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Domoic acid exposure and associated clinical signs and histopathology in Pacific harbor seals (*Phoca vitulina richardii*)

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ABSTRACT

Domoic acid (DA) is a potent neurotoxin that has caused strandings and mortality of seabirds and marine mammals off the California coast. Pacific harbor seals (*Phoca vitulina richardii*) are an abundant, nearshore species in California; however, DA exposure and toxicosis have not been documented for harbor seals in this region. To investigate DA exposure in harbor seals, samples were collected from free-ranging and stranded seals off California to assess exposure, clinical signs of toxicosis, and brain lesions in harbor seals exposed to DA. Domoic acid was detected in 65% (17/26) of urine samples collected from apparently healthy free-ranging seals, with concentrations of 0.4–11.7 ng/ml. Domoic acid also was detected in feces (2.4–2887 ng/g), stomach contents (1.4 ng/g; stranded only), milk (2.2 ng/ml; stranded only), amniotic fluid (9.7 ng/ml; free-ranging only), fetal meconium (14.6–39.8 ng/g), and fetal urine (2.0–10.2 ng/ml). Clinical signs indicative of DA toxicosis were observed in two live-stranded seals, and included disorientation, seizures, and uncoordinated movements. Histopathology revealed the presence of brain lesions consistent with DA toxicosis in two live-stranded seals, and one free-ranging seal that died during capture. Results indicated that harbor seals were exposed to DA, exhibited clinical signs and histological lesions associated with DA exposure, and that pups were exposed to DA in utero and during lactation via milk. Future investigation is required to determine the magnitude of impact that DA has on the health and mortality of harbor seals.

1. Introduction

Domoic acid (DA) is a potent neurotoxin produced by some diatom species of the genus *Pseudo-nitzschia*. It is an analog of the excitatory amino acid glutamate, and is known to affect the gastrointestinal, cardiac, and neurological systems in mammals and birds through binding to glutamate receptors on cell membranes (Bejarano et al., 2008a,b; Costa et al., 2010). Naturally occurring DA poisoning has been observed in humans (amnesic shellfish poisoning; Perl et al., 1990), birds (Fritz et al., 1992; Work et al., 1993), and marine mammals (Scholin et al., 2000). As harmful algal blooms are increasing in frequency worldwide (Van Dolah, 2000), there is an increasing potential for DA poisoning to impact humans and marine wildlife.

Along the California coast, DA poisoning was first reported in Brown Pelicans (*Pelecanus occidentalis*) and Brandt's Cormorants (*Phalacrocorax penicillatus*) in Monterey Bay in 1991 (Fritz et al., 1992; Work et al., 1993). Since 1991, DA has been the causative agent in unusual mortality events of California sea lions (*Zalophus californianus*; Lefebvre et al., 1999; Scholin et al., 2000; Torres de la Riva et al., 2009), and dolphin species (Torres de la Riva et al., 2009). Domoic acid toxicosis has been relatively well-studied in California sea lions because DA-related strandings have occurred almost annually since 1998, and live-stranded sea lions are accessible for observation and sampling (Bejarano et al., 2008a,b). Effects of DA poisoning in California sea lions include neurological symptoms (seizures, head weaving, ataxia, depression, and abnormal scratching) associated with brain lesions, reproductive

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failure, and degenerative cardiomyopathy (Brodie et al., 2006; Goldstein et al., 2008, 2009; Zabka et al., 2009).

Domoic acid exposure has not been well-documented in marine mammals other than California sea lions found along the California coast, and links between exposure and abnormal clinical signs and histopathology are limited. Exposure mainly occurs via ingestion of contaminated food, and potential vectors include northern anchovies (*Engraulis mordax*; Lefebvre et al., 1999), krill (Bargu et al., 2002), and other pelagic and benthic species (Lefebvre et al., 2002; Kvitek et al., 2008), although in utero exposure and lactational exposure via milk also are possible (Brodie et al., 2006). Between 2005 and 2009, approximately half of northern fur seals (*Callorhinus ursinus*) that stranded alive along the central California coast exhibited classic clinical signs of DA toxicosis (Lefebvre et al., 2010). Domoic acid was identified as the primary cause of death in four stranded southern sea otters (*Enhydra lutris nereis*), and may be a cause of cardiac disease in this species (Kreuder et al., 2003, 2005). Domoic acid also was detected in humpback (*Megaptera novaeangliae*) and blue (*Balaenoptera musculus*) whale fecal samples from Monterey Bay (Lefebvre et al., 2002), and a stranded minke whale (*Balaenoptera acutorostrata*) in southern California (Fire et al., 2010). The lack of data for some species of marine mammals is likely a combination of their distribution and life history characteristics, as some species occur primarily offshore, or spend their entire life at sea.

The Pacific harbor seal (*Phoca vitulina richardii*) is a small phocid that inhabits nearshore environments along the west coast of North America, and is widely distributed along the mainland coast, islands, and bays of California. Harbor seals are opportunistic predators that primarily feed on benthic and schooling fishes (Harvey et al., 1995; Orr et al., 2004). Despite the fact that harbor seals forage on similar prey and their habitat overlaps with California sea lions, there are no published data on DA exposure or toxicosis in Pacific harbor seals. Hall and Frame (2010) found that harbor seals in Scotland were exposed to DA, and suggested that this toxin may play a role in regulating their populations. Given that annual DA-producing harmful algal blooms in California coastal waters negatively impact California sea lions (Bejarano et al., 2008a,b), DA also may negatively affect health and cause mortality of harbor seals in this region.

The primary objectives of this study were to (1) document DA exposure in free-ranging and stranded harbor seals along the California coast, and (2) describe the clinical signs and histopathology findings in harbor seals exposed to DA.

2. Methods

2.1. Sample collection

Between June and July 2011, apparently healthy harbor seals were captured in San Francisco Bay ($n = 13$), Tomales Bay ($n = 8$), and Humboldt Bay ($n = 5$) with salmon nets, tangle nets, or a modified beach seine net (Jeffries et al., 1993; Fig. 1). Animals were sedated with IV diazepam to facilitate handling and sampling (dose rate of 0.2 mg/kg), and urine samples were collected from female seals via free-catch or urethral insertion of a urinary catheter. Age class was determined based upon length and mass (Bigg, 1969), body condition, and date of capture.

Additional samples were collected from three adult female seals that died during capture events in 2010 ($n = 1$; Elkhorn Slough) and 2011 ($n = 2$ of the 5 seals mentioned above; Humboldt Bay; Fig. 1). Full necropsies were performed and tissues collected for histopathology and DA analysis. The animal from Elkhorn Slough was pregnant, and feces, amniotic fluid, fetal meconium, and fetal urine were collected, whereas urine and bile were collected from the two seals from Humboldt Bay.

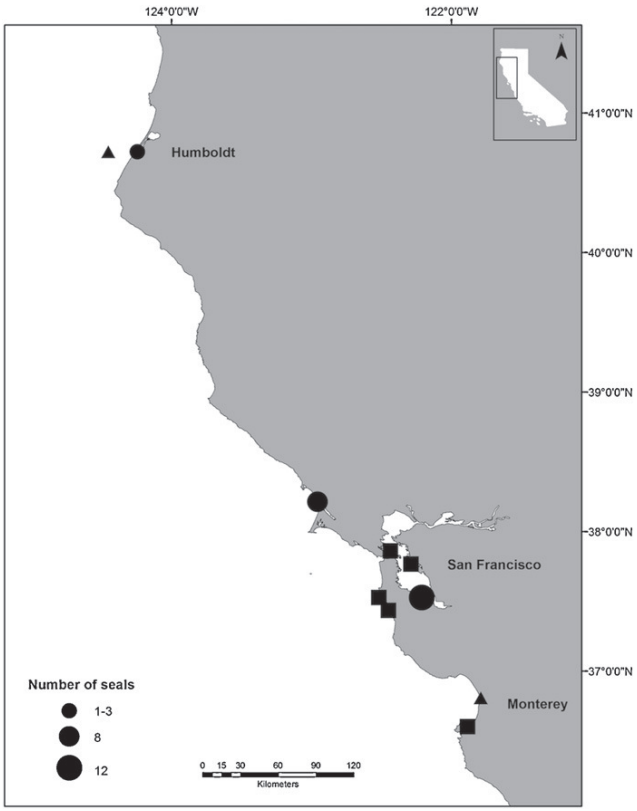

Fig. 1. Location of free-ranging (circles), died during capture (triangles), and stranded (squares) harbor seals sampled off central and northern California between 2009 and 2011. Size of the symbol (regardless of shape) corresponds to the number of individuals sampled at any given location.

Samples were collected from five harbor seals that stranded along the central California coast and were admitted to The Marine Mammal Center (TMMC) between 2009 and 2011 (Fig. 1). All seals died within 0–48 h of admission, and samples were collected at necropsy for histopathology and DA analysis. All samples for histopathology were fixed in 10% formalin, processed routinely for histopathology, and stained with H & E before examination.

2.2. Extraction and quantification of domoic acid

Samples analyzed for DA were extracted in 50% aqueous methanol at a ratio of 4 ml 50% methanol to 1 g sample. Fecal and stomach content extracts were homogenized for at least 60 s, and urine, milk, and amniotic fluid were sonicated for at least 45 s. Extracts were then centrifuged at 10,000 $\times$ *g* for 20 min at 4 °C. The supernatant was filtered and samples were stored at 4 °C until analysis, which was typically 1–3 days after extraction. Domoic acid quantification was carried out using Biosense® ELISA (Biosense® Laboratories, Bergen, Norway) according to the manufacturer's instructions, with modifications to the minimum extract dilutions to avoid matrix effects.

3. Results

3.1. Domoic acid levels

Domoic acid was detected in 65% (17/26) of urine samples collected from free-ranging seals, with concentrations ranging

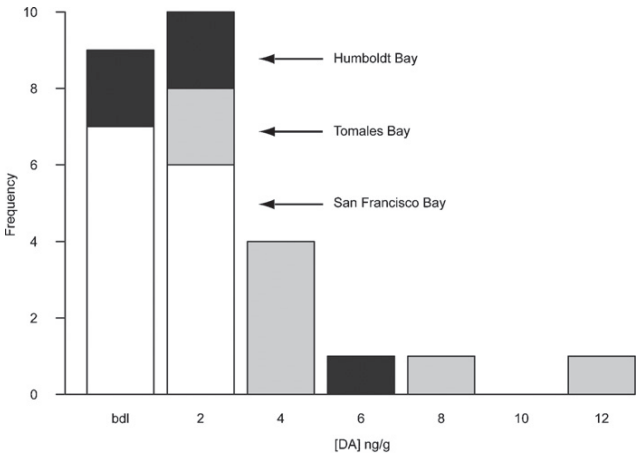

Fig. 2. Number of free-ranging harbor seals (n = 26) with domoic acid (DA; ng/g) in urine captured in San Francisco Bay (white), Tomales Bay (gray), and Humboldt Bay (black) between June and July 2011. Urine from nine seals was below the detectable limit (bdl).

from 0.4 to 11.7 ng/g (Fig. 2). All individuals from Tomales Bay had detectable levels of DA (8/8), whereas DA was detected only in 46% (6/13) of seals from San Francisco Bay, and 60% (3/5) of seals from Humboldt Bay. Domoic acid was not detected in either bile sample collected from the two seals that died in Humboldt Bay, despite detectable levels in urine.

All samples collected from the pregnant seal and her fetus that died during capture in Elkhorn Slough (WHS-2 and WHS-2F) tested positive for DA. The concentration of DA was greatest in feces (2887 ng/g), followed by fetal meconium (39.8 ng/g), fetal urine (10.2 ng/ml), and amniotic fluid (9.7 ng/ml). Necropsy revealed extensive congestion and acute hemorrhage within the brain, and severe acute congestion and hemorrhage with pulmonary edema in the lungs. The cause of death was likely associated with trauma to the head of unknown origin and DA toxicosis.

Samples were collected from five stranded harbor seals: four stranded alive and one was dead at stranding (premature carcass; Table 1). Levels of DA were greatest in fecal samples (1.4–275 ng/g), followed by urine (1.8–2.4 ng/ml) and stomach contents (1.4 ng/g; Table 1). Domoic acid was detected in the one milk sample collected (2.2 ng/ml), and the level was greater than the urine level (0.8 ng/ml) in this animal. Bile samples (n = 4) did not contain DA, despite detectable DA in feces of three of these animals.

3.2. Clinical signs and histopathology

Clinical signs associated with DA in body fluids included disorientation, seizures, and uncoordinated movements (Table 1). Histopathology revealed the presence of brain lesions consistent with DA toxicosis in two live-stranded seals that died following stranding (HS 2120 and HS 2171), and one free-ranging seal (WHS-2). HS 2120 had bilateral hippocampal neuronal necrosis primarily in CA2 through CA4 regions, with lesser involvement of CA1 (Fig. 3A). Other findings included exudate in the ear canal associated with fibrinosuppurative meningitis with Gram positive rods observed on histology, necrosuppurative bronchopneumonia with Gram negative rods, and rare small foci of myocardial necrosis. HS 2171 had bilateral hippocampal neuronal loss in CA3 and CA4 and moderate lymphocytic meningitis. In the left ventricle and septum of the heart, there were rare small foci of subacute myocardial necrosis and rare small foci of myocardial fibrosis (Fig. 3B). WHS-2 had edema and mild neuronal necrosis in the hippocampus that was most pronounced in the CA3 and CA4 regions of the hippocampus, and hemorrhage within the brain stem and cerebrum.

4. Discussion

To our knowledge, this is the first report of DA exposure in harbor seals along the west coast of North America, and the first evidence that harbor seals exhibit clinical signs and histological lesions associated with DA exposure. The detection of DA in samples from seals in this study likely represented recent exposure (days to weeks) because of relatively short prey retention times (mean retention time $\leq$30 h; Trumble et al., 2003), and because DA was rapidly excreted in urine (120 min; intravenous exposure; Suzuki and Hierlihy, 1993) and feces (0–48 h; oral exposure; Iverson et al., 1989) of laboratory animals following exposure. Differences in DA levels among individuals likely were a consequence of the time between exposure and sampling, the type of sample tested, and the dose ingested.

Toxic *Pseudo-nitzschia* spp blooms occur off the California coast every year and DA is routinely detected in seafood species (Lewitus et al., 2012). The detection of DA in samples from free-ranging

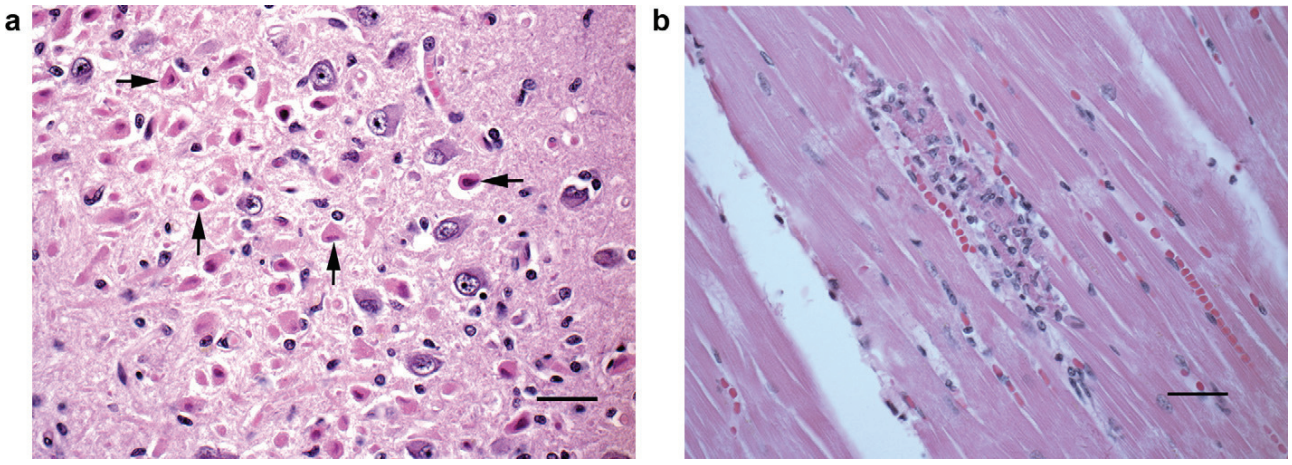

Fig. 3. (A) HS 2120 Numerous dead shrunken neurons in the CA2 hippocampal region (examples indicated with arrows), HE (HS 2120). Bar = 50 μm and (B) area of myocardial necrosis with dead fragmented muscle cells and macrophages, HE (HS 2171). Bar = 50 μm.

Table 1
Domoic acid (DA) concentrations (ng/g or ng/ml for fluids), clinical signs, and pathologic lesions in harbor seals that were examined post mortem. A combination of feces, bile, urine, aqueous humor (aqueous), stomach contents (stomach), amniotic fluid (amniotic), and milk were collected from male (M) and female (F), fetus, pup (P), subadult (SA), and adult (A) harbor seals.

Seal ID	Sex	Age	Samples	DA	Clinical signs	Histopathology
HS 1988	F	A	Feces	2.4	Dull, lethargic	Nonsuppurative meningoencephalitis, neuronal degeneration and edema in hippocampus
HS 1992	F	SA	Feces	8.2	Disoriented, seizures	Fungal encephalitis, myocardial edema
			Bile	*bdl*		
			Urine	1.8		
			Aqueous	*bdl*		
HS 1994	F	Fetus	Feces	14.6	Premature carcass	Brain edema
			Bile	*bdl*		
			Urine	2.0		
			Stomach	1.4		
HS 2120	M	A	Feces	275	Paralysis, seizures	Fibrinosuppurative meningitis with Gram positive rods, hippocampal neuronal necrosis in CA1-CA4, rare myocardial necrosis
			Bile	*bdl*		
			Urine	*bdl*		
HS 2171	F	A	Feces	*bdl*	Struggling in the surf, dazed and frothing at the mouth	Bilateral hippocampal neuronal loss, lymphocytic meningitis and rare mycocardial necrosis
			Urine	0.8		
			Bile	*bdl*		
			Milk	2.2		
Pv-062211	F	A	Urine	0.5	None	No significant findings
			Bile	*bdl*		
Pv-062311	F	A	Urine	0.4	None	No significant findings
			Bile	*bdl*		
WHS-2	F	A	Feces	2887	None	Neuronal necrosis in hippocampus, cerebral hemorrhage
			Amniotic	9.7		
WHS-2F	M	Fetus	Feces	39.8	In utero	None
			Urine	10.2		

harbor seals, including WHS-2, did not coincide with the detection of DA in any shellfish samples tested by the California Department of Public Health from northern or central California around the time seals were captured (California Department of Public Health, http://www.cdph.ca.gov/HealthInfo/environhealth/water/Pages/Shellfishreports.aspx). Despite this, *Psuedo-nitzschia* was abundant (>50% toxin producing phytoplankton) or present (1–10%) in water samples from the Monterey and San Francisco Bay areas during the month of and month proceeding collection of samples from harbor seals. Domoic acid was detected in one shellfish sample from the Santa Cruz Pier during the same month that HS 2120 stranded with paralysis and seizures; however, was not detected in central California around the same time when HS 2171 stranded with symptoms of DA toxicosis. Bloom and toxin data are not always spatially or temporally coincident with stranding data, but sea lions are likely exposed to DA in their prey throughout the year (Bargu et al., 2012). We do not have the density of data from harbor seals that exists for stranded sea lions, but suspect this is true for harbor seals as well.

Free-ranging seals in this study had DA concentrations in urine similar to those found in seals from Scotland (Hall and Frame, 2010), but concentrations were generally less than those reported for California sea lions that stranded with acute symptoms of DA toxicosis (10–3720 ng/ml; Goldstein et al., 2008). Domoic acid levels within the range associated with acute toxicosis in sea lions were detected in one seal, and the majority of seals had DA concentrations in urine within the range associated with chronic effects in California sea lions (2–11 ng/ml; Goldstein et al., 2008). The exposure of harbor seals to DA was not surprising given that the habitats of harbor seals and California sea lions overlap, and that northern anchovies, a DA vector, are an important component of harbor seal diet in this region (Gibble, 2011).

Clinical signs and histological lesions consistent with DA toxicosis were observed in several seals in this study; however, it is unclear how exposure levels relate to acute or chronic DA toxicosis. Although the CA2 hippocampal region is usually not affected in sea lions with DA toxicosis (Silvagni et al., 2005), one harbor seal in this study did have neuronal necrosis in this region. Two seals had small areas of myocardial necrosis, which has been described in sea lions with DA toxicosis (Silvagni et al., 2005; Zabka et al., 2009); however, one of these seals (HS 2120) also had bacterial bronchopneumonia, otitis, and meningitis that could have resulted in septicemia and hence the myocardial necrosis.

Exposure to DA not only affects the health of the individual, but may cause reproductive failure or lasting neurological and behavioral effects to pups exposed in utero or during lactation. Maternal transfer of DA has been reported in rats (Maucher and Ramsdell, 2007) and California sea lions (Brodie et al., 2006; Goldstein et al., 2009), but this was the first report of transplacental transfer of DA in harbor seals. The effects of prenatal exposure of marine mammals to DA have not been well-studied. Brain edema was the primary lesion found in aborted California sea lion pups with measurable DA concentrations, although the pathogenesis of the brain edema remains unknown (Goldstein et al., 2009). Brodie et al. (2006) found that maternal levels of DA in urine of California sea lions were at least an order of magnitude greater than levels in fetal urine. Domoic acid was detected in fetal fluids up to a week after the initial stranding, indicating that fetuses were acting as a reservoir for DA (Brodie et al., 2006). The effects of prenatal DA exposure have been investigated in laboratory species, and resulted in hippocampal damage, seizure disorders, and persistent behavioral changes (Dakshinamurti et al., 1993; Levin et al., 2005; Tiedeken et al., 2005). Postnatal exposure to DA also caused neurobehavioral changes, but changes were less pervasive than those reported from prenatal exposure (Levin et al., 2006). In our study, the level of DA in fetal meconium from WHS-2F was less than in maternal feces, and the DA level in the fetal urine was similar to levels detected in

the urine of California sea lion fetuses that were aborted as a result of maternal DA exposure (Brodie et al., 2006). Detection of DA in amniotic fluid, fetal urine and meconium, and milk indicate that harbor seal pups can be exposed to DA during gestation and lactation. The effects of this exposure on female reproductive success, and pup development and survival are unknown.

The apparent increase in DA-producing blooms off California in recent years highlights the need to understand the role of DA toxicosis in the health and population dynamics of marine mammals in this region. Whereas exposure and toxicosis have been relatively well-studied in California sea lions, this was the first report of exposure in harbor seals from this region. Harbor seals were exposed to levels great enough to cause acute and chronic effects, and exhibited clinical symptoms similar to those observed in other species. The presence of mid hippocampal lesions associated with DA toxicosis indicates that this region should be examined in suspected cases of DA toxicosis in harbor seals. Domoic acid toxicosis may be a cause of mortality in harbor seals and a contributing cause of stranding, but the magnitude of the impact remains unknown. Because adult and juvenile harbor seals rarely strand, examining exposure in free-ranging seals, and live- and dead-stranded pups may be a better indication of the role that DA plays in the health and mortality of harbor seals off California. The type of sample used to investigate DA effects in harbor seals will vary with sample collection logistics and project goals. We recommend testing urine and feces whenever practical. Feces may be the most appropriate sample for the detection of DA when using less sensitive detection techniques (Lefebvre et al., 2010) because it is relatively easy to collect from stranded animals and DA levels are greater in feces than blood or urine. Urine is sometimes easier to collect from free-ranging harbor seals, and may maximize the chances of detecting DA exposure because excretion of DA in feces may occur more rapidly as a result of toxin-induced diarrhea (Lefebvre et al., 1999).

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