular debris occurred in 56% of the cases (30/59). In some cases, the aspiration appeared substantial enough to compromise the initial expansion of the lungs and result in respiratory acidosis, had the pup survived. Extramedullary hematopoiesis was common in the liver; however, when the leukopoieses component occurred not only in the portal and centrilobular regions, as expected, but also in the sinusoids (where erythropoiesis occurs in nondiseased fetuses), then liver damage was also present. Overall, domoic acid was detected in samples from six premature pups with the brain edema, three of which had systemic bacterial infections and one of which had an unknown inflammatory condition.

In contrast to the varied findings in the premature fetuses from the rookery, those aborted from domoic acid–affected dams in rehabilitation showed minimal to no evidence of inflammation or infection.

TABLE 3. Histopathologic findings in 59 fresh premature pups examined on San Miguel Island in 2005 and 2006. The total affected is provided relative to the number of animals in which the tissues were present or adequate for appropriate histologic assessment of the respective lesion.

		Tissue affected						No. domoic acid positive ($n=9/59$)
Finding	Total	Brain	Liver	Lung	Vagina, conjunctiva	Placenta/ umbilicus	Other[a]	
Bacterial infection:	34/59							4
Local	3				1	2		1
Systemic	31		21	8	3	6	3	3
Inflammatory lesion[b]	26/59	11	5	16			6	1
Brain edema	19/55	19						6
Placental abruption	3/5					3		2
Aspiration significant	33/59			30				7

[a] Affected tissue types included heart, spleen, spinal cord, lymph nodes, urinary bladder, and diaphragm.

[b] Lesions do not include those of known bacterial origin listed in the first row.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

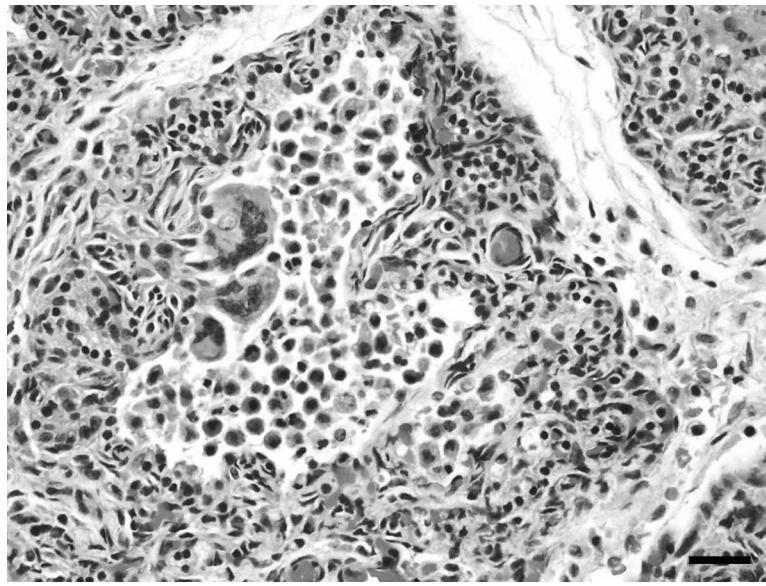


FIGURE 3. Lung from a premature California sea lion pup with lymphohistiocytic and eosinophilic pneumonia containing syncytial cells. H&E staining. Bar=30 μm.

Common histologic findings were placental abruption and moderate to severe brain edema. The brain edema was occasionally associated with widespread neuronal necrosis, including the laminar cortex, cerebellar Purkinje cells, cingulate to paracingulate gyrus, and all regions of the hippocampal formation.

The bacteriology results support the histopathologic results. Fifty bacterial isolates were obtained from liver, spleen, placenta, and stomach tissues (Table 4). The majority of the isolates was gram negative, and they were cultured most often from the stomach, followed by the liver and spleen; they included *Escherichia coli* spp. (15 isolates, 12% of fetuses), *Streptococcus phocae* (9 isolates, 12% of fetuses), and *Moraxella* spp. (8 isolates, 7% of fetuses). *Salmonella enteriditis* was cultured from placental tissue and stomach contents from one pup, and *Brucella* spp. were cultured from two placentas. *Campylobacter* spp. were not cultured from any fetuses. Results of molecular diagnostics for other infectious agents were varied. Polymerase chain reactions for Otarine herpesvirus-1, *Leptospira* spp., *Chlamydia* spp., *T. gondii*, and *S. neurona* were all negative. Marine caliciviral RNA was detected in umbilical tissues of five of the 59 animals. Two of these five animals did not have significant histologic lesions, one had hepatitis due to a systemic simple bacterial infection, and two had focal pleocellular encephalitis. Although *Brucella* was not detected in stomach content

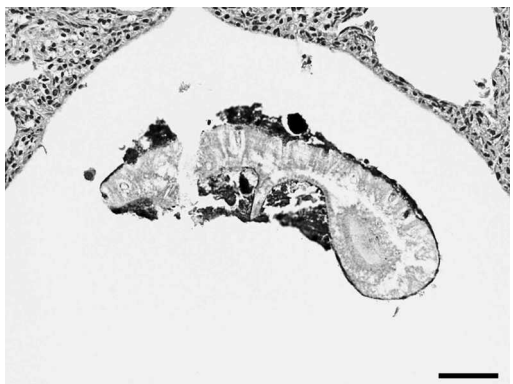


FIGURE 4. Lung from a premature California sea lion pup with histiocytic and granulocytic pneumonia. The alveolar space contains necrotic foreign material, approximately 300 μm long, which has semblance to a metazoan parasite. The tissue has slight autolysis. H&E staining. Bar=50 μm.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

TABLE 4. Bacterial isolates from swabs and tissues collected upon postmortem examination of premature pups from San Miguel Island.

Bacteria	Liver	Spleen	Stomach contents	Placenta	No. isolates	Prevalence of cases (no. cases)
Atypical *Escherichia coli*	5	4	6		15	12% (7)
Streptococcus phocae	2	3	4		9	12% (7)
Moraxella spp.	3	2	1	2	8	7% (4)
Psychrobacter spp.		1	2		3	5% (3)
Enterobaceteriacea	1	1	1		3	2% (1)
Brucella spp.				2	2	3% (2)
Lactococcus spp.	1	1	1		3	2% (1)
Micrococcus spp.		1	1		2	2% (1)
Salmonella enteriditis			1	1	2	2% (1)
Alpha hemolytic *Streptococcus*	1				1	2% (1)
Acinetobacter spp.	1				1	2% (1)
Coagulase negative *Staphylococcus* spp.		1			1	2% (1)
Total	14	14	17	5	50	59

samples by PCR or culture, *Brucella* DNA was detected in placental tissues in three of 59 cases, two of which were culture positive. Further characterization of this *Brucella* infection is ongoing (I. Sidor, pers. comm.). Lipid content in the 14 blubber samples varied from 18% to 35% (Table 1). Because of this variation, both wet weight and lipid normalized values for contaminants were calculated. The geometric means and 95% confidence limits were calculated as the pollutant residue concentrations were log-normally distributed. The arithmetic means and ranges were also calculated so that both values were available for comparison with other published data. Blubber residues of PCBs, DDTs, PBDE flame retardants, and the organochlorine pesticides HCHs, chlordanes, dieldrin, aldrin, mirex, endosulfan sulfate, and bile levels of PAH metabolites are presented in Table 5. Concentrations of hexachlorbenzene, aldrin, mirex, and endosulfan sulfate and some PCB (17, 18, 31, 33, 70, 156, 158, 171, 191, 195, 205, 206, 208, 209) and PBDE flame retardant (28, 49, 66, 85, 153, 154, 183) congeners and the chlordane isomer oxychlordane were less than the lower limit of quantitation. Most of the ΣDDT in these premature pups was present in the metabolite *p,p′*-DDE (ranging from 91% to 99%). Similarly, the dominant HCH isomer was β-HCH (73–100%), and, in most cases, the summed chlordanes consisted of trans nonachlor (40–80%). We examined the individual congeners comprising the ΣPCBs and ΣPBDEs and found that the dominant PCB congeners were 138 (12–19%) and 153 (15–27%) (Fig. 5), and the dominant PBDE congener was PBDE 47 (58–83%) (Fig. 6).

DISCUSSION

The results of this study indicate that there were multiple etiologies of abortion and premature parturition in California sea lions on San Miguel Island, some of which have been previously documented, as well as a novel one, domoic acid. *Pseudo-nitzschia* spp. were present both in the environment in water and rookery scat samples, and domoic acid was also detected in the latter. Although not all species of *Pseudo-nitzschia* found in the samples are known to be toxic and domoic acid levels were not measured in the phytoplankton samples collected off San Miguel Island, the widespread and elevated domoic acid concentrations in shellfish throughout southern California and the domoic acid–positive phytoplankton samples at coastal sites provide additional

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

TABLE 5. Concentrations of pollutant residues (geometric mean, lower and upper geometric confidence intervals [CI], arithmetic mean, standard deviation, range, sample size [*n*]) measured in blubber and bile samples from premature pups sampled on San Miguel Island.

Blubber contaminant	Geometric mean	Lower geometric CI	Upper geometric CI	Arithmetic mean	SD	Minimum	Maximum	*n*
ΣPCBs (ng g^{-1}, wet weight)	250	150	410	360	370	87	1,200	14
ΣPCBs (ng g^{-1}, lipid weight)	1,000	650	1,600	1,400	1,500	380	5,500	14
ΣDDTs (ng g^{-1}, wet weight)	960	530	1,700	1,700	2,000	290	6,200	14
ΣDDTs (ng g^{-1}, lipid weight)	4,000	2,300	6,900	6,600	8,100	1,300	29,000	14
ΣHCHs (ng g^{-1}, wet weight)	13	7	23	22	25	4	79	14
ΣHCHs (ng g^{-1}, lipid weight)	53	30	94	87	99	20	360	14
ΣPBDEs (ng g^{-1}, wet weight)	76	40	140	140	180	17	620	14
ΣPBDEs (ng g^{-1}, lipid weight)	320	170	580	570	770	75	2,900	14
Σchlordanes (ng g^{-1}, wet weight)	43	26	71	63	65	12	210	14
Σchlordanes (ng g^{-1}, lipid weight)	180	110	280	247	240	66	760	14
ΣOC pesticides (ng g^{-1}, wet weight)[a]	6	4	9	7	5	3	17	14
ΣOC pesticides (ng g^{-1}, lipid weight)[a]	26	18	36	30	19	11	74	14
Bile PAH metabolites:								
Benzo[*a*]pyrene equivalents (BaP, ng g^{-1}, wet weight)	95	73	120	130	150	18	890	33
Phenanthrene equivalents (PHN ng g^{-1}, wet weight)	14,000	13,000	17,000	15,000	6,800	1,600	32,000	33

[a] Organochlorine (OC) pesticides include aldrin, dieldrin, mirex, and endosulfan sulfate.

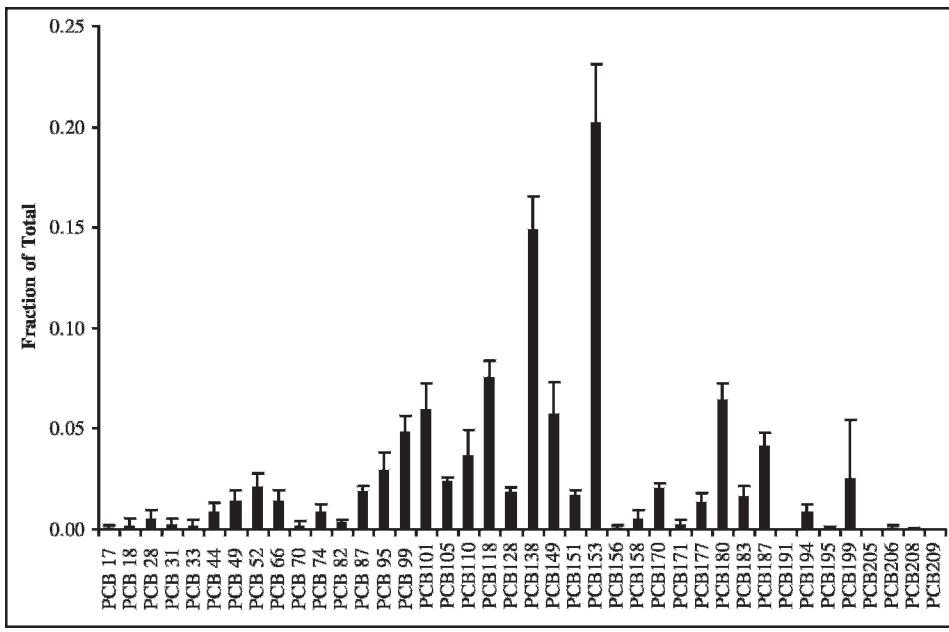


FIGURE 5. Mean percent PCB congener composition of the total PCB burden in California sea lion premature pup blubber samples from San Miguel Island ($n=14$). Error bars represent the standard deviation.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

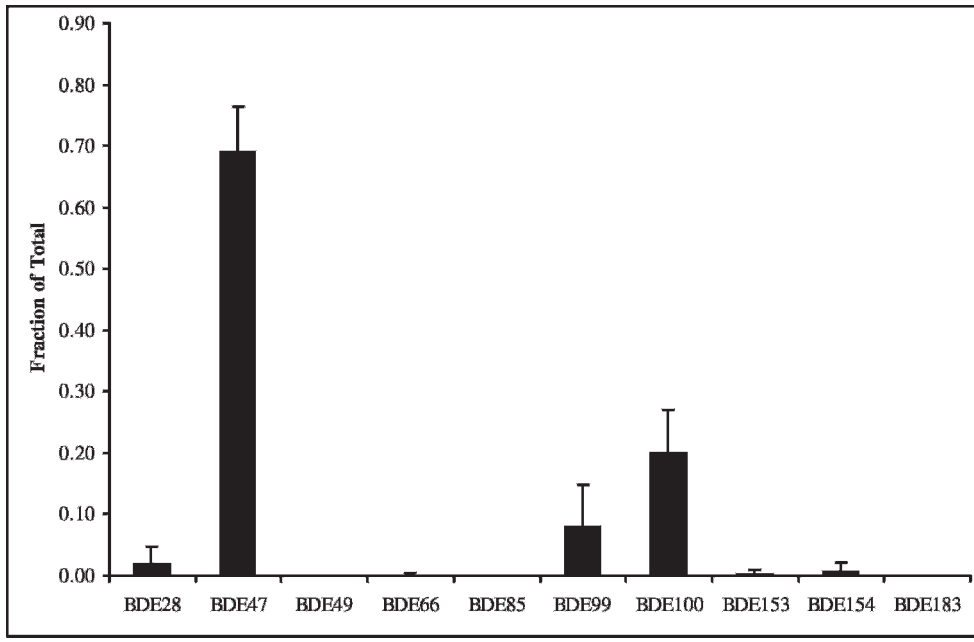


FIGURE 6. Mean percent PBDE congener composition of the total PBDE burden in California sea lion premature pup blubber samples from San Miguel Island ($n=14$). Error bars represent the standard deviation.

support for the presence of a toxic bloom off the coast. The concentrations of domoic acid measured in scat samples were comparable to those detected in feces of stranded sea lions seizing following domoic acid toxicosis (Scholin et al., 2000). These values are also comparable to ranges found in affected sea birds (500–25,300 ng/ml; Work et al., 1993) and other marine mammals species, including cetaceans, stranding off the coast of California (300–1,600 ng/ml; Ch'ng et al., 2000). Similarly, the concentrations detected in 17% of the premature pups on the rookery were comparable to those in fetuses from dams with acute domoic acid toxicosis in rehabilitation (Brodie et al., 2006). As with animals in rehabilitation, domoic acid was detected in fetal urine, amniotic fluid, and fetal stomach contents. The brain edema seen in approximately one third of the cases from San Miguel Island and the placental abruption in three of the five examined placentas were similar to those found in fetuses aborted from dams with acute domoic acid toxicosis in rehabilitation. Furthermore, five pups that tested positive for domoic acid did not have significant lesions other than brain edema or placental abruption, again as was observed in domoic acid–affected fetuses from rehabilitation. The fetal brain edema and placental abruption appear to be a manifestation of domoic acid–induced reproductive failure. Thus, based on the findings in rehabilitation, similar cases of reproductive failure from the rookery were attributed to domoic acid exposure.

The possible association of the brain edema with in utero hypoxia seems unlikely based on information available in human neonatology (Rees and Inder, 2005). In humans, in utero hypoxia-ischemia often manifests as subleptomeningeal and subependymal hemorrhage variably accompanied by intraventricular hemorrhage, periventricular leukoencephalomalacia, particularly at the level of the lateral ventricles, and apoptosis, particularly of oligodendroglia and the neurons in the periventricular region and cornu ammonis sector 1 of the hippocampal

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

formation, none of which was present in the fetuses in this study. Seizures of hippocampal origin have been associated with paroxysmal hypertension in humans, and hypertension is one risk factor for placental abruption (Ananth et al., 1999). Consequently, it is possible that domoic acid–induced seizures in the dam could result in placental abruption and perhaps translate to fetal hypertension and brain edema. The possibility of a direct interaction of domoic acid with receptors on the placenta seems less likely, as in the placenta, receptors for glutamate activity have not been identified, but rather the counteracting mechanisms of glutamate transporters and gamma-ammobutyric acid (GABA) inhibitory receptors have been identified (Mathews, 2005). However, the direct interaction of domoic acid with receptors in the brain could also explain the brain edema, being secondary to seizure activity. The effect of the toxin on the fetal brain and placenta of unborn fetuses is unclear, whether it is through a direct or indirect mechanism, and this warrants further investigation.

Bacterial infections were a common finding in these premature pups and often resulted in systemic lesions including hepatic necrosis and suppurative pneumonia. The nature and distribution of the lesions in the fetus and placenta (i.e., involvement of the lung, liver, vagina and/or conjunctiva, and stomach content with the suppurative inflammation of placenta associated with the umbilical epithelium, nonzonary chorion [fetal side], and amnion) are suggestive of a bacterial infection that originated from an ascending infection of the dam's reproductive tract, which then affected the amnion. Common bacteria isolated such as *E. coli* spp. and *Streptococcal* spp. have also been frequently cultured from the reproductive tract of female California sea lions (Johnson et al., 2006), suggesting that opportunistic ascending infections are likely. Although *Brucella* spp. were cultured from two cases, the most common *Brucella*-associated histologic lesion of necrosuppurative diffuse placentitis was not present in the small number of placental tissues examined (Walker, 2004). Nonetheless, the possible association of *Brucella* infection with reproductive failure in this species could be significant and warrants further investigation.

The inflammatory response of unknown etiology that affected more than one third of the pups was distinct in distribution and in morphology and had features suggestive of several etiologies. Differential diagnoses included parasite migration, as a suspect metazoan larva was seen in brain and lung; protozoal infection, as features consistent with congenital infections described in other marine mammals were present (Dubey et al., 2003) but were not confirmed by additional diagnostics; or viral infection, such as calicivirus or an unidentified virus. Although caliciviral RNA was detected, there was not a clear association of the infection with histologic findings. The anticipated caliciviral lesions of petechial hemorrhage described in aborted piglets (Smith et al., 1998) were not present in the sea lion fetuses.

Except for PCB and DDT concentrations, values measured for the other contaminants are not currently available in the literature for premature sea lion pups, and therefore the results presented here provide a baseline for future comparisons. Summed DDTs and PCBs measured in blubber of premature pups from San Miguel in the current study were lower than those measured in premature pups in the 1970s (DeLong et al., 1973), as well as those in fetuses aborted from domoic acid–affected dams in rehabilitation at the Marine Mammal Center (TMMC) from 1998 to 2002 (Greig et al., 2007). Geometric mean values reported in blubber tissues at TMMC for PCBs and DDTs were 760 ng/g wet weight and 2000 ng/g wet weight, respectively, both of which were more than two times higher than the geometric mean measured in these pups sampled in 2005. The data

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

available for the San Miguel samples from the 1970s were from brain (not blubber); therefore, a direct comparison cannot be made. Nonetheless, in 2005, blubber from premature pups from San Miguel had lower mean concentrations than brain from premature pups in the 1970s (450 ng/g wet weight PCB, 2,400 ng/g wet weight DDT) and similar concentrations to those measured in brain tissue from full-term pups (190 ng/g wet weight PCB, 1,200 ng/g wet weight DDT) on San Miguel in the 1970s. This suggests the decrease in total body burden over 30 yr is marked, as brain from marine mammals tends to have much lower concentrations of these organochlorines than blubber (Jenssen et al., 1996). The DDT metabolite *p,p′*-DDE and PCB congeners 138 and 153 were consistently dominant among the studies. The organochlorine pesticides hexachlorocyclohexanes were measured in premature sea lions in the current study and have been reported in neonatal and stillborn northern fur seals (*Callorhinus ursinus*) from Alaska (Mössner et al., 1994). The mean concentration (52.8 ng/g lipid weight) in the premature sea lions was four times lower than the mean value in the fur seal neonates (217 ng/g lipid weight). The dominant isomer β-HCH in blubber was consistent with other studies in marine mammals (Mössner et al., 1994). The PBDE flame retardants have been measured previously in blubber from stranded adult male sea lions (Stapleton et al., 2006). The geometric mean concentration measured in these premature pups of 320 ng/g lipid weight was an order of magnitude lower than the mean value of adult males (3,900 ng/g lipid weight). The dominant congener was PBDE 47 in both studies, as has been observed in other marine mammal species (Stapleton et al., 2006). Interestingly, the PAH levels in these premature sea lion pups were relatively high, with a mean over 13,000 ng/g bile. This level is comparable to those documented in harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*) that died after the *Exxon Valdez* oil spill (Calkins et al., 1994; Frost et al., 1994). These high levels could be a consequence of exposure to the natural oil seeps off the coast of Santa Barbara. However, it is unknown if exposure to PAHs could have contributed to the abortions or premature parturition of the sea lions in the current study. A study evaluating the effects of oil exposure on mink as a model for reproductive success in sea otters found oil-exposed females had reduced reproductive success, and their kits experienced poor survival to weaning (Mazet et al., 2001), indicating the potential for PAH exposure to contribute to reproductive failure in marine mammals.

Although there are similarities with the findings from the study on sea lion reproductive failure conducted in the 1970s, a wider range of potential causes for reproductive failure were found in the current study. As in the 1970s, calicivirus was present in premature pups, and although anticipated histopathology associated with caliciviral-induced abortion was not present, its contribution to reproductive failure could not be excluded. In contrast to the earlier study, evidence of *Leptospira* infection was not detected in any of the pups examined, although leptospirosis is still endemic in sea lions (Lloyd-Smith et al., 2007). Organochlorine compounds have decreased in tissues from premature pups since the 1970s, and PAH metabolites were measured in a subset of animals, but it is unknown if these compounds contributed to reproductive failure. Bacterial infections, especially those that commonly cause ascending infections of the reproductive tract, appear to be common in premature neonates, and a granulomatous infection of unknown etiology was also relatively common. The severity of inflammatory lesions was variable, and thus their contribution to abortion and premature parturition was uncertain in some instances. Finally, results indicated that domoic

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

acid was another factor playing a contributory role in reproductive failure in California sea lions on San Miguel Island. Continued monitoring will determine the magnitude of effect that the toxin and other pathogens will have on the population in the future.

ACKNOWLEDGMENTS

This study was supported by funds from the Oceans and Human Health Initiative grant number NA04OAR4600200 and through a Morris Animal Foundation Fellowship Training grant number D05ZO-401. This study was also supported by the West Coast Center for Oceans and Human Health (WCCOHH) as part of the National Oceanic and Atmospheric Administration Oceans and Human Health Initiative, WCCOHH publication no. 24. WCCOHH is part of the National Marine Fisheries Service's Northwest Fisheries Science Center, Seattle, Washington. The work was authorized under the US Marine Mammal Protection Act by Scientific Research Permits No. 932-1489-00 and No. 782–1446. Thanks are due to the biologists of the Marine Mammal Center and National Marine Mammal Laboratory for assistance with sample collection, the Cal-PreEMPT program for providing domoic acid concentrations for phytoplankton collected off the Cal Poly Pier, and many others for performing clinical diagnostics and sample analyses, especially S. Melin, T. Orr, H. Ziel, R. Jenkinson, E. Siek, T. Norris, T. Spraker, K. Colegrove, S. Orosco, D. King, S. Reid, K. Nielsen, W. Ling Yu, H. Nollens, R. Stoddard, P. Morris, G. Oliver, C. Field, and S. Fresca. We also thank B. Anulacion, D. Boyd, R. Boyer, C. Sloan, J. Bolton, K. Tilbury, and G. Yanagida from the Northwest Fisheries Science Center for contaminant and lipid analyses.

LITERATURE CITED

Ananth, C. V., G. S. Berkowitz, D. A. Savitz, and R. H. Lapinski. 1999. Placental abruption and adverse perinatal outcomes. Journal of the American Medical Association 282: 1646–1651.

Anderson, C. R., M. A. Brzezinski, L. Washburn, and R. Kudela. 2006. Circulation and environmental conditions during a toxigenic *Pseudonitzschia australis* bloom in the Santa Barbara Channel, California. Marine Ecology Progress Series 327: 119–133.

Bargu, S., C. L. Powell, S. L. Coale, M. Busman, G. J. Doucette, and M. W. Silver. 2002. Krill: A potential vector for domoic acid in marine food webs. Marine Ecology Progress Series 237: 209–216.

Brodie, E. C., F. M. D. Gulland, D. J. Greig, M. Hunter, J. Jaakola, J. St. Leger, T. A. Leighfield, and F. M. Van Dolah. 2006. Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). Marine Mammal Science 22: 700–707.

Bruckner, J. V., K. L. Khanna, and H. H. Cornish. 1973. Biological responses of the rat to polychlorinated biphenyls. Toxicology and Applied Pharmacology 24: 434–448.

Buckles, E. L., L. J. Lowenstine, C. Funke, R. K. Vittore, H.-N. Wong, J. A. St. Leger, D. J. Greigy, R. S. Duerr, F. M. D. Gullandy, and J. L. Stott. 2006. Otarine herpesvirus-1, not papillomavirus, is associated with endemic tumours in California sea lions (*Zalophus californianus*). Journal of Comparative Pathology 135: 183–189.

Calkins, D. G., E. F. Becker, T. R. Spraker, and T. R. Loughlin. 1994. Assessment of injury to Steller sea lions in Prince William Sound and the Gulf of Alaska. *Exxon Valdez* oil spill. *In* State Federal Natural Resource Damage Assessment Final Report. Marine Mammal Study Number 4, Anchorage, Alaska, pp. 119–230.

Ch'ng, M. M., T. A. Leighfield, M. A. Busman, F. Gulland, K. Matassa, M. Chechowitz, T. Rowles, and F. M. Van Dolah. 2000. Analysis of domoic acid involvement in marine mammal morbidities and mortalities on the West Coast of the US National Marine Fisheries. Technical Memorandum February–August 2000, Charleston, South Carolina, pp. 1–13.

Cloeckaert, A., M. Grayon, O. Grépinet, and K. Sidi Boumedine. 2003. Classification of *Brucella* strains isolated from marine mammals by infrequent restriction site-PCR and development of specific PCR identification tests. Microbes and Infection 5: 593–602.

Colagross-Schouten, A. M., J. A. K. Mazet, F. M. D. Gulland, M. A. Miller, and S. Hietala. 2002. Diagnosis and seroprevalence of Leptospirosis in California sea lions from coastal California. Journal of Wildlife Diseases 38: 7–17.

Dakshinamurti, K., S. K. Sharma, M. Sundaram, and T. Watanabe. 1993. Hippocampal changes in developing postnatal mice following intrauterine exposure to domoic acid. The Journal of Neuroscience 13: 4486–4495.

DeLong, R. L., W. G. Gilmartin, and J. G. Simpson. 1973. Premature births of California sea lions: Association with high organochlorine pollutant residue levels. Science 181: 1168–1170.

Dubey, J. P., R. Zarnke, N. J. Thomas, S. K. Wong, W. Van Bonn, M. Briggs, J. W. Davis, R. Ewing, M. Mense, O. C. H. Kwok, S. Romandi, and P. Thulliez. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystis*

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

canis–like infections in marine mammals. Veterinary Parasitology 116: 275–296.

FROST, K. J., C.-A. MANEN, AND T. L. WADE. 1994. Petroleum hydrocarbons in tissues of harbor seals from Prince William Sound and the Gulf of Alaska. *In* Marine mammals and the *Exxon Valdez*, T. R. Loughlin (ed.). Academic Press, Inc., San Diego, California, pp. 331–358.

FRY, D. M. 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. Environmental Health Perspectives 103 (Suppl): 165–171.

GILMARTIN, W. G., R. L. DELONG, A. W. SMITH, J. C. SWEENEY, B. W. DE LAPPE, R. W. RISEBROUGH, L. A. GRINER, M. R. DAILEY, AND D. B. PEAKALL. 1976. Premature parturition in the California sea lion. Journal of Wildlife Diseases 12: 104–115.

GOLDSTEIN, T., J. A. K. MAZET, T. S. ZABKA, G. LANGLOIS, K. M. COLEGROVE, M. SILVER, S. BARGU, F. VAN DOLAH, T. LEIGHFIELD, P. A. CONRAD, J. BARAKOS, D. C. WILLIAMS, S. DENNISON, M. A. HAULENA, AND F. M. D. GULLAND. 2008. Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): An increasing risk to marine mammal health. Proceedings of the Royal Society B 275: 267–276.

GREIG, D. J., G. M. YLITALO, A. J. HALL, D. A. FAUQUIER, AND F. M. D. GULLAND. 2007. Transplacental transfer of organochlorines in California sea lions (*Zalophus californianus*). Environmental Toxicology and Chemistry 26: 37–44.

GULLAND, F. M., M. HAULENA, D. FAUQUIER, G. LANGLOIS, M. E. LANDER, T. ZABKA, AND R. DUERR. 2002. Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): Clinical signs, treatment and survival. Veterinary Record 150: 475–480.

HARKIN, K. R., Y. M. ROSHTO, AND J. T. SULLIVAN. 2003. Clinical application of a polymerase chain reaction assay for diagnosis of leptospirosis in dogs. Journal of the American Veterinary Medical Association 222: 1224–1229.

HART, M. M., R. H. ADAMSON, AND S. FABRO. 1971. Prematurity and intrauterine growth retardation induced by DDT in the rabbit. Archives Internationales de Pharmacodynamie et de Therapie 192: 286–290.

HEWINSON, R. G., P. C. GRIFFITHS, B. J. BEVAN, S. E. KIRWAN, M. E. FIELD, M. J. WOODWARD, AND M. DAWSON. 1997. Detection of *Chlamydia psittaci* in DNA in avian clinical samples by polymerase chain reaction. Veterinary Microbiology 54: 155–166.

JEFFERY, B., T. BARLOW, K. MOIZER, S. PAUL, AND C. BOYLE. 2004. Amnesic shellfish poison. Food and Chemical Toxicology 42: 545–557.

JENSSEN, B. M., J. U. SKAARE, M. EKKER, D. VONGRAVEN, AND S. H. LORENTSEN. 1996. Organochlorine compounds in blubber, liver and brain in neonatal grey seal pups. Chemosphere 32: 2115–2125.

JOHNSON, S., L. LOWENSTINE, F. GULLAND, S. JANG, D. IMAI, F. ALMY, R. DELONG, AND I. GARDNER. 2006. Aerobic bacterial flora of the vagina and prepuce of California sea lions (*Zalophus californianus*) and investigation of associations with urogenital carcinoma. Veterinary Microbiology 114: 94–103.

KENNISH, M. J. 1998. Case study : Southern California Bight. *In* Pollution impacts on marine biotic communities. CRC Press LLC, Boca Raton, Florida, pp. 149-177.

KRAHN, M. M., L. K. MOORE, AND J. W. D. MACLEOD. 1986. Standard analytical procedures of the NOAA National Analytical Facility, 1986. Metabolites of Aromatic Compounds in Fish Bile. NOAA Technical Memorandum NMFS–F/NWC-102, Northwest Fisheries Science Center, NOAA Fisheries, Seattle, Washington, 25 pp.

LEFEBVRE, K. A., S. BARGU, T. KIECKHEFER, AND M. W. SILVER. 2002. From sanddabs to blue whales: The pervasiveness of domoic acid. Toxicon 40: 971–977.

LEVETTE, P. N. 2001. Leptospirosis. Clinical Microbiology Reviews 14: 296–326.

LEVIN, E. D., K. PIZARRO, W. G. PANG, J. HARRISON, AND J. S. RAMSDELL. 2005. Persisting behavioural consequences of prenatal domoic acid exposure in rats. Neurotoxicology and Teratology 27: 719–725.

LLOYD-SMITH, J. O., D. GREIG, G. GHNEIM, S. HIETALA, L. PALMER, J. ST. LEGER, B. T. GRENFELL, AND F. M. D. GULLAND. 2007. Cyclical changes in seroprevalence of leptospirosis in California sea lions: Endemic and epidemic disease in one host species? BMC Infectious Diseases 7: 125.

LLUCH, B. D. 1969. El lobo marino de California (*Zalophus californianus* Lesson, 1828) (Allen, 1880), en dos mamiferos marinos de Baja California. Institute Mexicano Recursos Naturales Renovables, Mexico, D. F., 69 pp.

MATE, B. 1977. Aerial censusing of pinnipeds in the Eastern Pacific for assessment of population numbers, migratory distributions, rookery stability, breeding effort, and recruitment. Report to the Marine Mammal Commission, Contract MM5AC001, Bethesda, Maryland, 68 pp.

MATHEWS, D. 2005. Expression of non-organelle glutamate transporters to support peripheral tissue function. *In* Glutamate receptors in peripheral tissue: Excitatory transmission outside the CNS, S. Gill and O. Pulido (eds.). Kluwer Academic/Plenum Publishers, New York, New York, pp. 3–23.

MAUCHER, J. M., AND J. S. RAMSDELL. 2005. Domoic acid transfer to milk: Evaluation of a potential

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

route of neonatal exposure. Environmental Health Perspectives 113: 461–464.

———, AND ———. 2007. Maternal-fetal transfer of domoic acid in rats at two gestational time points. Environmental Health Perspectives 115: 1743–1746.

MAZET, J. A. K., I. A. GARDNER, D. A. JESSUP, AND L. J. LOWENSTINE. 2001. Effects of petroleum on mink applied as a model for reproductive success in sea otters. Journal of Wildlife Diseases 37: 686–692.

MILLER, M. A., K. SVERLOW, P. R. CROSBIE, B. C. BARR, L. J. LOWENSTINE, F. M. GULLAND, A. PACKHAM, AND P. A. CONRAD. 2001. Isolation and characterization of two parasitic protozoa from a Pacific harbor seal (*Phoca vitulina richardsi*) with meningoencephalomyelitis. Journal of Parasitology 87: 816–822.

MÖSSNER, S., I. BARUDIO, T. S. SPRAKER, G. ANTONELIS, G. EARLY, J. R. GERACI, P. R. BECKER, AND K. BALLSCHMITER. 1994. Determination of HCHs, PCBs, and DDTs in brain tissues of marine mammals off different age. Fresenius Journal of Analytical Chemistry 349: 708–716.

ODELL, D. K. 1971. Censuses of pinnipeds breeding on the California Channel Islands. Journal of Mammalogy 52: 187–190.

O'HARA, C. M., M. P. WEINSTEIN, AND J. M. MILLER. 2003. Manual and automated systems for detection and identification of microorganisms. *In* Manual of Clinical Microbiology, 8th Edition, P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (eds.). ASM Press, Washington, DC, pp. 185–207.

ORR, A. J., A. S. BANKS, S. MELLMAN, H. R. HUBER, R. L. DELONG, AND R. F. BROWN. 2004. Examination of the foraging habits of Pacific harbor seal (*Phoca vitulina richardsi*) to describe their use of the Umpqua River, Oregon, and their predation on salmonids. Fisheries Bulletin 102: 108–117.

O'SHEA, T. J., AND R. L. BROWNELL. 1998. California sea lion (*Zalophus californianus*) populations and ΣDDT contamination. Marine Pollution Bulletin 36: 159–164.

REES, S., AND T. INDER. 2005. Fetal and neonatal origins of altered brain development. Early Human Development 81: 753–761.

REID, S. M., D. P. KING, A. E. SHAW, N. J. KNOWLES, G. H. HUTCHINGS, E. J. COOPER, A. W. SMITH, AND N. P. FERRIS. 2007. Development of a real-time reverse transcription polymerase chain reaction assay for detection of marine calciviruses (genus *Vesivirus*). Journal of Virological Methods 140: 166–173.

REIJNDERS, P. J. H. 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature 324: 456–457.

ROSS, P. S., R. L. DESWART, H. H. TIMMERMAN, P. J. H. REIJNDERS, J. G. VOS, H. VAN LOVEREN, AND A. D. M. E. OSTERHAUS. 1996. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. Aquatic Toxicology 34: 71–84.

SCHNETZER, A., P. E. MILLER, R. A. SCHAFFNER, B. A. STAUFFER, B. H. JONES, S. B. WEISBERG, P. M. DIGIACOMO, W. M. BERELSON, AND D. A. CARON. 2007. Blooms of *Pseudo-nitzschia* and domoic acid in the San Pedro Channel and Los Angeles harbor areas of the Southern California Bight, 2003–2004. Harmful Algae 6: 372–387.

SCHOLIN, C. A., F. GULLAND, G. J. DOUCETTE, S. BENSON, M. BUSMAN, F. P. CHAVEZ, J. CORDARO, R. DELONG, A. DE VOGELAERE, J. HARVEY, M. HAULENA, K. LEFEBVRE, T. LIPSCOMB, S. LOSCUTOFF, L. J. LOWENSTINE, R. MARIN, III, P. E. MILLER, W. A. MCLELLAN, P. D. MOELLER, C. L. POWELL, T. ROWLES, P. SILVAGNI, M. SILVER, T. SPRAKER, V. TRAINER, AND F. M. VAN DOLAH. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403: 80–84.

SILVAGNI, P. A., L. J. LOWENSTINE, T. SPRAKER, T. P. LIPSCOMB, AND F. M. D. GULLAND. 2005. Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). Veterinary Pathology 42: 184–191.

SLOAN, C. A., D. W. BROWN, R. W. PEARCE, R. H. BOYER, J. L. BOLTON, D. G. URROWS, D. P. HERMAN, AND M. M. KRAHN. 2005. Determining aromatic hydrocarbons and chlorinated hydrocarbons in sediments and tissues using accelerated solvent extraction and gas chromatography/mass spectrometry. *In* Techniques in aquatic toxicology, Volume 2, G. K. Ostrander (ed.). CRC Press, Boca Raton, Florida, pp. 631–651.

SMITH, A. W. 2000. Virus cycles in aquatic mammals, poikilotherms, and invertebrates. *In* Viral Ecology, C. J. Hurst (ed.). Academic Press, New York, New York, pp. 447–491.

———, T. G. AKERS, A. B. LATHAM, D. E. SKILLING, AND H. L. BRAY. 1979. A new calicivirus isolated from a marine mammal. Archives of Virology 61: 255–259.

———, D. E. SKILLING, N. CHERRY, J. H. MEAD, AND D. O. MATSON. 1998. Calicivirus emergence from ocean reservoirs: Zoonotic and interspecies movement. Emerging Infectious Diseases 4: 13–20.

STAPLETON, H. M., N. G. DODDER, J. R. KUCKLICK, C. M. REDDY, M. M. SCHANTZ, P. R. BECKER, F. GULLAND, B. J. PORTER, AND S. A. WISE. 2006. Determination of HBCD, PBDEs and MeOBDEs in California sea lions (*Zalophus californianus*) stranded between 1993 and 2003. Marine Pollution Bulletin 52: 522–531.

STODDARD, R. A., W. G. MILLER, J. E. FOLEY, J. LAWRENCE, F. M. D. GULLAND, P. A. CONRAD, AND B. A. BYRNE. 2007. *Campylobacter insulaenigrae* isolates from northern elephant seals

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use

(*Mirounga angustirostris*) in California. Applied and Environmental Microbiology 73: 1729–1735.

VAN DOLAH, F. M., T. A. LEIGHFIELD, B. L. HAYNES, D. R. HAMPSON, AND J. S. RAMSDELL. 1997. A microplate receptor assay for the amnesic shellfish poisoning toxin, domoic acid, utilizing a cloned glutamate receptor. Analytical Biochemistry 245: 102–105.

WALKER, R. L. 2004. *Brucella*. *In* Veterinary Microbiology, 2nd Edition, R. L. Walker, D. C. Hirsh, and N. J. MacLachlan (eds.). Blackwell Publishing, Ames, Iowa, pp. 105–113.

WORK, T. M., B. BARR, A. M. BEALE, L. FRITZ, M. A. QUILLIAM, AND J. L. C. WRIGHT. 1993. Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. Journal of Zoo and Wildlife Medicine 24: 54–62.

YLITALO, G. M., J. E. STEIN, T. E. HOM, L. J. JOHNSON, K. L. TILBURY, A. J. HALL, T. ROWLES, D. GREIG, L. J. LOWENSTINE, AND F. GULLAND. 2005. The role of organochlorines in cancer-associated mortality in California sea lions (*Zalophus californianus*). Marine Pollution Bulletin 50: 30–39.

ZENG, F. Y., AND K. TRAN. 2002. Distribution of chlorinated hydrocarbons in overlying water, sediment, polychaete, and hornyhead turbot (*Pleuronichthys verticalis*) in the coastal ocean, southern California, USA. Environmental Toxicology and Chemistry 21: 1600–1608.

Received for publication 11 February 2008.

Downloaded From: https://complete.bioone.org/journals/Journal-of-Wildlife-Diseases on 29 Mar 2024
Terms of Use: https://complete.bioone.org/terms-of-use