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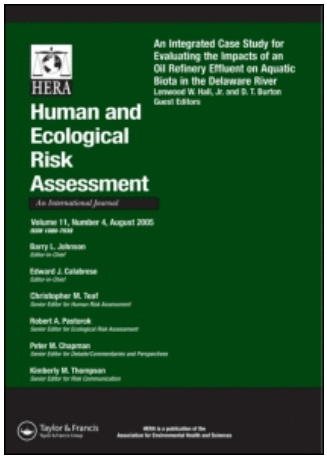

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Production and Toxicity of the Marine Biotoxin Domoic Acid and Its Effects on Wildlife: A Review

Adriana C. Bejarano [a]; Frances M. VanDola [b]; Frances M. Gulland [c]; Teresa K. Rowles [d]; Lori H. Schwacke [a]
[a] NOAA/NOS, Cooperative Center for Marine Animal Health, Hollings Marine Laboratory, Charleston, SC, USA
[b] NOAA/NOS, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC, USA
[c] The Marine Mammal Center, Sausalito, CA, USA
[d] NOAA Fisheries, Cooperative Center for Marine Animal Health, Silver Spring, MD, USA

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SCHOLARLY REVIEWS

Production and Toxicity of the Marine Biotoxin Domoic Acid and Its Effects on Wildlife: A Review

Adriana C. Bejarano,[1] Frances M. VanDola,[2] Frances M. Gulland,[3] Teresa K. Rowles,[4] and Lori H. Schwacke[1]
[1]NOAA/NOS, Cooperative Center for Marine Animal Health, Hollings Marine Laboratory, Charleston, SC, USA; [2]NOAA/NOS, Center for Coastal Environmental Health and Biomolecular Research, Charleston, SC, USA; [3]The Marine Mammal Center, Sausalito, CA, USA; [4]NOAA Fisheries, Cooperative Center for Marine Animal Health, Silver Spring, MD, USA

ABSTRACT

Domoic acid (DA), produced by marine diatom species in the genus *Pseudo-nitzschia*, is a potent excitotoxin linked since the late 1990s to massive marine mammal and seabird mortalities along the California coast. These and a previous incident involving human intoxication and deaths prompted many studies, some of which have unveiled the trophic transfer of DA from benthic invertebrates and planktivorous fish to top predators, demonstrating serious health risk to marine wildlife and humans. Top predator populations that may be more adversely affected by DA include those with narrow geographical distribution or those that are already in decline as a result of other environmental stressors or natural cyclic fluctuations. However, to date no studies have attempted to assess the population effects of recurrent exposures to DA on any of the affected wildlife species. Ecological risk assessment can help to identify DA effects on wildlife, but meaningful assessments require the integration of many types of information, often not available to conduct such studies. Hence, determining short- and long-term effects on marine wildlife populations

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Address correspondence to Adriana C. Bejarano, NOAA/NOS, Cooperative Center for Marine Animal Health, Hollings Marine Laboratory, 331 Fort Johnson Road, Charleston, SC 29412, USA. E-mail: BejaranoAC@gmail.com

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is rather challenging. The purpose of this review is to highlight recent research efforts and information gaps, and the need for interdisciplinary programs that allow collaborative wildlife population risk assessments of critical species.

Key Words: marine biotoxin, domoic acid, *Pseudo-nitzschia*, toxic algal blooms, wildlife.

INTRODUCTION

Certain marine diatom species in the genus *Pseudo-nitzschia* produce the biotoxin domoic acid (DA), an analog of the excitatory neurotransmitter glutamate. Binding of DA to glutamate receptors causes prolonged receptor activation and constant influx of cations into the neurons, leading to calcium toxicity. The potency of this biotoxin was first identified during an event involving the intoxication of 107 adults and the death of 3 elderly people (Perl *et al.* 1990). Ingestion of mussels containing up to 1.28 mg DA/g tissue produced gastrointestinal (vomiting, abdominal cramps, and diarrhea) and neurological disorders (short- and long-term memory loss), leading to the term amnesic shellfish poisoning (ASP; Perl *et al.* 1989). Since first identified as a human hazard in the late 1980s (Perl *et al.* 1989), and confirmed as a potent neurotoxin to California sea lions in the late 1990s (Scholin *et al.* 2000; Gulland 2002), DA has been shown to be intricately associated with the food web, thus posing a serious health risk to many top predators (Bargu *et al.* 2002; Lefebvre *et al.* 2001; Lefebvre *et al.* 2002a).

Furthermore, toxic *Pseudo-nitzschia* blooms not only have overlapped the geographical distribution of birds and marine mammals, but have also temporally coincided with the breeding season of marine wildlife (Brodie *et al.* 2006; Peery *et al.* 2006), further stressing this biotoxin as a pressing environmental threat. There is also an alarming increased occurrence of *Pseudo-nitzschia* blooms (*i.e.*, along the California coast) following a water temperature regime shift in the North Pacific Ocean (a.k.a. anchovy regime; Chavez *et al.* 2003). This shift, which appears to have occurred in the mid-1990s, favors cooler water, stronger upwelling, and increased nutrient input in the eastern Pacific, thus supporting greater phytoplankton productivity, and a larger northern anchovy (*Engraulis mordax*) population (Chavez *et al.* 2003). The multidecadal duration of this regime (~25-year) favors frequent toxic blooms along the California coast, with yet unknown consequences to many marine populations.

High trophic level wildlife species that have been affected by DA include California brown pelicans (*Pelecanus occidentalis*; U.S. Fish and Wildlife Service [USFWS] unpubl.), marbled murrelets (*Brachyramphus marmoratus*; Peery *et al.* 2006), California sea lions (*Zalophus californianus*; Gulland 2002; Brodie *et al.* 2006), common dolphins (*Delphinus delphis*; Torres de la Riva *et al. in review*), and Southern sea otters (*Enhydra lutris nereis*; Kreuder *et al.* 2005). Yet, no studies have attempted to assess the population level effects of recurrent exposures to DA on any of these species. Pressing concerns regarding the health of high trophic level wildlife and potential population implications are the main rational for conducting formal ecological risk assessments. Models and strategies for marine biotoxin risk assessments can be modified from previous experiences with persistent organic pollutants and pesticides (Ross

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and Birnbaum 2003; Solomon *et al.* 2001). However, multiple lines of evidence and multidisciplinary literature on the spatial and temporal occurrence of these toxic blooms, the trophic interactions of DA vectors with top predators, and all the intricate processes related to DA (*i.e.*, toxicity, production and effects to wildlife) are needed to support such efforts. In this review, we highlight recent research related to all levels pertinent to DA, identify information gaps and research needs, and stress the urgency for interdisciplinary programs with the objective of promoting wildlife population risk assessments for critical seabird and marine mammal species.

DIATOMS AND DOMOIC ACID PRODUCTION

Domoic acid (DA) is currently known to be produced by 11 species of pennate diatoms in the genus *Pseudo-nitzschia* (*P. multiseries*, *P. pseudelicatissima*, *P. delicatissima*, *P. australis*, *P. seriata*, *P. fraudulenta*, *P. pungens*, *P. turgidula*, *P. multistriata*, *P. calliantha*, *P. cuspidata* and *P. galaxiae*; Bates 2000; Bates and Trainer 2006), as well as a related species, *Nitzschia navis-varingica* (Kotaki *et al.* 2000). The biosynthetic pathway for DA in these toxigenic diatom species is not entirely resolved. Douglas *et al.* (1992) investigated DA biosynthesis by pulse labeling with [^{13}C] acetate and by analyzing the resultant molecules using nuclear magnetic resonance (NMR). This study suggested that DA is biosynthesized through the condensation of 3-hydroxyglutamate with an isoprenoid, probably geranyl pyrophosphate, followed by cyclization to produce a proline ring (Douglas *et al.* 1992). Smith *et al.* (2001) proposed that 3-hydroxyglutamate used in this reaction is actually derived from oxidative degradation of 3-hydroxyproline, a major cell wall component in diatoms, and found that DA levels in *Pseudo-nitzschia* indeed correlate inversely with free intracellular proline, consistent with its role as a precursor.

Although all toxigenic *Pseudo-nitzschia* species are planktonic and coastal, and most are distributed worldwide (Bates and Trainer 2006), *N. navis-varingica* has only been isolated from a brackish water shrimp pond (Kotaki *et al.* 2000). Although all species listed above are toxic, many non-toxic blooms of these species have occurred in various regions of the world, suggesting that toxicity is inducible by environmental conditions. In culture, cellular DA production is generally low during the exponential growth phase and increases as cells enter late exponential phase when cell division rates decrease as a result of nutrient depletion. Both silicon (Si) and phosphorus (P) depletion were shown to correlate with increased DA production following prolonged senescence (Pan *et al.* 1996a,b; Bates 1998). Because Si is essential for diatom cell wall (frustule) formation, its depletion limits cell division and increases DA production (see Bates 1998). Log phase cells grown under low iron (Fe) conditions can also induce toxin production, resulting in the excretion of DA to the medium and subsequent increase in Fe uptake (Maldonado *et al.* 2002). Unlike centric diatoms, *Pseudo-nitzschia* spp. are capable of adapting to growth under low Fe conditions, where DA plays a role as a chelator (Rue and Bruland 2001). Thus, the currently proposed functional role of DA is that it serves to chelate both extracellular Fe and copper (Cu), the latter of which is necessary for the function of high affinity Fe transporters that support growth under Fe-limiting conditions (Wells *et al.* 2005).

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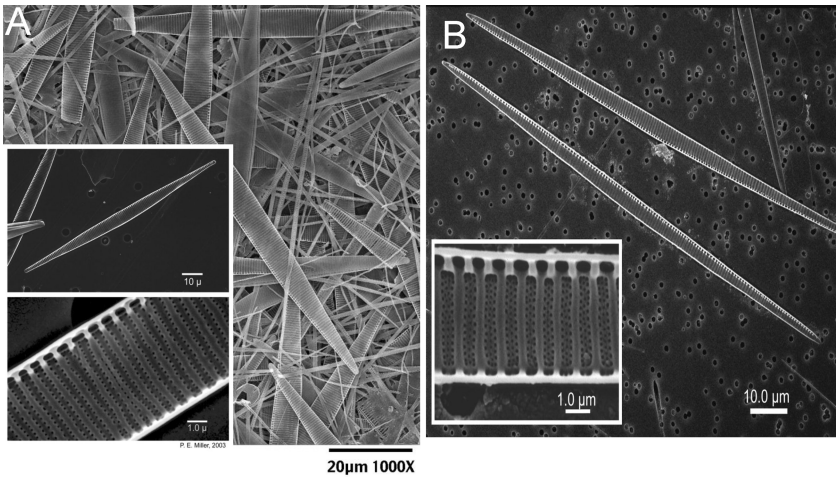

Figure 1. Scanning electro microscopy of domoic acid producing diatoms. A. *Pseudo-nitzschia australis* and B. *Pseudo-nitzschia multiseries* frustules. Photos courtesy of P. Miller, USCSC.

Not all *Pseudo-nitzschia* species appear to produce DA, and among the known toxic species, different levels of production are observed. For example, clonal cultures of *P. multiseries* from the Washington coast produced particulate and dissolved DA up to 70.4 nmol/L and >5 nmol/L, respectively, whereas *P. australis* produced particulate and dissolved DA up to 3.2 nmol/L and 4.3 nmol/L, respectively. In contrast, *P. delicatissima*, *P.* cf. *pseudodelicatissima*, *P. pungens*, and *P. fraudulenta* isolates from the same region produced low or undetectable levels of DA under the same culture conditions (Baugh *et al.* 2006). Not surprisingly, *P. multiseries* and *P. australis* (Figure 1) have been the most abundant DA producers in multiple toxic blooms along the California coast (CDHS 2000, 2002, 2005).

One epigenetic contributor to expressed toxicity may be the presence of different bacterial flora in association with different culture isolates. Bates *et al.* (1995) demonstrated that bacterial strains can enhance *P. multiseries* synthesis of DA between 2- to 95-fold compared to bacteria-free cultures, but the bacteria from these cultures do not themselves appear to produce DA (Bates *et al.* 2004). Bacteria growing epiphytically on *P. multiseries* may provide metabolic precursors that facilitate diatom production of DA while in turn benefiting from extracellular nutrient release from the diatoms (Bates *et al.* 2004; Kaczmarska *et al.* 2005). However, the mechanism by which bacteria enhance DA production in some *Pseudo-nitzschia* species is still unknown (Bates 1998).

On a larger scale, the environmental conditions that favor diatom blooms vary among geographic regions depending on mesoscale oceanographic and climatic conditions. Upwelling regions such as the west coast of North America, Chile, Spain, and Portugal support recurrent blooms of several toxic *Pseudo-nitzschia* species (Bates *et al.* 1998; Bates and Trainer 2006). However, blooms are also found in regions with much different hydrographic characteristics as the Gulf of Mexico and the Bay of Fundy (Martin *et al.* 1990; Pan *et al.* 2001). Freshwater nutrient runoff (i.e., rich

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in phosphates, nitrates, and silicates) has been proposed as one of the triggering factors of *Pseudo-nitzschia* blooms in areas along the Pacific coast of North America and the Gulf of Mexico (see Trainer *et al.* 2000). Trainer *et al.* (2000) also suggested that nutrient inputs from upwelling, combined with wind transport of cells, are important factors in the initiation of toxigenic algal blooms.

Along the California coast, algal blooms are initiated during spring and maintained through summer by nutrient-rich upwelled water (Horner *et al.* 1997). Between late summer and fall the concentration of DA-producing diatoms is at its highest, coinciding with coastal upwelling subsidence and nutrient depletion (see Kudela *et al.* 2005; Trainer *et al.* 2000; Walz *et al.* 1994). These toxic *Pseudo-nitzschia* blooms have been inconsistent in their annual geographical occurrence, and are not restricted to coastal waters, as cellular concentration of DA (up to 6300 ng/L) has been found within 20 km of the coast (Trainer *et al.* 2000). In the Monterey Bay area, where *Pseudo-nitzschia* blooms have occurred frequently (see below), large-scale features including floor topography, water circulation, and proximity to a major coastal upwelling may be favorable to the development and maintenance of algal blooms, as they influence the spatial distribution of phytoplankton (Ryan *et al.* 2005). In this area, *P. australis* abundance has ranged from 10^4 to 10^7 cells/L (September 1991, Walz *et al.* 1994; May-June 1998, Scholin *et al.* 2000; June 1998, Trainer *et al.* 2000), with intracellular DA ranging from 0.1 to 78 pg DA/cell (Scholin *et al.* 2000; Trainer *et al.* 2000). In the nearby Santa Barbara Channel, high densities of *P. australis* (2×10^6 cells/L) and elevated particulate and dissolved DA concentrations were found in May of 2003, in the center of a silicate-depleted cyclonic eddy (Anderson *et al.* 2006). Although to date it is unknown whether the physical conditions within eddies favor toxigenic blooms, these currents can retain and transport phytoplankton to adjacent waters (Anderson *et al.* 2006).

Despite several efforts aimed at characterizing toxic, large-scale *Pseudo-nitzschia* blooms in California, the occurrence of these events is inconsistent in relation to specific oceanographic and environmental conditions (i.e., terrestrial runoff, nutrients, temperature; Kudela *et al.* 2004). The complexity of these toxic algal blooms and the various environmental scenarios under which they occur further hinders a clear correlation between *Pseudo-nitzschia* density, and intracellular DA and DA concentration in the water column (K Lefebvre and G Langlois, pers. comm.), limiting our ability to predict the severity and location of these blooms. These toxic blooms, however, almost annually over the last decade, have left behind many poisoned birds and marine mammals owing to the toxic potency of DA.

DOMOIC ACID MODE OF TOXICITY

Domoic acid (DA) is toxic primarily to animals with complex central nervous systems. This biotoxin is an analog of the amino acid glutamic acid and the excitatory neurotoxin kainic acid. The structural similarity of DA to these compounds allows tight binding to glutamate receptors and displacement of both chemicals from binding sites, accentuating its toxic potency. Glutamic acid is one of the main excitatory neurotransmitters of the central nervous system, present in nearly 40% of all neuronal synaptic sites (Coyle and Puttfarcken 1993). At synaptic sites glutamate binds to ionotropic glutamate receptors, ligand-gated ion channels embedded

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in pre- and post-synaptic nerve cell membranes, including α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), kainate receptors (Na^+-permeable ion channels; Sommer and Seeburg 1992), and N-methyl-D-aspartate receptors (NMDA; Ca^{++}, Na^+ and K^+- permeable ion channels; Nakanishi 1992). Binding of glutamate to these receptors causes differential voltage potential in the membrane, resulting in the transmission of the nerve impulse to adjacent nerve cells.

At high concentrations, glutamate acts as a toxic molecule over-stimulating nerve cells through the activation primarily of NMDA receptors (Berman and Murray 1997), causing prolonged elevated intracellular calcium, and thus leading to calcium toxicity. Domoic acid mimics glutamate by binding to kainate and AMPA receptors, but with approximately 100-fold higher affinity than glutamate. Unlike glutamate, DA binding causes partial opening of the ion conducting pore and simultaneous disruption of the desensitization process, resulting in prolonged receptor activation and constant influx of cations into the neurons (Hampson and Manalo 1998). However, the extreme potency of DA results from the integrative action of its effects on glutamate receptors on both sides of the synapse (reviewed by Ramsdell 2007). Its action on presynaptic glutamate receptors results in extensive glutamate release, whereas its consequences on the post-synaptic membrane results from the sequential activation of kainate and NMDA receptors, voltage gated calcium channels, and reversal of the Na^+/Ca^{2+} exchanger that together result in sustained elevated calcium and to calcium toxicity (Xi and Ramsdell 1996; Berman and Murray 1997; Berman *et al.* 2002). One of the most critical aspects of the toxicity of DA is its induced neuronal degeneration. Glutamate receptors are most highly concentrated in the hippocampus, a region of the brain where DA induces the most significant damage (*see* Ramsdell 2007 for details). Because of high density of kainate receptors the areas CA3 and CA1 within the hippocampus, are highly prone to DA-induced neurodegeneration (Debonnel *et al.* 1989). Damage on the area CA3 leads to the disruption of learning and memory formation, an induced toxicity that has profound ecological implications (*see* later).

Aside from the localized effects of DA in the brain, exposed rats have shown irreversible developmental effects. Intrauterine exposure of mice to subacute levels of DA caused severe neuronal and progressive hippocampal damage (function and morphology; Dakshinamurti *et al.* 1993). In rats, such exposure compromises the ability of adult rats to perform memory tasks and impairs hormonal imprinting of sex differences in the hippocampus, which would normally define sexual behavioral traits later in life (*see* Ramsdell 2007; Levin *et al.* 2005). Neonate rats exposed during brain development to low doses of DA (5 or 20 μg/Kg bw) also exhibit permanent behavioral changes, and visible anatomical changes characteristic of repeated seizures and hippocampal hyperexcitability (Doucette *et al.* 2004). Increased sensitivity of neonates to DA likely results from limited renal clearance, leading to high biotoxin levels in the blood stream (*see* Ramsdell 2007).

Glutamate receptors are also found outside the central nervous system (*see* Ramsdell 2007). Studies in the heart of rats and monkeys found evidence of GluR5/6/7 and NMDAR1 subtypes, receptors that can mediate the excitatory effects of DA (Gill *et al.* 2000; Mueller *et al.* 2003). These findings are consistent with the myocardial lesions found in wild California sea lions and southern sea otters exposed to DA (*see* later; Gulland 2000; Kreuder *et al.* 2005). Glutamate receptor subtypes that

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can mediate the toxic action of DA are also present in kidney, liver, lung, spleen, and testis (Gill *et al.* 2000); however, their involvement in DA toxicity has not been demonstrated.

The large body of literature presented here shows the complex interactions of DA with receptors in vital organs. This complexity is further amplified by considering biotoxin interactions with a larger ecological system: the food web.

DOMOIC ACID: VECTORS AND EFFECTS ON WILDLIFE

Invertebrates

Benthic and filter-feeding invertebrates are perhaps some of the most important vectors of DA in the environment. Nutrient depletion causes *Pseudo-nitzschia* cells to become less buoyant and to sink onto the sediment (Trainer *et al.* 2000), where the biotoxin is readily available to invertebrates such as clams and oysters. Domoic acid accumulates primarily in the digestive gland of shellfish, with no apparent toxicological effects, but with negative consequences to consumers. Shellfish vectors include mussels (*Mytilus* spp), razor clams (*Siliqua patula*), Dungeness crabs (*Cancer magister*; Wekell *et al.* 1994), and sand crabs (*Emerita analoga*; Ferdin *et al.* 2002; Powell *et al.* 2002), species that are important from a regulatory standpoint as they serve as sentinels of environmental quality. For instance, the California Marine Biotoxin Monitoring Program (California Department of Health Services) surveys several areas along the California coastline for marine biotoxins in several species (razor clam, spiny lobster, Pacific oyster, sea mussels, among others). Routine quantification of DA in various species results in warnings or quarantines that ensure that shellfish are safe for human consumption. Of these vectors, the sand crab, one of the most common intertidal invertebrates along the California coast, has been proposed as a reliable indicator for DA, as it mimics internally the abundance of *Pseudo-nitzschia* in the water column (Ferdin *et al.* 2002).

Other important vectors have been studied by Costa *et al.* (2003, 2004, 2005a) along the coast of Portugal. One of the most abundant species along the Portuguese–Spanish continental shelf (Fariña *et al.* 1997) is the swimming crab (*Polybius henslowii*), a potential DA vector (Costa *et al.* 2003) to the yellow-legged gull *Larus cachinnans* (Munilla 1997). In the swimming crab, the maximum measured DA concentration of 323.1 μg DA/g tissue was found in July of 2002, with concentrations receding to 106.8 μg DA/g tissue two months later. In this species, viscera, followed by gonad, had the highest DA concentrations (max: 571.6 μg DA/g tissue and $<$120 μg DA/g tissue, respectively), whereas gill and muscle tissues had between 4 to 12 times less DA than viscera. Elevated DA in viscera and relatively low levels in tissues supports previous observations of low gastrointestinal biotoxin assimilation (*see* Ramsdell 2007). The same researchers also analyzed levels of DA in several carnivorous cephalopods (Costa *et al.* 2004, 2005a,b). A two year study evaluated the occurrence of *Pseudo-nitzschia*, and the distribution and accumulation of DA in the common cuttlefish (*Sepia officinalis*) (Costa *et al.* 2005a), an important prey for Mediterranean monk seals (*Monachus monachus*) and Risso's dolphin (*Grampus griseus*) (Clarke *et al.* 1985; Salman *et al.* 2001). The digestive gland of the cuttlefish contained high concentration of DA (241.7 μg DA/g tissue) two weeks after a *Pseudo-nitzschia*

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bloom (9.8×10^4 cells/L; June, 2003), and high biotoxin levels (132.4 μg DA/g tissue) nearly a year later and despite low *Pseudo-nitzschia* densities (9×10^2 cells/L) (Costa *et al.* 2005a).

Delayed and long-lasting accumulation of DA in the cuttlefish may indicate a slow biotransfer of DA in herbivores and omnivores, and may result in delayed impacts to high trophic levels. In this species, branchial hearts also contained measurable amounts of DA derivatives (epi-DA and iso-DA) of known lower neurotoxicity than the parent compound (*see* Costa *et al.* 2005a), suggesting the presence of a detoxification pathway for this biotoxin. Costa *et al.* (2005b) also quantified DA in two cephalopod species (*Eledone cirrhosa* and *Eledone moschata*) collected simultaneously and found that whereas in one species (*E. moschata*) the concentration of DA in the digestive gland reached levels as high as 100 μg DA/g tissue, the other species (*E. cirrhosa*) had either non-detectable or low levels of DA (Costa *et al.* 2005b). These findings highlight the implications of feeding ecology on the relevance of cephalopods as DA vectors. Elevated concentrations of DA in the digestive gland of cephalopods imply that when ingested whole these vectors pose a great risk to top predators. Despite high DA concentration in the invertebrates presented herein, these species showed little or no toxicological response to this biotoxin.

Other invertebrate species regarded as important DA vectors to top predators are Euphausiids (krill) (Bargu *et al.* 2002). These keystone pelagic crustaceans are an important prey source for invertebrates, fish, birds, seals, and baleen whales (Baker *et al.* 1990). In Monterey Bay, krill samples with elevated DA levels (44 μg DA equiv/g krill) (Bargu *et al.* 2002) were detected simultaneously with high densities of *P. australis* (10^6 cells/L). Tangible effects of dissolved DA in krill included reduced grazing rates (control $= 12 \pm 2.5 \times 10^4$ cells/mg dw/d *vs.* $\leq$1000 mg DA/d treatment $\leq 6.8 \pm 1.6 \times 10^4$ cells/mg dw/d) possibly due to toxicity of the filtering apparatus or avoidance due to a chemoreceptor mechanism (Bargu *et al.* 2006). However, the larger ecological implications of reduced grazing rates in krill are currently unknown.

Most research with invertebrates has focused on their role as DA vectors, whereas fewer have documented toxicological effects resulting from exposure. Jones *et al.* (1995) studied the immune response of the Pacific oyster (*Crassostrea gigas*) to DA (1.6 pg DA/cell) by evaluating changes in the phagocytic activity of haemocytes, one of the main defense mechanism against foreign material. During exposure to DA, oysters showed increased haemocyte density and fluctuations in haemocyte chemiluminescence, with both endpoints returning to normal levels after a 48 h clearance period. This study, as well as the previously presented research on invertebrates, has not found lethal effects resulting from exposure to DA, suggesting either a low sensitivity to the biotoxin or yet unnoticed negative effects. Given their importance in the marine food web, studies on the toxicological effects of DA to invertebrates are essential, as well as research on their role as bioindicators and as vectors to upper level consumers.

Fish

Schooling fish whose diets are composed partially or totally of marine diatoms (*i.e.*, planktivorous and omnivorous fish) are important DA trophic vectors to wildlife

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as these fish can accumulate DA and *Pseudo-nitzschia* frustules in their gastrointestinal tract (Fritz *et al.* 1992; Scholin *et al.* 2000). The planktivorous northern anchovy (*Engraulis mordax*) and Pacific sardine (*Sardinops sagax*) are known DA vectors to higher trophic-levels including salmon, seabirds, and marine mammals (Nettleship *et al.* 1984; Fritz *et al.* 1992; Lefebvre *et al.* 1999, Scholin *et al.* 2000). Although numerous studies have identified planktivorous fish as vectors of DA (Fritz *et al.* 1992; Scholin *et al.* 2000; Lefebvre 2002a), few have evaluated its subacute toxicity. Lefebvre *et al.* (2001) found that DA administered directly into the abdominal cavity of anchovies (intracoelomic (*i.c.*) 1–14 μg DA/g fish) crossed the blood-brain barrier and exerted severe neurological toxicity (*i.e.*, disorientation, spinning, inability to school) comparable to that observed in marine mammals. However, direct injection of this biotoxin into body cavities (*i.e.*; or intraperitoneal (*i.p.*)) may overestimate its toxicological effects as DA can be more quickly absorbed into the blood stream and at volumes higher than via oral administration. In fact, oral administration of DA to fish has shown no toxicological effects demonstrating a relatively low gastrointestinal absorption of the biotoxin (Lefebvre *et al.* 2001). This in turn may explain the low amounts of DA found in brain from freshly collected anchovies during a *Pseudo-nitzschia* bloom. Consistently, high levels of DA in viscera (up to 1175 μg/g), but normal fish behavior, have been found in field DA exposed animals (Lefebvre *et al.* 2001). In comparison, Schaffer *et al.* (2006) found no histological or behavioral (swimming and feeding) changes in juvenile leopard sharks (*Triakis semifasciata*) exposed to DA doses as high as 27 mg/Kg (*i.c.*), despite the presence of glutamate receptors (GluR 5/6/7) in shark brains. The presence of an endogenous ligand for kainic acid receptors may be effectively competing with DA for binding sites, preventing neurological toxicity in sharks (Schaffer et. al. 2006).

The effects of DA in fish have also been studied at the neuronal level. Immediate early response genes, like c-fos, are genes that activate immediately after cellular stimulation with DA (Peng and Ramsdell 1996). These genes encode for transcription factors that initiate the expression of downstream effector proteins (*see* Salierno *et al.* 2006). C-Fos protein expression in killifish (*Fundulus heteroclitus*) brains was used to characterize patterns of neuronal activation following exposure to DA (Salierno *et al.* 2006). A 60-min exposure to DA (*i.p.* injection, 5 mg/Kg) not only increased c-fos expression in the anterior optic lobe, but also caused behavioral changes indicative of neuronal alterations (*i.e.*, hyperactivity, disorientation, loss of balance). To date, no other studies have evaluated c-fos induction in fish following exposure to DA.

Despite their importance as DA vectors, only a handful of published literature exists on continuous DA quantification in fish, information that can be used to estimate acute and subacute doses in top predators. From a health assessment perspective, continuous spatial and temporal monitoring of multiple recognized and suspected vectors is also vital to determine their relative risk to susceptible species.

Seabirds

Incidents of large and unusual seabird mortalities linked to biotoxins have been largely under-reported (Shumway *et al.* 2003). The first anecdotal account of a

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massive seabird toxicosis potentially caused by DA dates back to 1961, when a large flock of sooty shearwaters (*Puffinus griseus*) exhibiting neurological disorders typical of DA intoxication appeared along the Santa Cruz (California) coast (Garrison and Walz unpubl; Mestel 1995). Later, in 1991 DA intoxication was first confirmed as the cause of death of 95 Brandt's cormorants (*Phalacrocorax penicillatus*) and 43 brown pelicans (*Pelecanus occidentalis*) (Fritz at al. 1992; Work *et al.* 1993a,b). During this event, dozens of surviving animals exhibited DA-induced behavioral symptoms (head weaving, scratching, and vomiting; Fritz at al. 1992), whereas dead birds typically had 40–50 μg DA/g wet weight (ww) in stomach contents (max: 150 μg DA/g ww). Schools of anchovies located within a mile of these seabirds roosting areas were the primary biotoxin vectors (whole fish $\leq$100 μg DA/g , viscera $\leq$ 190 μg DA/g, and flesh$\leq$ 40 μg DA/g) in this event (Work *et al.* 1993b; Fritz *et al.* 1992). In Baja California a few years later, DA caused >150 brown pelican deaths and decimated 50% of the colony (Sierra-Beltrán *et al.* 1997). Pelican tissues had a quantifiable amounts of DA (37.2 μg/g) in the digestive track, and although mackerel (*Scomber japonicus*) and sardines (*Sardinops sagax*) were the suspected biotoxin vectors, only stomach contents of mackerel tested positive for DA (142.85 μg/g). Surviving seabirds showed subacute DA symptoms including reduced motor coordination and disorientation, agitation, as well as swimming and flying impairment (Sierra-Beltrán *et al.* 1997).

These and other reports (USFWS unpubl.) of DA-induced toxicosis in seabirds have involved the California brown pelican, a species that since 1970 has been listed as endangered. These frequent massive mortalities combined with other environmental stressors may be hampering the recovery that this population has experienced over the last decade. Likewise, the 1998 *Pseudo-nitzschia* bloom in central California may have contributed to the reduced 1998–1999 interannual survival of the marbled murrelet (*Brachyramphus marmoratus*; Peery *et al.* 2006). The actual impacts of DA on this population, and perhaps on other bird populations, are largely unknown given the difficulties associated with animal and carcass recovery (Peery *et al.* 2006), and positive identification of this biotoxin in body fluids and tissues.

The assessment of DA impacts on seabird populations requires information on the population(s) at risk including estimates on biotoxin-induced mortality rates and compromised survival skills (reduced foraging efficiency). In addition, coupling of population dynamics with DA exposure assessment studies may prove useful to evaluate potential long-term effects. We further underscore the importance of research on feeding ecology and reiterate previous recommendations by Shumway *et al.* (2003).

Marine Mammals

Some of the marine mammal adaptations to diving may increase their vulnerability to DA exposure and intoxication. During diving, marine mammals reduce oxygen consumption by slowing the heart rate and restricting blood flow to tissues and organs except for vital organs (heart and brain) (Butler and Jones 1997; Fuson *et al.* 2003). Restricted blood flow to the kidneys, which decreases glomerular filtration rate (reducing blood detoxification), and increased blood flow to the heart and brain, may facilitate localized exposure to this biotoxin. Not surprisingly, marine mammals are often the most conspicuous animals affected by DA.

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Marine mammal mass mortalities and live strandings along the California coast, involving hundreds of animals within a relatively short period of time, have been documented since the 1970s (Gulland 2000). However, DA was first recognized as the primary cause of some of these events in the late 1990s following a large scale stranding and mortality of California sea lions (*Zalophus californianus*; Brodie *et al* 2006; Scholin *et al.* 2000). Between May and June 1998, more than 400 stranded sea lions suffered neurological dysfunctions (*i.e.*, disorientation, seizures, ataxia and scratching behavior; Gulland 2000; Silvagni *et al.* 2005) consistent with DA's interaction with neurotransmitter receptors. Visual confirmation of DA's role in this event was also obtained from clinical signs and histopathological examination of the brain, which showed hippocampal atrophy and ischemic neuronal necrosis (Figure 2) and explained the observed neurological disorders (Gulland 2000). Exposure to DA also resulted in pallor of the myocardium and fibrinous pericarditis, bronchopneumonia, and death due to pregnancy-related complications (Gulland 2000; Scholin *et al.* 2000; Gulland *et al.* 2002; Silvagni *et al.* 2005).

Brodie *et al.* (2006) later suggested that the elevated reproductive failure seen in 1998 among females admitted to California marine wildlife rehabilitation centers (n = 209; abortion, premature parturition of pups and death of pregnant sea lions) was primarily caused by exposure to DA. The biotoxin was detected in maternal urine, fetal feces, urine and gastric fluid, as well as in amniotic fluid, and at least 14% of those females showed lesions consistent with exposure to DA. Although to date, the mechanisms by which DA causes reproductive toxicity are unclear, it is likely the result of uterine excitation and the synergy between DA and oxytocin (Ramsdell 2007). Potential effects in sea lion pups exposed to DA during gestation and/or lactation are also of concern as these routes of exposure have been confirmed in experimental animals (mice and rats; Levin *et al.* 2005, 2006; Maucher and Ramsdell 2005).

Over the last 6 years, half of the 500+ sea lions treated in rehabilitation facilities for DA toxicosis have exhibited chronic neurological effects lasting more than 2 weeks post-exposure (Goldstein *et al.* 2005). Furthermore, released animals treated for DA toxicosis are more likely to restrand than animals admitted for other causes (Goldstein *et al.* 2005), and many have displayed long-term behavioral changes including male aggression and severe disorientation (Goldstein *et al.* 2005; T. Goldstein pers. comm.). These long-lasting effects may compromise an individual's post-rehabilitation survival and reproductive potential, as debilitating and likely irreversible neurological alternations may lead to reduced foraging and mating efficiency. However, further research is needed to elucidate the ecological implications of DA-induced behavior changes in sea lions.

Other marine mammal mortalities along the California coast have also been linked to DA. Recently, Kreuder *et al.* (2003) reported the first four cases of DA intoxication in southern sea otters (*Enhydra lutris nereis*). Although DA toxicosis represented only 4.2% of the total causes of sea otter mortality from 1998–2001, confirmation of DA cases will increase with improved diagnostic methods and continuous monitoring (Kreuder *et al.* 2003). Kreuder *et al.* (2005) later evaluated the risk factors associated with cardiac diseases in sea otters finding that exposure to DA increased the probability of myocarditis and dilated cardiomyopathy (11 and 31.5 times, respectively), with myocarditis advancing to dilated cardiomyopathy with recurring or

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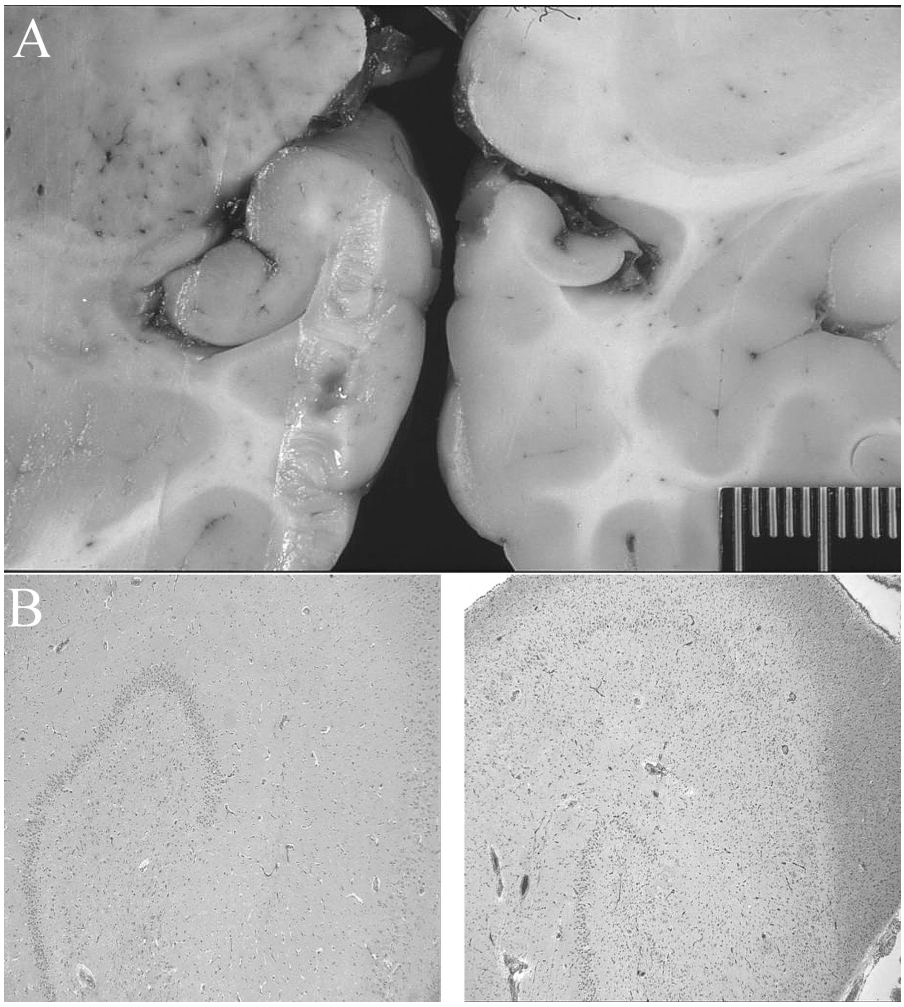

Figure 2. Typical lesions induced by domoic acid (DA) in California sea lion hippocampus compared to unexposed animals. A. Normal hippocampus (left) *versus* hippocampus atrophy (right), and B. Normal neuronal density (left) *versus* DA induced ischemic neuronal necrosis (right). Photos courtesy of TMMC.

prolonged exposure to DA (Kreuder *et al.* 2005). This research also found variations in the prevalence of DA cases across stranding locations along the California coast, with the southern most animals (Morro Bay) being 55 times more likely to die from DA-induced myocarditis than unexposed sea otters from other areas (Kreuder *et al.* 2005). These researchers further suggested that the excitotoxic effects of DA on preganglionic neurons, intracranial ganglia, and the interconnecting plexus alter the cardiac rhythm, leading to myocardial necrosis and inflammation. Sea otters with dilated cardiomyopathy were often emaciated and showed bite wounds, indicating that

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this condition could compromise successful foraging and adequate defense against territorial males (Kreuder *et al.* 2005).

Domoic acid has also been linked to multispecies marine mammal mortality events. In the second largest unusual mortality event in the United States along the southern California coast in 2002, many stranded species tested positive for this biotoxin (*see* later). These included California sea lions, long-beaked common dolphins (*Delphinus capensis*), southern sea otters, as well as grey pelicans (*Pelecanus occidentalis*) (Heyning 2003). Although mortalities/strandings of humpback (*Megaptera novaeangliae*) and blue whales (*Balaenoptera musculus*) in this event were not definitively linked to DA exposure, low levels of DA (10 to 207 μg/g) and *Pseudo-nitzschia* frustules were also found in fecal samples from both species (Lefebvre *et al.* 2002b).

A similar multispecies stranding and mortality event occurred in 2004 along the Gulf of California. Although inconclusive, this event may have been caused by DA as *P. pseudodelicatissima* was the dominant phytoplankton species in the surrounding waters (Sierra-Beltrán *et al.* 2005). In total, 103 long-beaked and 9 short-beaked dolphins, 195 sea lions and 9 grey pelicans (*Pelecanus occidentalis*) were found dead or stranded, whereas nearly 20 tons of sardines (*Sardinops* ssp.) were found dead floating offshore. Although DA was detected in dolphin samples, this mass mortality event was not conclusively attributed to DA due to limited analysis performed during the event.

Although DA has also been detected in samples from marine mammals on both the East and Gulf coasts of the United States, it has not yet been directly linked to any marine mammal mortality in these areas. Low levels of DA were found in 2 of 15 dead humpback whales on Georges Bank in 2003 (NMFS 2005), and has been found over a 4-year period in feces of Northern right whales (*Eubalaena glacialis*) in the Bay of Fundy (Doucette *et al.* 2006). Similarly, samples (*i.e.*, stomachs contents, urine, and feces) collected during a massive bottlenose dolphin (*Tursiops truncatus*) mortality event along the panhandle of Florida contained low amounts of DA, between one and two times lower than those of marine mammal counterparts of the California coast (NMFS 2004). This dolphin mortality event, however, was attributed to brevetoxins as the primary factor. Following this 2004 event, the National Marine Fisheries Service (NMFS) emphasized the importance of establishing baseline information on the concentration of biotoxins, including DA, in marine mammal populations (NMFS 2004).

A better understanding of the feeding requirement and ecology of marine mammals and a temporal evaluation of vital health parameters are essential to produce species specific DA assessments, and to determine long-term effects on marine mammal populations.

ISSUES AND CURRENT EFFORTS

Potential Effects of Domoic Acid on Wildlife Populations

For many marine species, such as the California sea lion, the risk of adverse effects from biotoxins at an individual level is incontrovertible. Hundreds of individual sea lion cases have been investigated and the findings of high levels of DA in body fluids, as well as symptoms and histopathology consistent with the neurotoxic effects of DA

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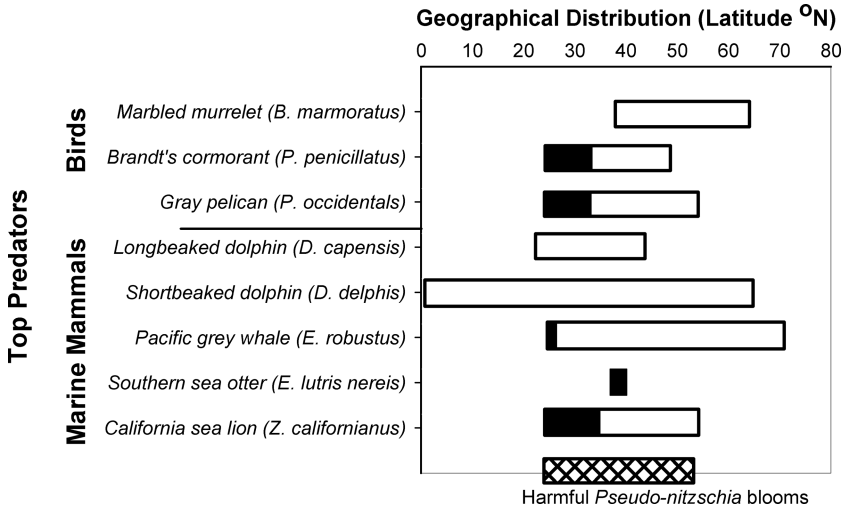

Figure 3. Overlap between the geographical distribution of top predators (sources: The Sanctuary Integrated Monitoring Network and NatureServe) and *Pseudo-nitzschia* blooms. Only distribution in the northern hemisphere was included. The black bar represents the distribution breeding grounds, when known.

seen in experimental studies, leave little doubt of DA as a cause of mortality and morbidity. The more important question, and the one that is much more difficult to answer, is whether or not there will be long-term consequences for the populations. Population effects are certainly of concern given the geographical and temporal scale of toxic *Pseudo-nitzschia* blooms that have often coincided with the spatial distribution and breeding season of many top predators (Figure 3; Brodie *et al.* 2006; Peery *et al.* 2006). Exposure of seabirds to DA could have devastating population consequences, given their reproductive strategy (*i.e.*, low reproductive rate and delayed sexual maturity; Shumway *et al.* 2003). Despite the large California sea lion die-offs, the viability of this population, which has seen an exponential growth over the past several decades (Lowry and Maravilla-Chavez 2005), is not likely to be immediately threatened by DA. However, large-scale blooms that have occurred prior to the pupping season (May–June; DeLong *et al.* 1973), have caused fatalities, primarily of adult females (Gulland 2002), and have been associated with increased reproductive failure rates (Brodie *et al.* 2006). Thus, given repeated events over time, the potential long-term risk for this population is also worth investigating.

Populations that may exhibit the greatest risk of adverse effects by DA include those with narrow distribution ranges and with breeding grounds that overlap the geographical occurrence of toxic blooms. A specific population that may be at risk is that of the southern sea otter (Figure 3), a species with a narrow distribution covering only a couple of hundred miles (Point Año Nuevo, Santa Cruz to Purisima Point, Santa Barbara) and that is listed as threatened under the Endangered Species Act of 1973. The area encompassing the sea otter's distribution range has certainly seen toxic *Pseudo-nitzschia* blooms over the last years. Other populations at risk also

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include species with wider distribution ranges that have experienced severe population declines such as the brown pelican. If top predator populations are indeed affected by DA, this may lead to changes in the structure of marine communities by shifting the structure of the food web. Hence, assessing potential effects on populations of top level predators is a crucial first step towards assessing potential ecosystem impacts.

Information Needs for Risk Assessment: An Example with California Sea Lions

Due to the repeated California sea lion morbity/mortality events involving DA in recent years, substantial efforts are being directed toward understanding potential population level impacts and we use this species as an example to illustrate risk assessment data needs and current knowledge gaps. Following the standard tiered paradigm established for ecological risk assessment for chemical contaminants, we examine the types of information required to support the various components of the risk assessment process (Figure 4).

Effects characterization

Despite massive mortalities resulting from DA intoxication, we do not know the actual oral DA doses that cause acute or subacute neurotoxic effects in marine mammals. Only a handful of data on incidental and quantitative mammalian toxicology exist for DA. The data provided by Perl *et al.* (1990) on the 1987 incident involving human intoxication, suggests a non-lethal oral dose of 1.24 mg DA/Kg (*i.e.*, gastrointestinal symptoms) and a minimum lethal dose of 2.71 mg DA/Kg (*i.e.*, admission to the intensive care unit), for an average 70 Kg individual. In the Cynomolgus monkeys, Truelove *et al.* (1997) found a no observable adverse effect levels (NOAEL) of 0.75 mg/ Kg and a non-lethal oral dose of 1 mg/Kg. Estimating oral DA doses for marine mammals, for which experimental data do not exist, is less straight forward. Lefebvre *et al.* (2002a) estimated the oral DA dose for humpback whales assuming a diet exclusively based on schools of anchovies. Based on the weight and feeding rate of whales, and on the concentration of DA and energy density of anchovies, a minimum daily oral dose of 1.1 mg DA/Kg was estimated for this species. Similar estimations were made for blue whales feeding on krill, resulting in an oral dose of 0.62 mg DA/Kg whale (Bargu *et al.* 2006). These doses, relative to estimated toxic oral doses in humans, suggest potential neurotoxic effects in whales (Lefebvre *et al.* 2002a). However, we do not know how the sensitivity to DA compares between marine wildlife and primates.

More detailed efforts have been concentrated on estimating acute and subacute doses in California sea lions via computer modeling assuming ingestion of anchovies and sardines (Bejarano *et al.* 2007). The sea lion bioenergetic model incorporated information on energy requirements, prey energy density, and DA concentrations measured in sea lion prey during a bloom event and used Monte Carlo simulation to derive distributions of likely DA oral doses. The model results were then compared to available DA oral toxic thresholds to assess the risk of non-lethal and lethal toxic effects on an individual sea lion. Assuming a realistic daily anchovy diet (10%), the estimated DA daily doses ranges from a low 0.81 mg/Kg bw in adult males to a high of 4.01 mg/Kg bw in female pups. In contrast, a daily sardine diet (10%) would

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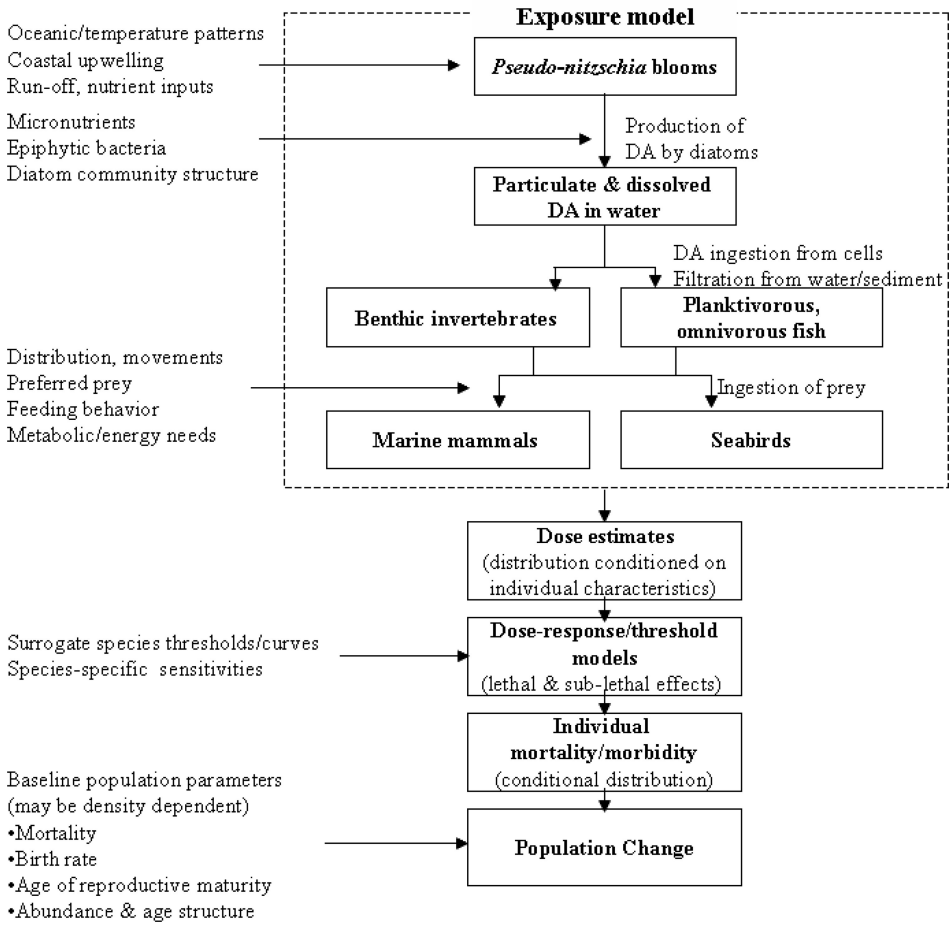

Figure 4. A conceptual model for conducting ecological risk assessments in seabirds and marine mammals exposed to domoic acid (DA).

result in DA daily doses at least 60% lower than those estimated for an anchovy diet. These findings suggest that anchovies are a more potent DA vector to sea lions than sardines, results that may have larger implications under the current multidecadal anchovy regime (Chavez *et al.* 2003).

Although the estimated thresholds extrapolated from experimental studies are informative, actual species-specific acute and subacute doses for California sea lions would require more intensive sample collection and analysis. Specifically, samples collected as a time series for DA analysis across several compartments (feces, urine, serum, and other fluids) would need to be collected starting immediately upon stranding. This information is required to estimate the minimum DA doses that results on sublethal or lethal effects.

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Exposure characterization

Although understanding the species-specific sensitivities for DA dose-response relationships are certainly important, the more daunting task for assessing long-term population risks for marine species, and in particular California sea lions, lies in the characterization of exposures. Characterizing potential DA exposures is immensely difficult, partially because of the complex spatial and temporal dynamics of *Pseudo-nitzschia* blooms. This is particularly true when toxic blooms of *Pseudo-nitzschia* occur offshore where our capabilities to detect effects on birds and marine mammals are limited. This spatial and temporal complexity is further amplified by the intricate relationship between *Pseudo-nitzschia* and the marine food web (Figure 4). For example, the introduction of DA into the food web is not solely dependent on the occurrence of blooms, but also influenced by the diatom community structure and presence of epiphytic bacteria and specific micronutrients that promote DA production.

Furthermore, because certain prey species are indicated as more potent DA vectors and the abundance of such prey are spatially and temporally variable, the variable prey distribution and availability in concert with the feeding ecology of receptor species must be considered. California sea lions in particular have been reported to vary in their feeding behaviors both seasonally and between reproductive classes. For example, Melin (1995) reported that forage habitat differed for female sea lions during breeding and non-breeding seasons, with females foraging further offshore during the non-breeding season. Melin (1995) further reported that choice of forage area was influenced by the female's lactation status, with females foraging closer to the breeding colony during lactation and closer to the mainland coast during the post-lactation period. In contrast, adult males reportedly often forego at-sea foraging trips during the breeding season in order to remain at the colony, guarding their potential mates (Francis and Heath 1991). This variability in feeding behavior and habitat use related to sex, age-class, and reproductive status has likely influenced the structure of past mortality events, which have had a disproportional effect on adult females, and illustrates how feeding ecology of the receptor species adds yet another layer complexity to the characterization of DA exposures.

The multitude of environmental factors which influence DA production, the seasonal and long-term cyclic movements and abundance of a number of prey species, and the seasonal movements of sea lions themselves with varying choices of foraging habitat, create a complex system requiring information on a large number of parameters if modeling for risk assessment/forecasting are to be implemented. In addition, to reliably characterize potential long-term population risks requires a capability to forecast with some degree of certainty the environmental factors, such nutrient inputs, water temperature and the long- and short-term oceanic cycles that may drive the frequency, intensity, duration, and characteristics of *Pseudo-nitzschia* blooms over the next several decades.

The Importance of Database Repositories: An Example with Marine Mammals

Assessing the effects of DA and other biotoxins on wildlife also relies on adequate documentation of large-scale events. In particular, having centralized databases for reporting of wildlife mortality, for instance the Marine Mammal National Stranding

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Database mandated under the Marine Mammal Protection Act (MMPA; 16 U.S.C. 1421f), are critical to support analysis of trends and correlations of unusual mortality with specific environmental events. Of the total number of marine mammals (ca. 2796 individuals, 18 species) reported dead or stranded along the California coast in 2002, an estimated 13% showed signs consistent with DA intoxication (seizures and disorientation; ca. 377 individuals, 7 species) (NMFS stranding records 2002). In most of these cases DA was not directly quantified and postmortem examination to confirm hippocampal lesions was not conducted. Short-beaked dolphins followed by sea lions and long-beaked dolphins had the highest percentage of suspected DA cases (60%, 18%, and 14%, respectively), most of which were male dolphins (62%) and female sea lions (82%). The stranding data also indicated that most suspected DA cases were reported between March and June with peak strandings (most of which were sea lions) occurring in late April. A similar analysis of this stranding event by Mazet *et al.* (2005), ruled out DA as the major cause of stranding or death for humpback whales, harbor seals (*Phoca vitulina*), and northern elephant seal (*Mirounga angustrirostris*) based on species-specific ecological data, previous stranding patterns and analysis of the stranding demographics. Analyses similar to those undertaken by Mazet *et al.* (2005) and Torres de la Riva *et al.* (in review), and involving the evaluation of stranding trends related to season, species, and sex- and age-class are crucial for detecting and quantifying the potential impacts of DA to populations. Expanding data repositories to include consistent information on pathologies, measured biotoxin levels, and causes of death will also allow for large scale epidemiological studies to investigate factors in marine mammal mortality events.

CLOSING REMARKS

Understanding the connection among all the intricate processes related to DA production and its effects on wildlife populations, and the elaboration of comprehensive ecological risk assessments for specific species further stresses the need for multidisciplinary efforts and scientific collaborations. An example of a multidisciplinary effort that will likely improve bloom detection as well as identification of potentially impacted areas is the recently established *Rapid Analysis of Pseudo-nitzschia and DA, Locating Events in near-Real Time* (RAPDALERT) a research project part of the Monitoring and Event Response for Harmful Algal Blooms (MERHAB, NOAA; D. Caron pers. comm.). This early warning system uses new technology (satellite sensing and *in-situ* sensor networks) providing near-real time spatial and temporal information at a resolution high enough to aid the detection of algal blooms along Southern California. This system can be further utilized to establish sampling strategies (*i.e.*, vector species) and to advise increased monitoring efforts to ensure rapid response and increased animal recovery in critical areas (*i.e.*, seabirds and marine mammals). These and similar efforts could be also expanded to prevent human exposure to DA via shellfish vectors.

The information presented in this review highlights the challenges associated with the current knowledge and understanding of diatom production of DA, bloom development, and assessment of effects through the food web. These aspects are interconnected and information at any of these levels will help understand the dynamic interactions of DA with the surrounding abiotic and biotic environments. Research

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at all levels of DA interactions will be beneficial for future wildlife population health assessments, as well as to address potential human health concerns.

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