Spring 2016

Year-Round Algal Toxin Exposure in Free-Ranging Sea Lions: Implications of Trophic Exposure for Declining Populations

Adrianne M. (Adrianne Monet) Akmajian
akmajia@students.wwu.edu

Follow this and additional works at: https://cedar.wwu.edu/wwuet

Part of the Biology Commons

Recommended Citation

YEAR-ROUND ALGAL TOXIN EXPOSURE IN FREE-RANGING SEA LIONS:

IMPLICATIONS OF TROPHIC EXPOSURE FOR DECLINING POPULATIONS

By

Adrianne Monet Akmajian

Accepted in Partial Completion

of the Requirements for the Degree

Master of Science

Kathleen L. Kitto, Dean of the Graduate School

ADVISORY COMMITTEE

Chair, Dr. Alejandro Acevedo-Gutiérrez

Dr. Suzanne Strom

Dr. Brian Bingham

YEAR-ROUND ALGAL TOXIN EXPOSURE IN FREE-RANGING SEA LIONS:

IMPLICATIONS OF TROPHIC EXPOSURE FOR DECLINING POPULATIONS

A Thesis

Presented to

The Faculty of

Western Washington University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

By

Adrianne Monet Akmajian

May 2016

ABSTRACT

Harmful algal bloom (HAB) toxins have led to illness and mortality of many species of marine mammals and seabirds, including species with declining populations. On the US West Coast, the two most common HAB toxins affecting both humans and wildlife are domoic acid and saxitoxin. In an effort to document baseline concentrations and to investigate factors that affect exposure to HAB toxins, I measured concentrations of domoic acid and saxitoxin in scats from Steller sea lions *Eumetopias jubatus* (n = 383 scats) and California sea lions *Zalophus californianus* (n = 125 scats) in Washington State over a two-year period. Toxin concentrations in the scat were compared to the prey remains in the scat and to concentrations in nearshore bivalves. Saxitoxin was detected in 45 % and domoic acid was detected in 17 % of all scats tested, and both toxins were detected in all seasons and months of the year. Saxitoxin in scat was variable by season, year, and location, whereas domoic acid levels were consistently higher in the summer and at the southern-most haulout complex. Both toxins were detected in scat in winter when it was not detected in nearshore bivalves, confirming for the first time that marine mammals can be exposed to algal toxins through their prey outside periods of active algal blooms, most likely through benthic to pelagic food web transfer of precipitated cells and resting cysts. This study also found that prey with low occurrence in the sea lions' diet, including walleye pollock *Theragra chalcogramma*, may act as vectors of significant algal toxin transfer up the food chain, a finding that could have profound implications for the endangered western distinct population segment of Steller sea lions because pollock are a dominant prey species in their diet. A variety of planktivorous, benthic, and pelagic

iv

fish were significantly associated with toxins in sea lion scat suggesting that multiple

pathways through the marine food web lead to HAB toxin exposure in these top

predators. In the face of increasing HABs worldwide, the finding that generalist

predators, like sea lions, can be exposed to algal toxins year-round via multiple prey

species may signal disproportionate impacts on declining populations already enduring

multiple stressors.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GENERAL INTRODUCTION

Harmful algal blooms (HABs) are increasing worldwide and have led to mortalities of both humans and marine wildlife, including several species of marine mammals (Fire & Van Dolah 2012). This trend is particularly concerning for declining and depleted wildlife populations (Forcada et al. 1999, Durbin et al. 2002, Shumway et al. 2003, Shearn-Bochsler et al. 2014), providing the need to understand what factors most influence exposure to these toxins. Despite recent mortality events related to HAB exposure and other pathogens, the US population of California sea lions *Zalophus californianus* is estimated at nearly 300,000 individuals (Carretta et al. 2014). Conversely, the Steller sea lion *Eumetopias jubatus* experienced an 80% reduction in abundance in US waters from the 1970s through 1998 (Miller et al. 2005). The decline in the population was driven by declines in the western distinct population segment (DPS) (Western Alaska through Russia; west of 144° W) whereas during the same time period the eastern DPS (Southeast Alaska through California; east of 144° W) increased at 3% per year (Pitcher et al. 2007). Currently the western DPS is listed as endangered and the eastern DPS was recently delisted (NMFS 2008, 2013). Though no mortalities related to algal toxins have been reported in Steller sea lions, monitoring to assess the threat of biotoxins is listed as a priority in the recovery plan of the western DPS (Goldstein et al. 2005, NMFS 2008) and for post-delisting monitoring of the eastern DPS (NMFS 2013).

This study assessed the year-round exposure of Steller and California sea lions in Washington State, USA, to two common algal toxins, domoic acid and saxitoxin, by measuring the concentrations of the two toxins in sea lion scat. To understand what

factors put marine mammals most at risk of marine algal toxin exposure, I review the

history of HAB toxins in North America and their impacts on human health as well as the

oceanographic conditions and life history features that lead to their occurrence. To more

specifically understand the impacts of algal toxins on marine wildlife, I review our

knowledge of illness and mortalities in marine mammals, potential routes of exposure

through the marine food web, and sea lion food habits and foraging behavior.

HABs and Human Health

Toxins are produced by some species of bloom-forming marine algae in events

referred to as harmful algal blooms (HABs) or red tides (Van Dolah 2000). Although not

all HABs result in production of algal toxins, these toxins have perhaps become most

well known for their contamination of shellfish and their impacts on human health (Van

Dolah et al. 2001, James et al. 2010). On the US West Coast, the two most common

outcomes of HABs that affect human and animal health are paralytic shellfish poisoning

and amnesic shellfish poisoning (Horner et al. 1997, Trainer 2002).

Paralytic shellfish poisoning is caused by dinoflagellates of the genera

Alexandrium, *Gymnodinium*, and *Pyrodinium* (Backer & McGillicuddy 2006), though on

the West Coast only species of *Alexandrium* have been documented (Trainer 2002).

These dinoflagellates produce saxitoxin and a suite of congeners, of which saxitoxin is

the most toxic (Wang 2008, James et al. 2010). The primary producer of saxitoxin in

Washington is *Alexandrium catenella* (Trainer 2002). Saxitoxin binds to site 1 of voltage-

gated sodium channels embedded in the plasma membranes of nerve and muscle cells, effectively blocking neurotransmission by inhibiting sodium ion release and creation of an action potential, leading to paralysis (Bricelj & Shumway 1998, Wang 2008). In humans, saxitoxin initially causes numbness, particularly around the lips, mouth, and face, and in more severe cases can lead to a lack of motor coordination and respiratory paralysis (Backer & McGillicuddy 2006, Wang 2008). The lethal oral dose of saxitoxin in humans is $1 - 4$ mg; however, because saxitoxin is cleared through the urine in <24 hours, deaths are rare when medical treatment is given (Van Dolah et al. 2001, Backer & McGillicuddy 2006, James et al. 2010). Although the minimum dosage causing toxicity in humans is around 200 µg saxitoxin 100 g^{-1} tissue (2000 ng g^{-1}), the regulatory limit for shellfish harvesting in the US is set conservatively at only 80 µg saxitoxin 100 g^{-1} tissue (800 ng g^{-1}) (Wekell et al. 2004).

Amnesiac shellfish poisoning is primarily caused by diatoms of the genus *Pseudo-nitzschia* that produce the neurotoxic amino acid domoic acid (Landsberg 2002). At least ten species of *Pseudo-nitzschia* have been identified along the Washington coast (Trainer et al. 2009), of which *P. pseudodelicatissima*, *P. australis*, *P. cuspida*, and *P. multiseries* have been associated with high levels of domoic acid (Trainer et al. 2007, 2009). Domoic acid mimics the excitatory neurotransmitter glutamate (Landsberg 2002). The toxin binds to glutamate receptors in neural tissue, primarily located in the hippocampus, causing continuous activation of the receptor and release of Ca^{2+} ions until the cell dies, ultimately leading to atrophy of the hippocampus (Landsberg 2002, Bejarano et al. 2008). Clinical symptoms of amnesiac shellfish poisoning in humans include gastrointestinal distress, such as vomiting and diarrhea, and neurological

3

symptoms such as disorientation, seizures, and permanent memory loss (James et al. 2010, Fire & Van Dolah 2012). The minimum lethal oral dose estimated for an average 70 kg adult is 200 to 300 mg of domoic acid, or ingesting $0.7 – 4$ mg domoic acid kg^{-1} body weight (Wekell et al. 2004). Federal US regulators implemented a conservative regulatory limit of 20 µg g^{-1} (20,000 ng g^{-1}) in the tissue of shellfish consumed by humans using the lowest observed dose to cause symptoms (50 mg), the assumption that a normal meal of shellfish is 200 g, and a safety factor of 12 (Wekell et al. 2004).

Outbreaks of both amnesiac and paralytic shellfish poisoning cause temporary and season-wide closures of shellfish harvest on Washington beaches (Trainer 2002, Dyson & Huppert 2010). The length of harvest closure depends in part on toxin depuration rates that vary among the species sampled from a period of days to weeks or months (Shumway 1990; Whyte et al. 1995). Blue mussels *Mytilus edulis* and California mussels *Mytilus californianus* are good indicators of saxitoxin in the environment because they display toxins weeks before other co-occurring bivalves (Bricelj & Shumway 1998). Saxitoxin has few reported toxic effects on the mussels themselves, allowing them to accumulate the toxins quickly at high concentrations and to depurate the toxins from their tissues within several weeks (Shumway 1990, Bricelj & Shumway 1998). Similarly, domoic acid has no known toxic effects on mussels and they are able to completely clear the toxin from their body in two weeks or less (Novaczek et al. 1992, Whyte et al. 1995). Conversely, Pacific razor clams *Siliqua patula* hold domoic acid in their tissues for as long as six to 18 months with relatively few toxic effects (Wekell et al. 1994, Trainer & Bill 2004). The length and timing of harvest closure and the impact of algal blooms on

4

human and wildlife health also depends on the oceanographic conditions and plankton

life histories that result in bloom production.

Oceanography and Plankton Life History

HABs occur when the right oceanographic conditions and life history stages of

the phytoplankton coincide (Van Dolah 2000). Relatively few species of phytoplankton

produce toxins (Van Dolah 2000) and the ecological reasons for toxin production are not

well understood (Turner & Tester 1997). Toxicity varies based on many factors including

plankton species and strain, growth phase, salinity, temperature, nutrient limitation, and

presence of bacterial flora (Anderson 1998, Van Dolah et al. 2003, Bejarano et al. 2008).

Increased monitoring and surveillance of HABs has substantially increased our

understanding of the species involved and their clinical effects (Landsberg 2002,

Anderson et al. 2008).

The apparent increase in HABs in recent years is in part attributed to increases in

bloom-favorable conditions. Eutrophication and changes in climate and oceanographic

conditions can enhance bloom growth and lengthen the bloom season (Anderson et al.

2008, Moore et al. 2008). Furthermore, bloom-forming species may disperse to new areas

via storms and currents (Anderson et al. 2002) and if climate change increases storm

frequency or modifies currents it could increase dispersal of toxin-producing species

(Moore et al. 2008). The unnatural dispersion of phytoplankton species via transport in

ship ballast water may also be increasing the dispersal of toxin-producing plankton (James et al. 2010).

The northwest Washington coast is a unique marine environment created by the confluence of oceanic currents with outflow from the Strait of Juan de Fuca, wind- and topographic-induced upwelling, and nutrient inputs from the Fraser and Columbia River outflows (Hickey & Banas 2003). A cold-water gyre, known as the Juan de Fuca Eddy, is formed seasonally off the mouth of the Strait of Juan de Fuca, spanning 50 km in diameter, and characterized by cold surface waters, low dissolved oxygen, and high nutrients (Hickey & Banas 2003, Marchetti et al. 2004, MacFadyen et al. 2005). The eddy is initiated in the spring by southward wind-driven currents that combine with the outflow of the Strait of Juan de Fuca and the westward flow of the coastal Vancouver Island Current (MacFadyen et al. 2005). Located at the northernmost extent of the California Current System, nutrient rich waters of the California Undercurrent are brought up through the Juan de Fuca Canyon (>400 m) to the mouth of the Strait of Juan de Fuca, then returned offshore via the outflow of the Strait (Hickey & Banas 2003). Topographic upwelling in the Juan de Fuca Canyon and at Cape Flattery, as well as wind-driven upwelling beginning in the spring, make the eddy a nutrient-rich area with high primary productivity and phytoplankton biomass, particularly around its margins (MacFadyen et al. 2008). In the fall and winter, the eddy dissipates with the shift to northward, downwelling-favorable winds (Tweddle et al. 2010).

High levels of domoic acid in nearshore shellfish along the Washington coast are thought to originate offshore in the Juan de Fuca Eddy, which has been identified as a hotspot for *Pseudo-nitzschia* and domoic acid production (MacFadyen et al. 2008,

6

Trainer et al. 2009). Although *Pseudo-nitzschia* cells have been found nearshore, the species assemblage differs from that found in the offshore eddy region and typically does not include the species known to produce toxin (Trainer et al. 2009). Waters from the eddy may be transported to the Washington coast as thin filaments that escape the southeastern margin of the eddy during southward, upwelling-favorable winds (MacFadyen et al. 2005, 2008, Trainer et al. 2009). During northward wind shifts, such as periods of low winds or storm events, eddy waters and associated *Pseudo-nitzschia* or domoic acid are brought onshore (MacFadyen et al. 2005, 2008, Trainer et al. 2002, 2009). During such events, very high concentrations of domoic acid have been measured in Washington razor clams, leading to beach closures (Trainer et al. 2002).

     Pseudo-nitzschia bloom when surface water begins to cool and mix across the thermocline, allowing them to outcompete dominant summer plankton, such as dinoflagellates, that require more stratified waters (Mos 2001). As such, the eddy concentrations of *Pseudo-nitzschia* and particulate domoic acid (pDA, the toxin within the plankton cells) are high in the late summer or early fall (Marchetti et al. 2004, Trainer et al. 2009). While domoic acid production has been frequently associated with nutrient and trace metal depletion in both laboratory and field studies (Mos 2001, Anderson et al. 2006, Bejarano et al. 2008, Trainer et al. 2012), high concentrations of *Pseudo-nitzschia* and pDA in the eddy have been associated with high nutrient concentrations (Trainer et al. 2009). High concentrations of domoic acid may be attributed to larger, sexually-produced *Pseudo-nitzschia* (Mos 2001). As asexual reproduction occurs, each daughter cell is successively smaller and when the cells reach a certain threshold, sexual reproduction is induced to return cells to normal size (Mos 2001, Trainer et al. 2012).

7

Sexual reproduction in *Pseudo-nitzschia* has been associated with high concentrations of domoic acid detected in harvested Pacific razor clams (Holtermann et al. 2010).

Hotspots for *Alexandrium* production in Washington are primarily reported in inland waters including Sequim Bay and Discovery Bay in the eastern Strait of Juan de Fuca (Trainer et al. 2009). Despite closures of shellfish harvest due to high concentrations of saxitoxin (Trainer 2002), there is little published information on *Alexandrium* distributions or occurrence of blooms on the Washington outer coast. *Alexandrium* species typically proliferate in calm, highly stratified waters, such as nearshore embayments, undergoing diel migrations to locate nutrients throughout the water column (Nishitani & Chew 1984, Anderson 1998, Trainer et al. 2003). *Alexandrium* blooms may occur with initial spring or summer warming, (in Washington the threshold is about 13° C, Nishitani & Chew 1984), and re-bloom in the fall when temperatures start to decline (Anderson 1998, Weise et al. 2002). Offshore occurrence of *Alexandrium*, including reports of deep-water cyst germination, has been reported in other areas and may in part be the result of vegetative cells or cysts transported from inshore to offshore waters via river outflow or coastal currents (Anderson 1998, Townsend et al. 2001). Marchetti et al. (2004) detected *Alexandrium* species in low concentrations in the Juan de Fuca Eddy, however, little is known about offshore occurrence of *Alexandrium* in the region.

Alexandrium are meroplankton, undergoing both sexual and asexual reproduction, including a vegetative, motile form and a dormant, resting cyst form (Wyatt & Jenkinson 1997, Anderson 1998). Gametogenesis leads to formation of zygotes that remain motile for 1-2 weeks before either producing new gametes or forming cysts, which settle to the

8

bottom and remain dormant until conditions for germination are right (Wyatt &
Jenkinson 1997, Anderson 1998). Saxitoxin is produced by the bloom-forming vegetative
cells, with the highest saxitoxin concentrations detected during the highest cell densities
(Wyatt & Jenkinson 1997). However, dormant cysts may hold the toxin and are often
more toxic than the vegetative cells (Wyatt & Jenkinson 1997, Bricelj & Shumway
1998). Even in relatively low abundance, *Alexandrium* may produce saxitoxin, as
evidenced by detection of saxitoxin in nearshore shellfish despite *Alexandrium* cells not
being detected in nearshore waters (Tweddle et al. 2010). While shellfish toxicity in the
summer may be due to the vegetative cells, winter toxicity in shellfish is primarily
attributed to cysts (Wyatt & Jenkinson 1997, Bricelj & Shumway 1998). *Alexandrium*
cysts can be upwelled into the water column and taken up by nearshore shellfish
(Schwinghamer et al. 1994, Tweddle et al. 2010, Butman et al. 2014). When these algal
blooms or cyst hotspots overlap with the prey and foraging areas of marine mammals,
there is the potential for toxin exposure and serious illness or mortalities.

HABs and Marine Mammals

Harmful algal bloom (HAB) toxins have been suspected or implicated in mass
strandings of multiple species of marine mammals including dolphins, seals, sea lions,
sea otters *Enhydra lutris*, West Indian manatees *Trichechus manatus*, and large whales
(Bossart 2011, Fire & Van Dolah 2012). HAB-related strandings of marine mammals
were first documented in the US in the 1980s (Anderson & White 1989) and have since

become increasingly frequent (Fire & Van Dolah 2012). Today, the majority of marine mammal mass stranding events in the US have been attributed to HAB toxins (Fire et al. 2010). The specific concentrations that cause toxicity and mortality are largely unknown, but the unique biology of marine mammals may make them more susceptible to the toxins than humans or terrestrial wildlife (Anderson & White 1989).

Saxitoxin binds to marine mammal neural tissue with high affinity and specificity (Trainer & Baden 1999). The paralytic nature of saxitoxin may put marine mammals at particular risk of toxicity. As part of the mammalian dive response, marine mammals shunt blood to their vital tissues such as the lungs and brain and away from detoxifying tissues such as liver and kidney (Anderson & White 1989). Sublethal effects, such as loss of equilibrium or respiratory distress typical of paralytic shellfish poisoning in humans, could quickly incapacitate marine mammals and lead to death (Anderson & White 1989). Although symptoms of chronic exposure have not been documented, Durbin et al. (2002) suggest that repeated exposure to saxitoxin could affect diving capabilities. The authors suggest that longer surfacing time after a dive, as observed in North Atlantic right whales *Eubalaena glacialis*, could be the result of saxitoxin exposure and inhibition of respiratory or muscle coordination and could increase the possibility of ship strike (Durbin et al. 2002).

Saxitoxin has caused mass mortalities of humpback whales *Megaptera novaeangliae* and endangered Mediterranean monks seals *Monachus monachus* (Anderson & White 1989). In 1987, 14 humpback whales died in Cape Cod due to eating Atlantic mackerel *Scomber scombrus* containing high concentrations of saxitoxin (Geraci et al. 1989). The whales appeared in good body condition and were observed actively

feeding up to 90 minutes before death (Geraci et al. 1989). In 1997, saxitoxin was suspected in the deaths of 117 Mediterranean monk seals off the coast of Western Sahara (Hernández et al. 1998). Similar to the case of the humpback whales, the seals appeared in good body condition, but exhibited a lack of motor coordination and paralysis, and many were found floating with congested lungs indicative of drowning (Hernández et al. 1998). In addition to these mortality events, saxitoxin exposure has also been documented in a variety of other pinniped and cetacean species (Durbin et al. 2002, Doucette et al. 2006, Jensen et al. 2015, Lefebvre et al. 2016).

In general, little is known about the concentrations of saxitoxin that may cause health effects in marine mammals, and exposure has primarily been documented through testing feces, urine, and body fluids or testing prey species or stomach contents (Geraci et al. 1989, Durbin et al. 2002, Doucette et al. 2006). For terrestrial mammals, the lethal oral dose of saxitoxin ranges from 200 to 600 μg 100 g^{-1} (6,000 ng g^{-1}) (Anderson & White 1989). In the case of the stranded humpback whales, it was estimated that the concentration of saxitoxin in the whale's prey was high enough that whales would obtain the lethal dose of saxitoxin before consuming their daily average amount of food (Anderson & White 1989). Although the majority of saxitoxin is cleared rapidly through the urine (Van Dolah et al. 2001), saxitoxin has been detected in low concentrations in body tissues such as liver, kidney, muscle and brain in Mediterranean monk seals (Hernández et al. 1998, Reyero et al. 1999). Because feces was not collected from these monk seals, it unknown how concentrations in the scat compared to accumulation in the body tissues.

11

Cases of marine mammal strandings due to domoic acid have been much more extensive than those associated with saxitoxin. Mortalities have included California sea lions (Gulland 2000, Scholin et al. 2000, Bejarano et al. 2008, Goldstein et al. 2008), long-beaked common dolphins *Delphinus capensis* (Torres de la Riva et al. 2009), a common minke whale *Balaenoptera acutorostrata* (Fire et al. 2010), and sea otters (Torres de la Riva et al. 2009). Exposure to domoic acid has confirmed in a wide variety of other species (Fire et al. 2009, Lefebvre et al. 2010, 2016, Twiner et al. 2011, McHuron et al. 2013, Jensen et al. 2015). Both acute and chronic pathologies of domoic acid toxicity have been described in marine mammals (Goldstein et al. 2008, Zabka et al. 2009). Acute symptoms include head weaving, muscle tremors, seizuring, and ataxia (Goldstein et al. 2008). Chronic symptoms are epilepsy with intermittent seizures and abnormal behaviors such as stranding in unnatural locations (e.g. roadways), obsessive repetitive behaviors, or abnormal aggression (Goldstein et al. 2008). Both acute and chronic pathologies may be detected through atrophy of neurons and distinct lesions in the hippocampus (Goldstein et al. 2008, Buckmaster et al. 2014). Chronic toxicity may compromise foraging behavior and navigation, causing sea lions to decrease diving ability and travel out of their normal range (Thomas et al. 2010). Domoic acid is also linked to reproductive failure, causing abortion, premature parturition, and stillbirth in California sea lions (Brodie et al. 2006, Goldstein et al. 2009).

Due to its water-soluble nature, domoic acid is primarily detected in feces, urine, and other fluids and has not been identified in other body tissues such as kidney, liver, muscle, and brain (Gulland 2000). Domoic acid is rapidly excreted from the body through both urine and feces and is thus commonly highest in these products (McHuron

et al. 2013). While concentrations in feces indicate exposure, detection of domoic acid in the urine or serum indicates absorption through the digestive tract and into the blood stream (Fire et al. 2010, Lefebvre et al. 2010). Still, it is not well known what concentrations induce toxicity. In sea lions exhibiting clinical symptoms of acute domoic acid toxicity, domoic acid was not always detected in feces, urine, or other fluids (Gulland 2000, Goldstein et al. 2008). The toxin concentrations detected in feces of sea lions exhibiting acute symptoms may be as low as 10 ng ml^{-1} (Goldstein et al. 2008) to >120,000 ng g^{-1} (Lefebvre et al. 1999).

HABs and the Marine Food Web

Given the breadth of species with known exposure to HAB toxins and the wide variety of feeding habits of these species, it is likely that algal toxins transfer up the marine food web by many pathways. Domoic acid and saxitoxin have been detected in a variety of fish species (Lefebvre et al. 2002, Vigilant & Silver 2007, Jensen et al. 2015), suggesting that generalist foragers such as sea lions may be exposed to toxins through multiple prey items. The mechanism of marine mammal dietary exposure to algal toxins has been primarily investigated through sampling of prey or fecal remains of actively foraging or live-captured animals (Durbin et al. 2002, Lefebvre et al. 2002, Jensen et al. 2015) and sampling the feces or stomach contents of ill or deceased animals (Geraci et al. 1989, Lefebvre et al. 1999, Fire et al. 2010).

Several mass strandings of marine mammals due to HAB toxins have been linked to consumption of a specific prey species, while other studies have found more complex spatial and temporal trends. Stranded California sea lions and a common minke whale had eaten Northern anchovy *Engraulis mordax* contaminated with high concentrations of domoic acid (Lefebvre et al. 1999, Fire et al. 2010). In a mass stranding of humpback whales due to saxitoxin toxicity, the whales' normal prey item at that time of year, sand lance *Ammodytes* spp., was absent, leading the whales to travel farther north looking for prey and resulting in consumption of contaminated Atlantic mackerel (Anderson & White 1989). Spatial overlap of foraging areas and algal blooms is of particular concern for adult female California sea lions, which strand at a greater frequency from domoic acid toxicity compared to other demographic groups due to foraging in offshore locations that overlap with the major algal blooms (Silvagni et al. 2005, Bejarano et al. 2008).

Several pathways for trophic exposure in the marine food web exist. Zooplankton and planktivorous fish are a major source of algal toxins to marine mammals and seabirds (Bargu et al. 2002, Lefebvre et al. 2002). Zooplankton, such as krill and copepods, have exposed humpback and blue whales *Balaenoptera musculus* to domoic acid (Lefebvre et al. 2002) and North Atlantic right whales to saxitoxin (Durbin et al. 2002). Domoic acid and saxitoxin have been detected in anchovy, sardine *Sardinops sagax*, and Pacific herring *Clupea pallasii* (Lefebvre et al. 2001, Costa & Garrido 2004, Jester et al. 2009). Saxitoxin in Pacific sand lance *Ammodytes hexapterus* has led to deaths of several marine birds (Shumway et al. 2003, Shearn-Bochsler et al. 2014). Toxins primarily accumulate in the gut or viscera of fish, with little accumulation in the muscle, and do not appear to cause direct toxic effects on the fish (Lefebvre et al. 2001, 2007, Jester et al. 2009).

Domoic acid and saxitoxin have also been detected in a variety of both benthic and pelagic fish species (Lefebvre et al. 2002, Vigilant & Silver 2007, Jensen et al. 2015). Vigilant et al. (2007) propose two possible pathways for exposure of benthic fish to these toxins. Bentho-pelagic feeding fish may eat planktivorous fish or zooplankton that are directly feeding on the toxic phytoplankton (Vigilant & Silver 2007). The second pathway for benthic feeding fish is feeding on plankton or fish fecal pellets containing the toxin when they sink to the bottom (Vigilant & Silver 2007). HABs toxins are also found higher in the marine food web in fish such as albacore tuna *Thunnus alalunga* (Lefebvre et al. 2002).

Toxicological risk in marine mammals is frequently estimated using the known concentrations of toxins in fish tissues and calculating toxin exposure based on feeding rates (Fire et al. 2010, Jensen et al. 2015) and bioenergetics models (Bejarano et al. 2007). In a somewhat novel approach, Jensen et al. (2015) used the otoliths found in harbor seal *Phoca vitulina* feces to selectively sample fish species to test for domoic acid and saxitoxin and estimate oral dosages from eating these common prey species. Bejarano et al. (2007) used a bioenergetics model including toxin concentrations measured in Northern anchovy and Pacific sardine to estimate the relative impact and toxicity of these two species on California sea lions. The authors concluded that the risk of domoic acid exposure is higher if sea lions ingest anchovy compared to sardine (Bejarano et al. 2007). However, both prey availability and the migration and foraging patterns of the predator itself will further influence the risk of exposure to algal toxins.

Sea Lion Food Habits and Foraging Behavior

Steller and California sea lions are considered generalist predators that feed on seasonally and locally abundant prey and that may migrate in response to seasonally predictable dense prey aggregations such as herring spawns and salmon returns (Weise 2000, Sinclair & Zeppelin 2002, Womble et al. 2005, 2009, Bredesen et al. 2006, Sigler et al. 2009). A more diverse diet could reduce the potential for acute HAB toxicity from ingesting a single contaminated prey species, however the detection of HAB toxin in a variety of pelagic and benthic fish (Lefebvre et al. 2002, Vigilant & Silver 2007, Jensen et al. 2015) suggests that generalist predators may be at risk of exposure from multiple vectors. Further, although sea lions may feed on a large number of prey species, diets are often dominated by only a few species (Sigler et al. 2009, Riemer et al. 2011).

The overlapping range of eastern DPS Steller sea lions and California sea lions leads to an overlap in diet, suggesting the two species could have a similar risk of exposure to algal toxins. Steller sea lions of all demographic groups are present on the Washington coast year-round and in recent years classic breeding structure and low levels of pupping have been observed(Jeffries et al. 2000, NMFS 2013, Wiles 2015). California sea lions breed in southern California and the Gulf of California, Mexico, and males disperse north as far as Alaska in the non-breeding season (Maniscalco et al. 2004). California sea lions occur in Washington year-round, with peak abundance in the non-breeding season (Antonelis & Fiscus 1980, Jeffries et al. 2000, Edgell & Demarchi 2012). Typically only males are seen in Washington waters (Jeffries et al. 2000), with few known cases of adult females (Thomas et al. 2010).

Diet in sea lions differs by age (Orr et al. 2011), sex (Trites & Calkins 2008), season, year, and location (Sinclair & Zeppelin 2002, McKenzie & Wynne 2008, Riemer et al. 2011, Scordino et al. 2013) and is influenced by abundance and distribution of prey (Sigler et al. 2009, Womble et al. 2009). Steller sea lion food habits vary greatly by study region, but top prey species include walleye pollock *Theragra chalcogramma* and Atka mackerel *Pleurogrammus monopterygius* in Alaska (Merrick et al. 1997, Sinclair & Zeppelin 2002) and Pacific hake *Merluccius productus* in California, Oregon, and Washington (Scordino 2010, Riemer et al. 2011). In Washington, the primary prey items observed are skates (Rajidae family), rockfish *Sebastes* sp., Pacific salmon *Onchorynchus* sp., unidentified clupeids (Clupeidae family), and Pacific spiny dogfish *Squalus suckleyi* (Scordino et al. 2013). Other important prey species consumed in multiple regions include Pacific sand lance, Pacific herring, Pacific salmon, and Pacific cod *Gadus macrocephalus* (Merrick et al. 1997, McKenzie & Wynne 2008, Trites & Calkins 2008, Riemer et al. 2011). Food habits of California sea lions have primarily been described in California (Lowry et al. 1991, Weise 2000, Orr et al. 2011) and include Northern anchovy, Pacific sardine, market squid *Doryteuthis opalescens*, and Pacific hake. In Washington, the top prey species observed are Pacific salmon, Pacific herring, unidentified clupeids, rockfish, and dogfish (Scordino et al. 2013).

Differences in diet also stem from differences in foraging area by age, sex, and season (Merrick et al. 1994, Merrick & Loughlin 1997). Adult female Steller sea lions forage over the continental shelf, closer to shore with shorter trip durations in the summer (17 km) compared to winter (133 km) (Merrick & Loughlin 1997, Trites & Calkins 2008), whereas the foraging behavior of adult male Steller sea lions has not been studied

(Trites & Calkins 2008). This shift in female foraging distance is in part attributed to females with dependent pups in the summer limiting forage trip distance and due to shifts in prey distribution in the winter (Merrick & Loughlin 1997). Adult male California sea lions typically forage over the continental shelf, but in a warm water El Niño event, sea lions foraged longer distances (averaged 124 km compared to 48 km in pre-El Niño years) and further offshore (Weise et al. 2006). Differences in diet and foraging area could result in different risk of exposure to algal toxins. In California sea lions, adult males forage close to rookeries during the breeding season and then migrate north during the non-breeding season, whereas adult females remain in California waters throughout the year and forage in offshore locations that overlap with the major algal blooms (Silvagni et al. 2005, Bejarano et al. 2008). Consequently, adult female California sea lions are the primary demographic affected by annual toxic domoic acid blooms (Silvagni et al. 2005, Bejarano et al. 2008).

Little is known about the specific foraging areas and behavior of sea lions in Washington. Satellite-tagged California sea lions frequently occurred over the continental shelf and slope and, in Washington, spent time in the area of the Juan de Fuca Canyon and Juan de Fuca Eddy (Wright et al. 2010). South of the study area on the Washington coast, Oleson et al. (2009) reported Steller and California sea lions both nearshore and near the shelf break, with sea lions occurring closer to shore in the summer. Given the overlap between the occurrence of HAB blooms on the Washington coast and the range of these two sea lion species, there is a potential for exposure to HAB toxins through the marine food web.

Research Goals and Purpose

In this study, I used fecal analysis to assess the year-round exposure of sea lions in Washington State to domoic acid and saxitoxin by measuring the concentrations of the two toxins in scat samples. There were three research goals in this study. The first was to determine to what degree sea lions in Washington are exposed to HABs and to assess the temporal and spatial factors affecting the occurrence (presence/absence) of HAB toxins in scat. The second goal was to identify potential routes of trophic exposure to algal toxins by looking for differences in the food habits of sea lions with and without detectable toxins in the scat and to investigate whether particular prey are more often associated with the presence of toxin than others. Lastly, I attempted to compare the presence and concentrations of toxins in sea lion scat to those measured in nearshore bivalves over the same time period to determine whether bivalves could be used to predict HAB exposure in sea lions.

To answer these questions, Steller and California sea lion scats were collected from haulout sites on the northwest Washington coast from spring 2011 through winter 2013. Subsamples from each scat were analyzed by enzyme-linked immunosorbent assays (ELISA) for domoic acid and saxitoxin. Prey remains were removed from the fecal material and identified to the lowest taxonomic group possible. I used logistic regression to examine temporal and spatial variables influencing toxin exposure by comparing the presence/absence of detectable toxin in scats to collection season, year, and haulout location. I used non-metric multidimensional scaling and analysis of similarity to determine if there was a difference in diet between sea lions with and

without measureable levels of toxins. To determine if particular prey species were more associated with toxin, I used Chi-Squared and Fisher's Exact tests to compare the presence or concentration level of toxin to the presence of prey species in the scat. Lastly, I graphically compared the presence of toxins in the scat to observed concentrations of toxins in California mussel and Pacific razor clam to determine if a nearshore indicator can be used to predict exposure of a top predator. This study addresses research needs identified in the recovery plan of the western DPS (Goldstein et al. 2005, NMFS 2008) and for post-delisting monitoring of eastern DPS Steller sea lions (NMFS 2013) by assessing current exposure and threat of biotoxins. To the best of my knowledge, this is the first study documenting year-round exposure of domoic acid and saxitoxin in free-ranging sea lions on the US West Coast.

YEAR-ROUND ALGAL TOXIN EXPOSURE IN FREE-RANGING SEA LIONS: IMPLICATIONS OF TROPHIC EXPOSURE FOR DECLINING POPULATIONS

INTRODUCTION

Marine wildlife is frequently exposed to a variety of pollutants through ingestion of contaminated prey. These pollutants may result from human activities, such as organochlorine pesticides and industrial organic chemicals (Tanabe 2002, Law et al. 2003, Islam & Tanaka 2004) or from elevated levels of essential and non-essential elements (Sydeman & Jarman 1998, Law et al. 2003, Scheuhammer et al. 2007). Pollutants may also be naturally occurring, such as toxins produced by bloom-forming marine algae (Van Dolah 2000). Harmful algal bloom (HAB) toxins have led to mass mortalities of fish and seabirds (Work et al. 1993, Sierra Beltrán et al. 1997, Van Dolah 2000, Shumway et al. 2003) and are suspected or implicated in mass strandings of many species of marine mammals (Geraci et al. 1989, Lefebvre et al. 1999, 2010, Reyero et al. 1999, Gulland 2000, Kreuder et al. 2003, Torres de la Riva et al. 2009, Fire et al. 2015). A number of studies have examined algal toxin exposure in live, free-ranging marine mammals including several species of cetaceans (Durbin et al. 2002, Lefebvre et al. 2002, Doucette et al. 2006) and pinnipeds (Jensen et al. 2015, Lefebvre et al. 2016); however, none have directly examined the potential for year-round exposure through the food web.

Two phytoplankton taxa implicated in marine mammal mortalities are diatoms of the genus *Pseudo-nitzschia* and dinoflagellates of the genus *Alexandrium*, which produce

domoic acid and saxitoxin, respectively (Van Dolah et al. 2001). In humans, domoic acid causes amnesiac shellfish poisoning (ASP), while saxitoxin, and its many congeners, cause paralytic shellfish poisoning (PSP) (James et al. 2010). On the US West Coast, algae blooms leading to unsafe levels of domoic acid and saxitoxin cause annual closures of shellfish harvests (Horner et al. 1997, Trainer 2002, Trainer et al. 2007). Domoic acid has led to mass mortalities of California sea lions *Zalophus californianus* and Northern fur seals *Callorhinus ursinus* along the California coast (Gulland 2000, Lefebvre et al. 2010). Saxitoxin was recently implicated in the death of Kittlitz's murrelet *Brachyramphus brevirostris* nestlings in Alaska (Shearn-Bochsler et al. 2014), but has not yet been linked to strandings of West Coast marine mammals. Both toxins, however, were recently documented in a variety of marine mammals in Alaska, including some cases of concurrent exposure having both toxins detected (Lefebvre et al. 2016).

HABs appear to be increasing in frequency and severity worldwide (Van Dolah 2000) giving rise to the need to understand what factors most influence exposure to HAB-related toxins in marine wildlife, particularly in declining populations (Forcada et al. 1999, Durbin et al. 2002, Shumway et al. 2003, Shearn-Bochsler et al. 2014). The Washington coast is a unique marine environment for studying domoic acid and saxitoxin, which both impact state shellfish fisheries (Trainer et al. 2002, 2003). A hotspot for *Pseudo-nitzschia* blooms on the Washington coast is the Juan de Fuca Eddy, a seasonally formed, cold-water gyre located offshore the mouth of the Strait of Juan de Fuca (Trainer et al. 2002). The eddy forms in the spring with southward upwelling-favorable winds and dissipates in the fall (MacFadyen et al. 2005, 2008). Some waters escape from the southeastern margin of the eddy bringing their associated toxins to the

nearshore environment (MacFadyen et al. 2005, 2008, Trainer et al. 2009). In contrast, *Alexandrium* blooms occur nearshore, typically in embayments with highly stratified waters, after water temperatures begin warming in the spring (Nishitani & Chew 1984, Anderson 1998, Trainer et al. 2003). These differences in bloom dynamics may manifest as different food web pathways to top predators, such as sea lions, depending on the specific prey vectors, predator foraging area, and seasonal and annual changes in both.

Despite mortality events related to HAB exposure and other pathogens, the US population of California sea lions is estimated at nearly 300,000 individuals (Carretta et al. 2014). Conversely, the Steller sea lion *Eumetopias jubatus*, experienced an 80% reduction in abundance from the 1970s through 1998 in US waters (Miller et al. 2005). The decline in the population was driven by declines in the western distinct population segment (DPS) (Western Alaska through Russia; west of 144° W) whereas during the same time period the eastern DPS (Southeast Alaska through California; east of 144° W) increased at 3% per year (Pitcher et al. 2007). Currently the western DPS is listed as endangered while the eastern DPS was recently delisted (NMFS 2008, 2013). Though no mortalities related to algal toxins have been reported in Steller sea lions, monitoring to assess the threat of biotoxins is listed as a priority in the recovery plan of the western DPS (Goldstein et al. 2005, NMFS 2008) and for post-delisting monitoring of the eastern DPS (NMFS 2013). The overlapping range of eastern DPS Steller sea lions and California sea lions suggests the two species could have a similar risk of exposure to algal toxins through contaminated prey.

Illness and mortality of marine mammals due to HAB toxins are frequently linked to consumption of specific prey species, but some studies have found more complex

spatial and temporal trends. Northern anchovy *Engraulis mordax* was named the primary vector of toxin exposure in the deaths of California sea lions and a common minke whale *Balaenoptera acutorostrata* that died due to domoic acid toxicity (Lefebvre et al. 1999, Scholin et al. 2000, Fire et al. 2010). In a mass stranding of humpback whales *Megaptera novaeangliae* due to saxitoxin toxicity, the whales' normal prey item at that time of year, sand lance *Ammodytes* spp., was not present, leading the whales to travel farther north looking for prey and resulting in consumption of contaminated Atlantic mackerel *Scomber scombrus* (Anderson & White 1989, Geraci et al. 1989). Spatial overlap of foraging areas and algal blooms is of particular concern for adult female California sea lions, which strand at a greater frequency due to domoic acid toxicity compared to other demographic groups (Silvagni et al. 2005, Bejarano, Gulland, et al. 2008). Adult male California sea lions forage close to rookeries during the breeding season and then migrate north during the non-breeding season, but adult females remain in California waters throughout the year and forage in offshore locations that overlap with the major algal blooms (Silvagni et al. 2005, Bejarano, Gulland, et al. 2008). Thus, a combination of prey availability and the migration and foraging patterns of the predator itself influence the risk of exposure to algal toxins.

Sea lions are considered generalist predators that change their diet based on seasonally and locally abundant prey and may migrate in response to seasonal prey fluctuations (Weise 2000, Sinclair & Zeppelin 2002, Womble et al. 2005, 2009, Bredesen et al. 2006, Sigler et al. 2009). In other areas the diet of Steller sea lions (Merrick et al. 1997, Sinclair & Zeppelin 2002, Bredesen et al. 2006, Sigler et al. 2009, Riemer et al. 2011) and California sea lions (Lowry et al. 1991, Weise & Harvey 1999, Orr et al. 2011)

is often dominated by only a few species, which is not the case on the Washington coast where diet specialization in both Steller and California sea lions is low (Scordino et al. 2013). Sea lion diets are diverse, with as many as 76 prey identified (McKenzie & Wynne 2008) and as many as 25 different prey found in a single scat (Riemer et al. 2011). Domoic acid and saxitoxin have been detected in both benthic and pelagic fish species (Lefebvre et al. 2002, Vigilant & Silver 2007, Jensen et al. 2015), suggesting that while a more diverse diet could lessen the effects of acute toxicity experienced by ingesting a single infected prey species, generalist predators are at risk of exposure from multiple prey items. Previous studies have attempted to examine the question of marine mammal exposure to algal toxins through sampling of prey and fecal remains of actively foraging or live-captured animals (Durbin et al. 2002, Lefebvre et al. 2002, Doucette et al. 2006, Jensen et al. 2015), through sampling of plankton and nearshore bivalves, such as the blue mussel *Mytilus edulis*, in areas near mortality events as indicators of blooms and toxin production (Scholin et al. 2000, Bargu et al. 2010), and through sampling the feces or stomach contents of ill or deceased animals (Geraci et al. 1989, Lefebvre et al. 1999, Fire et al. 2010).

In this study, I assessed the year-round exposure of free-ranging sea lions in Washington State to domoic acid and saxitoxin through comparing the toxin concentrations measured in feces to the prey remains in the scat and to toxin concentrations measured in nearshore bivalves collected over the same time period. The goals of this study were to 1) determine year-round baseline levels of HAB toxins ingested by both sea lion species and to identify factors associated with toxin exposure, including season, year, and haulout location 2) determine if there was a difference in diet

between sea lions with and without measureable levels of toxins and whether particular

prey were more associated with toxin exposure, and 3) compare the presence and

concentration of toxins in the scat to that in nearshore bivalves to determine how well

these nearshore indicators predicted toxin exposure of top predators. To the best of my

knowledge, this is the first study to document domoic acid and saxitoxin in free-ranging

sea lions on the US West Coast.

METHODS

Study Area

The study area was located at the northernmost extent of the California Current on the northwest coast of Washington State (MacFadyen et al. 2008). The region is characterized by high phytoplankton biomass and primary productivity, driven by wind and topographic induced upwelling, as well as the seasonally formed Juan de Fuca Eddy, a cold water gyre located off the mouth of the Strait of Juan de Fuca, and nutrient inputs from the Fraser and Columbia River outflows (Horner et al. 1997, Trainer et al. 2002, Hickey & Banas 2003). Both Steller sea lions *Eumetopias jubatus* and California sea lions *Zalophus californianus* can be found in the study area throughout the year. Steller sea lions of all demographic groups live and forage on the Washington coast year-round (Jeffries et al. 2000, NMFS 2013, Wiles 2015), while California sea lions migrate into Washington waters seasonally, with pulses in abundance in spring and fall (Antonelis & Fiscus 1980, Jeffries et al. 2000, Edgell & Demarchi 2012).

Scats were collected from sea lion haulout sites on the northwest Washington coast. Due to the proximity of the individual haulout sites (between 0.5 km and 1.3 km), the haulouts were grouped into three haulout complexes: Tatoosh Island complex, Bodelteh Islands complex, and Carroll/Sea Lion Rock complex (Figure 1). Scats from the Tatoosh Island complex included collections from a rock located to the east of the main island (Tatoosh East, N 48° 23.59, W 124° 43.89) and one location on the north side of the island (Tatoosh West Reef, N 48° 23.56, W 124° 44.59). Scats from Bodelteh Island

27

complex included collections from three haulouts (East Bodelteh, N 48˚ 10.57 W 124˚ 45.15; West Bodelteh, N 48˚ 10.75 W 124˚ 46.20; Guano Rock, N 48˚ 10.90 W 124˚ 44.52). Scats from the Carroll/Sea Lion Rock complex were collected from Carroll Island (N 48˚ 00.23 W 124˚ 43.67) and Sea Lion Rock (N 47˚ 59.58 W 124˚ 43.45).
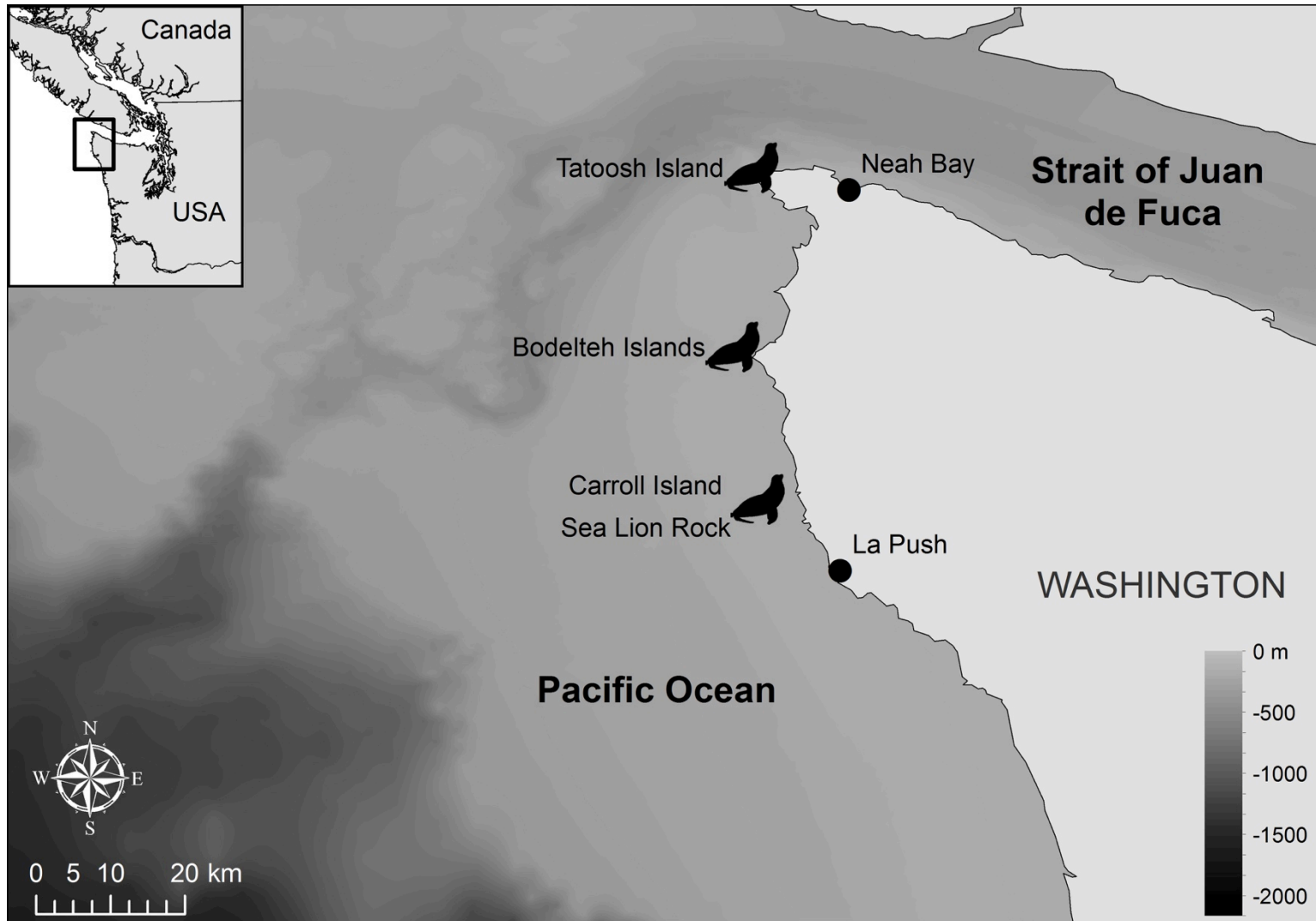
Figure 1. Locations of sea lion haulout complexes on the northwest coast of Washington State, USA, with 10-m digital elevation map bathymetry.

Scat Collection

Steller sea lion and California sea lion scats were collected from March 2011 through February 2013. Seasons were defined by month rather than calendar date as spring: March, April, May; summer: June, July, August; fall: September, October, November; and winter: December, January, February.

Scats were collected from sites where sea lions were hauled out at the time of approach. The sea lions were disturbed off the rock by the boat approaching and personnel landing on the rock. Scats were classified as either Steller or California sea lion scat if ≥95 % of sea lions at the collection site were of a single species. Scats were collected using a metal spoon to scoop the sea lion feces into individual plastic Whirl-Pak™ bags for storage. Because domoic acid and saxitoxin are water-soluble, only fresh scats were selected for use in this study. Fresh scats were estimated to be <48 hours old based upon whether the scat appeared moist and not weathered or dried out.

The haulout complexes and the specific haulout within a complex where samples were collected varied by season and by where the sea lions were hauled out on the sampling day. Winter samples (collected from Steller sea lions only) were only collected at the Tatoosh Island complex because ocean conditions made landings on the southern haulout sites dangerous. Summer samples for Steller sea lions were primarily collected from the Carroll/Sea Lion Rock complex, due to the majority of sea lions in the summer using the southern haulout sites and because sea lions using northern sites in the summer often haul out at locations inaccessible for boat approach and personnel landing. Summer collections at the Carroll/Sea Lion Rock complex targeted areas of the rocks that were

dominated by adult and subadult males in order to minimize disturbance to females and newborn pups. California sea lion scats were only collected from the Bodelteh Islands complex due to the large number of animals that use the Bodelteh Island complex during both the spring and fall (3,000-5,000; Scordino and Akmajian 2013).

Toxin Analysis

After collection, scats were processed fresh or were frozen and later thawed for processing. I subsampled the scats for toxin analysis by placing approximately 4 g of each scat in a 15 ml centrifuge tube. To ensure that no prey remains were lost during subsampling, the 4 g of fresh scat was pushed through either 1) a nylon, fine mesh, paint strainer bag (0.25 mm), or 2) through a 0.5 mm sieve to be collected for toxin analysis. If the scats were frozen prior to subsampling, I collected as much of the liquid as possible from the scat as it was pushed through the mesh in an attempt to capture the water-soluble toxins. I weighed, labeled, and placed each centrifuge tube in a labeled Whirl-Pak bag then frozen at -20˚ C. At least 2 g of feces were necessary for the analysis of each toxin. When scats were small and had insufficient material for analyzing both toxins, I prioritized the analysis of domoic acid because it has caused strandings and mortalities of sea lions on the US west coast (Scholin et al. 2000) and there is particular interest in determining the exposure of Washington sea lions to this toxin.

Scat subsamples were sent to Dr. Elizabeth Frame at the Wildlife Algal Toxin Research and Response Network at NOAA's Northwest Fisheries Science Center in

Seattle, WA, where they were analyzed using enzyme-linked immunosorbent assays (ELISA). The extraction methods for the toxin assays followed methods described in Lefebvre et al. (2016). Scat samples were thawed and split into two 15-ml falcon tubes for domoic acid and saxitoxin analysis respectively, with approximately 2 g in each tube. For domoic acid analysis, the sample was extracted using 50 % methanol and 50 % water. For saxitoxin, the extraction solvent was 80 % ethanol and 20 % water. Samples were homogenized and centrifuged at 4˚C and the resulting supernatant was filtered and spun in a microcentrifuge. Domoic acid was analyzed using ASP direct cELISA kits made by Biosense Laboratories (Norway). Saxitoxin was analyzed using Saxitoxin (PSP) ELISA kits made by Abraxis Laboratories (Pennsylvania). Samples were diluted using a 10 % methanol buffer at a 1:100 dilution for domoic acid analysis and a 1:50 dilution for saxitoxin analysis. The lower quantification limits for the ELISA's are 3 ng g^{-1} for saxitoxin and 4 ng g^{-1} for domoic acid.

The highest concentration of domoic acid measured in this study (672.2 ng g^{-1}) was detected in a California sea lion scat. Due to an error in sample processing, this same concentration was reported in two consecutive scats that appeared to be a single sample erroneously processed twice for domoic acid and recorded with two different identifications. Because I was unable to identify the sample, it was discarded from statistical analyses and diet comparisons.

Food Habits Analysis

After subsampling for the toxin analysis, the remaining fecal material was processed for food habits analysis. For the majority of samples, I followed the methods of Orr et al. (2003), using a commercial washing machine to clean the fresh fecal material from the prey remains (cephalopod beaks and bones and other hard parts from fish). Fresh or thawed scats were washed from the Whirl-Pak™ bags into nylon, fine mesh (<0.05 mm) paint strainer bags. The paint strainer bags were washed in a washing machine with cold water, on a regular cycle, using a small amount of detergent to help break down the oily fecal material. Following washing, the prey remains were emptied from the mesh bags into a 0.5-mm sieve and hard parts were picked out using forceps. Samples with gravel were washed by hand through nested sieves of 2 mm, 1mm, and 0.5 mm, following methods described by Lance et al. (2001), so that the gravel would not damage prey remains during cleaning (Orr et al. 2003). When processed through sieves, prey remains were collected from each sieve. Washed prey remains were placed into labeled plastic scintillation vials filled with ~50 % alcohol solution for preservation. All samples were dried completely prior to shipping for identification.

Prey remains were identified by Susan Reimer at Oregon Department of Fish and Wildlife in Medford, OR. Remains were examined using a dissecting microscope and identified to the lowest taxonomic group possible using a comparative reference collection of fish from the northeast Pacific Ocean (see Reimer et al. 2011). Prey hard parts identified included bones (e.g. otoliths, vertebrae, teeth, gill rakers, etc.), cartilaginous structures, and cephalopod beaks. Several fish species (e.g. rockfishes and

salmonids) can only be identified to species using otoliths or molecular techniques (Lance et al. 2012), therefore higher taxonomic classifications were used in these cases. Salmon *Oncorhynchus* spp. were identified during food habits analysis by size and grouped as "juvenile salmon" (smolts) and "non-juvenile salmon" (all other age classes) by comparing to references of different species and age classes. Remains that could not be identified to a taxonomic group were recorded as unidentified bony fish (class Osteichthyes). Prey taxa recorded as present/absent for each sample were converted to percent frequency of occurrence (FO) in sea lion diet, where FO of a particular taxon is equal to the number of scats having that taxon divided by the total number of scats with any identifiable prey.

Bivalve Samples

Biotoxin results measured in nearshore bivalves from January 2011 through March 2013 were provided by the Washington Department of Health's (WDOH) Biotoxin Monitoring Program and included data from eight locations in the western Strait of Juan de Fuca and northern Washington coast (Figure 2). Shellfish were collected and analyzed for monitoring of paralytic, amnesiac, and diuretic shellfish toxins (okadaic acid) year-round on a weekly to bi-weekly basis (Trainer et al. 2002). Contributors to this dataset included the Makah and Quileute Tribes and the Washington Department of Fish and Wildlife, who perform monitoring for subsistence and recreational harvest.
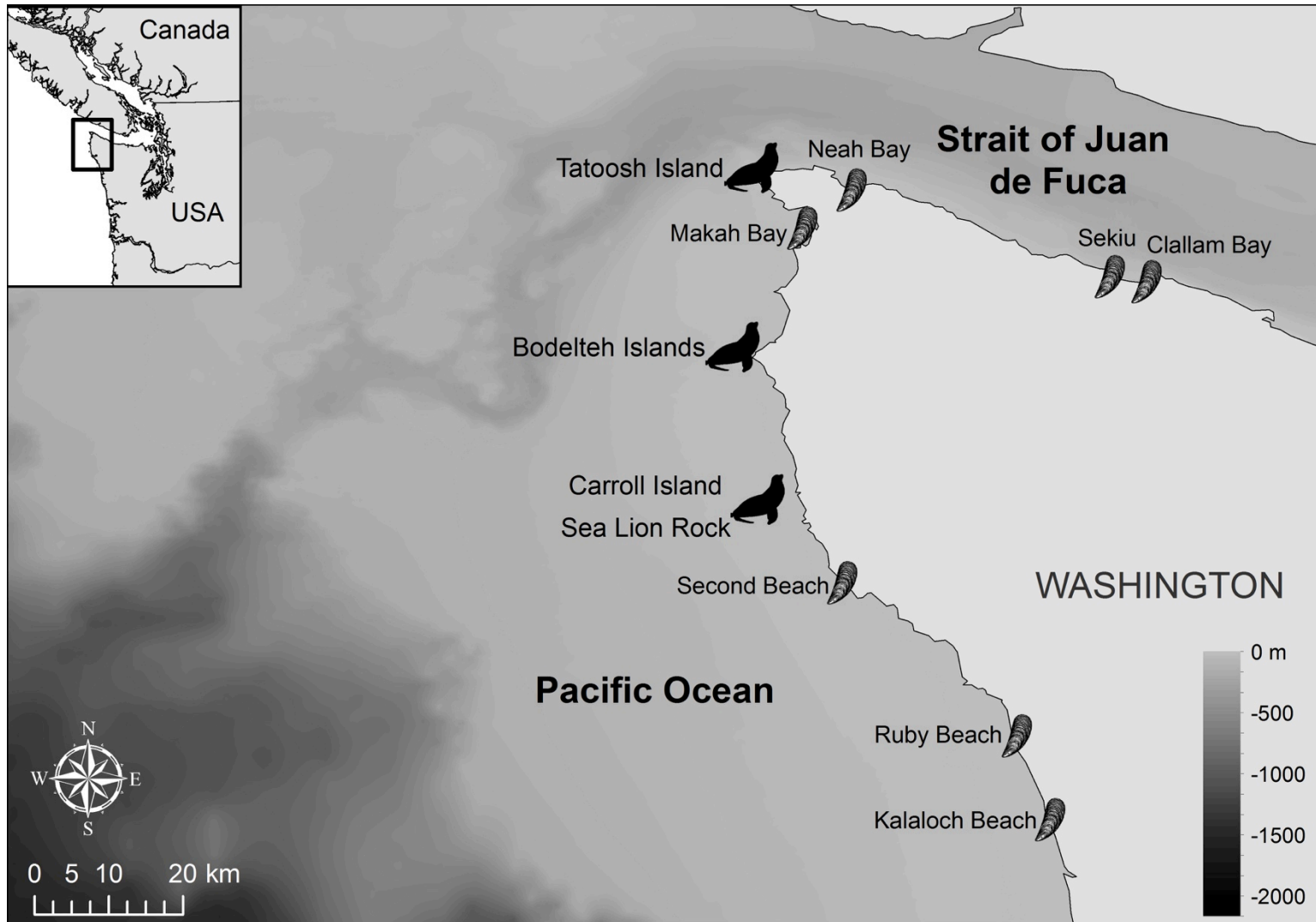
Figure 2. Locations of bivalve and scat sample locations along the western Strait of Juan de Fuca and northwest Washington coast. Parentheses refer to sites having more than one sample location.

Analysis of shellfish was performed by the WDOH Public Health Laboratory in Seattle, WA, using the standardized mouse bioassay for detection of saxitoxin (AOAC 1965, APHA 1980, NSSP 2013) and high-performance liquid chromatography for detection of domoic acid (Quilliam et al. 1995). Saxitoxin is reported in µg saxitoxin equivalents per 100 g of tissue with a regulatory limit for human consumption of 80 ug 100 g^{-1}, or 800 ng g^{-1} (Wekell et al. 1994). Domoic acid is reported in µg domoic acid g^{-1} of tissue with a regulatory limit of 20 µg g^{-1}, or 20,000 ng g^{-1} (Wekell et al. 1994). Samples reported by WDOH as not detected for both toxins are reported here as "0". For saxitoxin, concentrations below 38 µg 100 g^{-1} are reported by WDOH as "<38" and are reported here conservatively as 380 ng g^{-1}. This value was chosen because no true concentrations were measured at 380 and because I do not know where between zero and 380 the true concentration lies. For domoic acid, WDOH reports concentrations of both <1 µg g^{-1} and 1 µg g^{-1} (1000 ng g^{-1}), hence I substituted <1 µg g^{-1} for 0.90 µg g^{-1}, or 900 ng g^{-1}. This number was chosen to differentiate between a true concentration of 1000 and <1000 and because I do not know where between zero and 1000 the true concentration lies. Only two species of shellfish are included in this analysis: California mussel *Mytilus californianus* and Pacific razor clam *Siliqua patula*. California mussels were the dominant species collected at all sites except Kalaloch Beach (Figure 2) where razor clams were dominant.

Nearshore bivalve toxin content was graphically compared to the concentrations in sea lion scats to examine the relationship between presence and concentration of toxin in bivalves to that in the scats. Bivalves harvested for human consumption are sampled on a weekly or bi-weekly time scale based on blooms that peak and decline in short time

36

periods (Horner and Postel 1993; Trainer 2002) and toxin depuration rates of days to weeks in the species monitored (Shumway 1990; Whyte et al. 1995). California mussels depurate saxitoxin in a span of three weeks to >12 weeks (Bricelj and Shumway 1998), whereas they depurate domoic acid in as little as two weeks (Whyte et al. 1995). Although high concentrations of saxitoxin have been detected in razor clams (Bricelj and Shumway 1998; Trainer 2002), razor clams typically accumulate only low levels of saxitoxin (Anderson et al. 2002, Trainer et al. 2003) and depuration rates for the toxin have not been reported. Razor clams hold domoic acid in their tissues for as long as 6-18 months (Wekell et al. 1994); therefore, the detection of domoic acid in razor clams over multiple months does not necessarily reflect individual blooms (i.e. multiple repeated exposures). Comparisons of toxin levels in bivalves to sea lion scat were based on the date of scat sampling and any bivalve samples collected within the span of the minimum depuration time for the toxin (i.e. three weeks before or after the scat sample date). Based on the low occurrence of domoic acid in mussels over this time period and the inability to distinguish multiple exposures in razor clam, I performed only limited comparisons of domoic acid concentrations between bivalves and scats.

Statistical Analysis

Influence of season, year, and location

I used binary logistic regression models in the statistical program R (R Core Team 2015) to examine which factors best predicted the presence or absence of toxin in sea lion scats. The dependent variable was the presence/absence of either domoic acid or saxitoxin in the scat. All explanatory variables were modeled as categorical, including haulout complex, season, and year, which was defined as "year 1" representing samples from March 2011 through February 2012 and "year 2" representing samples from March 2012 through February 2013. I used Akaike's Information Criterion (AIC) to determine which model gave the best, most parsimonious, fit with my data.

For Steller sea lions, I performed four total analyses, running two analyses for each toxin. Due to low sample collection at Bodelteh Islands (n = 12 scats), the first analysis pooled samples from all complexes to predict the presence/absence of saxitoxin (n = 373 scats) or domoic acid (n = 383 scats) from season, year, and the additive and interaction effects of those two variables. The second analysis added haulout complex as a model variable, using samples from Tatoosh Island and Carroll/Sea Lion Rock only. This model only included the spring and fall seasons because Tatoosh Island was not sampled in summer both years and because Carroll/Sea Lion Rock was not sampled in the winter. Thus, the second analysis predicted the presence/absence of saxitoxin (n = 190 scats) or domoic acid (n = 197 scats) based on the predictors of season, year, haulout complex and all possible interactions of those variables.

For California sea lions, I performed a single logistic regression for each toxin (n = 119 scats for saxitoxin and n = 123 scats for domoic acid) modeling presence or absence of toxin as a factor of season (spring, summer, and fall) and year. I was unable to include the interaction in the model because I did not have samples from all seasons for both years. I did not use regression analysis to compare toxin prevalence between the two sea lion species because the scats were not collected at the same haulout complexes; known spatial variability in toxin concentrations and plankton cell counts in this study area (Trainer et al. 2009) would make it difficult to discern whether any differences in toxin loads in the two sea lion species were species-based or location-based.

Influence of diet

I used nonmetric multi-dimensional scaling (NMDS) and analysis of similarity (ANOSIM) in the Community Ecology Package (vegan) of R (Oksanen et al. 2015) to investigate whether there was a difference in diet between sea lion scat with detectable levels of toxins versus scats with no detectable toxins. I only included prey taxa that had a total frequency of occurrence (FO) in the diet (averaged over all seasons) ≥5 % and discarded any scats that only contained unidentified bony or cartilaginous fish. Prey taxa were defined by the lowest taxonomic classification identified and includes individual species and unidentified species grouped by a higher taxonomic level (i.e. family, order, or class). Salmon were grouped as "juvenile" and "non-juvenile" salmon.

For the two sea lion species, I ran NMDS and ANOSIM for each toxin separately. I used the vegdist function with binary Jaccard distance matrix, employed to compare presence/absence of species (Riemer et al. 2011), to calculate the dissimilarity matrix. For NMDS, I used the metaMDS function, which can handle zero distance where two points (two scats) are identical (Oksanen et al. 2015). Each scat represents a single point in the NMDS and each individual prey item was input as present/absent. The plotted points were labeled first by the presence/absence (Yes/No) of toxin measured in the scat, then in a second MDS by categorical levels, "no", "low", "med", and "high" toxin.

Designation of toxin levels was based on natural breaks in the concentrations measured in scats and not intended to be indicative of toxicological effects. For both toxins, "no" represents all samples below detection limit, "low" between 0-20 ng g^{-1}, "med" between >20-50 ng g^{-1}, and "high" for concentrations >50 ng g^{-1}. MDS ordination was evaluated by stress values as defined by Kruskal (1964). I tested for significant differences in diet based on presence/absence of toxin using ANOSIM, where $p < 0.05$ indicated a significant difference.

To determine the relationship between specific prey items and toxin exposure in sea lions, I used 2 x 2 and 2 x 4 contingency tables to compare the presence/absence of individual prey items to either presence/absence of toxin (2 x 2) or categorical toxin levels as defined above (2 x 4). I used Chi-squared tests when expected values of at least 80 % of the cells were ≥5 and Fisher's Exact tests when expected values were <5 (McHugh 2012). I evaluated standardized residuals from the 2 x 2 and 2 x 4 Chi-squared tests to determine whether presence, or categorical level, of toxin was significantly higher (as indicated by a positive residual) or lower (as indicated by a negative residual) than

expected values based on presence or absence of each prey item. Residual values $>|1.96|$ were considered significant at $p<0.05$ based on z scores (Agresti 2007). For Fisher's Exact tests, I used odds ratios and confidence intervals to evaluate positive or negative relationships of significant p-values. For the Fisher's Exact 2 x 4 tables, I performed post-hoc testing of pairwise comparisons of each possible 2 x 2 pair, using Bonferroni-corrected p-values where the corrected alpha was calculated as 0.05 divided by the number of pairwise comparisons.

RESULTS

Toxin Detection

A total of 508 scats were collected; 383 scats were from Steller sea lions *Eumetopias jubatus* and 125 scats were from California sea lions *Zalophus californianus* (Table 1). Saxitoxin was detected in 45 % of all scat samples and domoic acid was detected in 17 % of samples. In Steller sea lions, saxitoxin was detected in more scats from year 1 (56 %) than year 2 (30 %), with the highest percent detected in spring (47 %) compared to summer (43 %), fall (33 %), and winter (39 %). Domoic acid was detected in more scats from year 2 (22 %) than year 1 (12 %), with the highest percent detected in the summer (30 %) compared to spring (13 %), fall (17 %), and winter (6 %). In California sea lion scats, saxitoxin was detected in more scats from year 2 (31 %) than year 1 (23 %) and in summer (32 %) and fall (29 %) compared to spring (15 %). Domoic acid was similar between years (15 % in year 1 and 17 % in year 2) and was detected most often in summer (26 %) compared to spring (10 %) and fall (15 %). The two toxins were detected concurrently in only 26 scats from Steller sea lions and 6 scats from California sea lions.

Table 1. Number of scats collected per season at each haulout complex from Steller sea lions *Eumetopias jubatus* and California sea lions *Zalophus californianus* and the total number of scats analyzed for domoic acid (DA) and saxitoxin (STX).

Species	Season/Year	Tatoosh Island complex	Bodelteh Island complex	Carroll Island/ Sea Lion Rock complex	Total scat
E. jubatus	Spring 2011	36	–	14	50
	Summer 2011	–	–	46	46
	Fall 2011	11	–	39	50
	Winter 2011/2012	37	–	–	37
	Spring 2012	24	8	18	50
	Summer 2012	8	4	38	50
	Fall 2012	26	–	29	55
	Winter 2012/2013	45	–	–	45
	Total DA	*187*	*12*	*184*	*383*
	Total STX	*185*	*12*	*176*	*373*
Z. californianus	Spring 2011	–	20	–	20
	Fall 2011	–	45	–	45
	Summer 2012	–	19	–	19
	Fall 2012	–	41	–	41
	Total DA	–	*125*	–	*125*
	Total STX	–	*121*	–	*121*

While all California sea lion scats were from a single site (Table 1), I was able to compare Steller sea lions samples between the haulout complexes. Saxitoxin was detected more often at the Tatoosh Island complex (43 %) than the Carroll/Sea Lion Rock complex (39 %), whereas domoic acid was less common at Tatoosh (6 %) compared to Carroll/Sea Lion Rock (28 %). Only 12 scats from Steller sea lions were collected at the Bodelteh Islands complex; eight scats had detectable levels of saxitoxin, while only three scats had detectable levels of domoic acid.

For samples where toxin was detected, saxitoxin concentrations were found in similar ranges between sea lion species, ranging from 3.5 to 273.6 ng g^{-1} in Steller sea lions and from 4.7 to 258.6 ng g^{-1} in California sea lions. Concentrations of domoic acid in Steller sea lions ranged from 4.2 to 423 ng g^{-1} and in California sea lions ranged from 4.5 to 672.2 ng g^{-1}. The highest concentrations of saxitoxin in both sea lion species were detected in year 1 (Figure 3) and in spring (Figure 4), while domoic acid was higher in year 2 (Figure 3) and in summer in Steller sea lions and in fall in California sea lions (Figure 4). In Steller sea lions, the highest concentrations of saxitoxin were from the Tatoosh Island complex, while the highest concentrations of domoic acid were from Carroll/Sea Lion Rock complex (Figure 5).
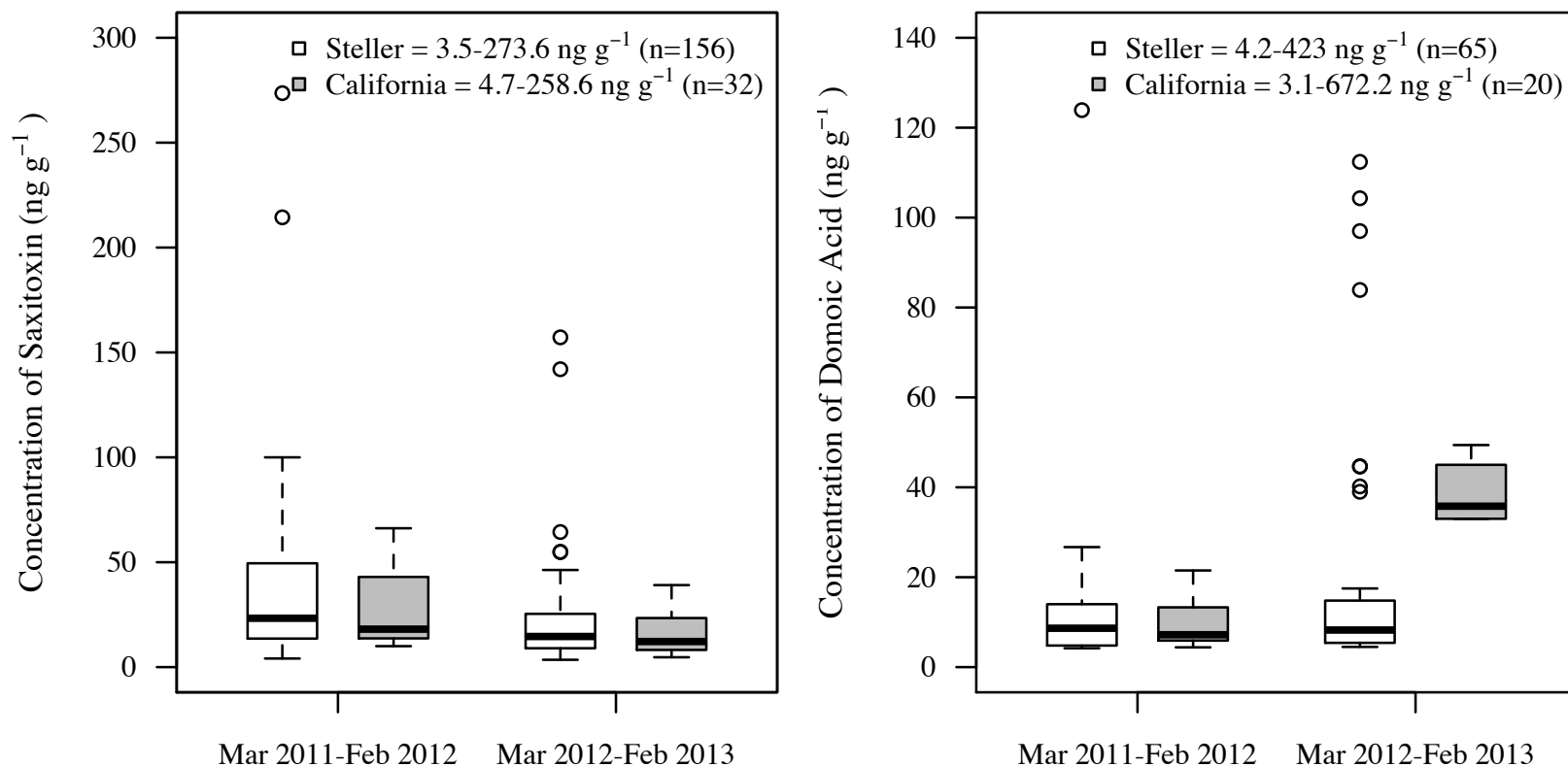
Figure 3. Concentrations of saxitoxin (left) and domoic acid (right) above detection limit in Steller sea lion *Eumetopias jubatus* (white) and California sea lion *Zalophus californianus* (gray) scats by collection year. Boxplots display the median (bold line), with the first quartile represented in the box below the median and the third quartile represented in the box above the median. Points above and below the whiskers represent outliers. Maximum domoic acid concentrations (423 and 672.2 ng g^{-1}), both from year two (Mar 2012 - Feb 2013), are not displayed in the figure.
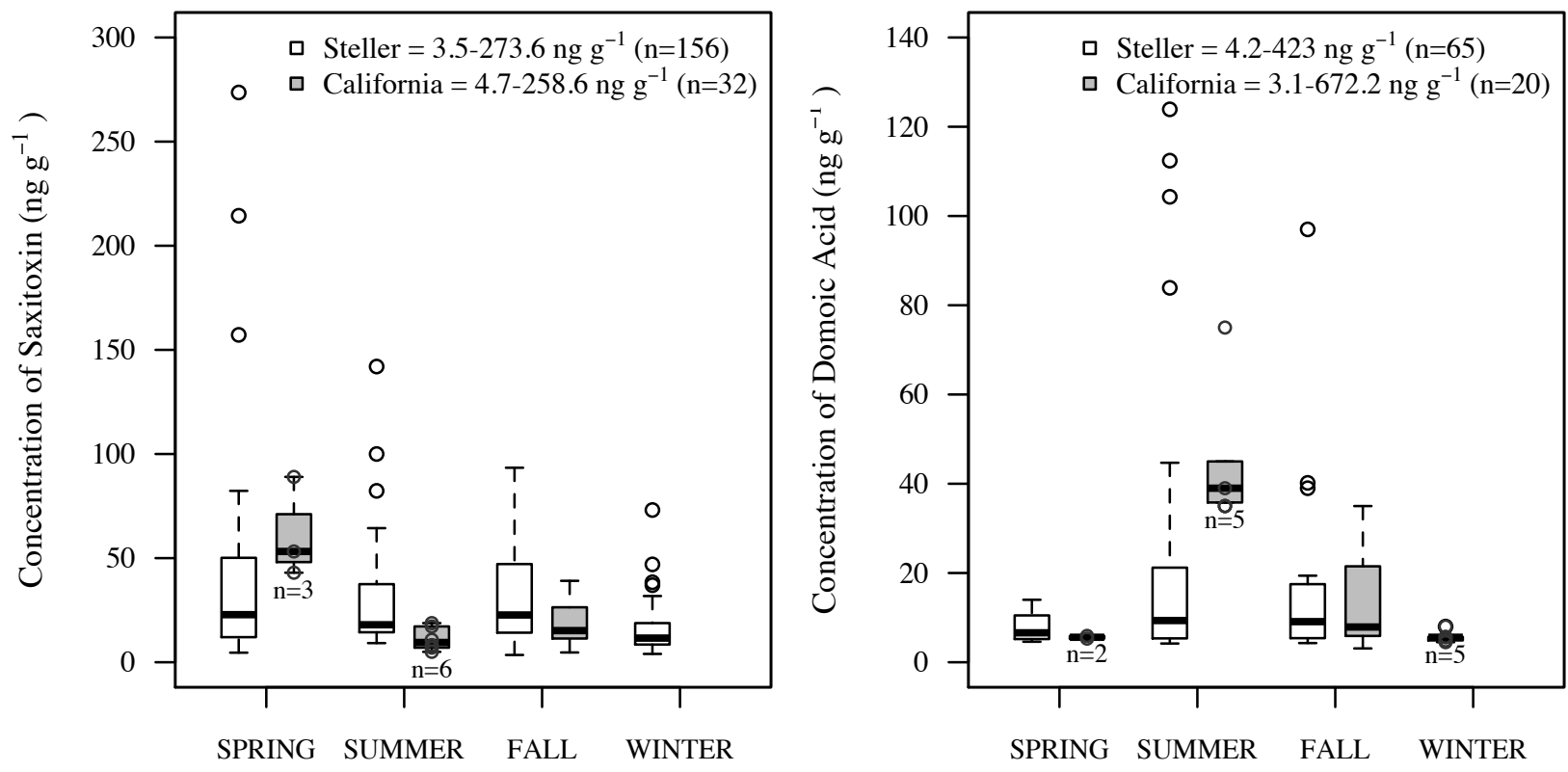
Figure 4. Concentrations of saxitoxin (left) and domoic acid (right) above detection limit in Steller sea lion *Eumetopias jubatus* (white) and California sea lion *Zalophus californianus* (gray) scats by season. Points shown inside the boxes and whiskers represent the actual concentrations measured and are presented in the case of sample sizes <10, as denoted by *n* values. Maximum domoic acid concentrations (423 and 672.2 ng g^{-1}), collected in summer (Steller sea lions) and fall (California sea lions), are not displayed in the figure.
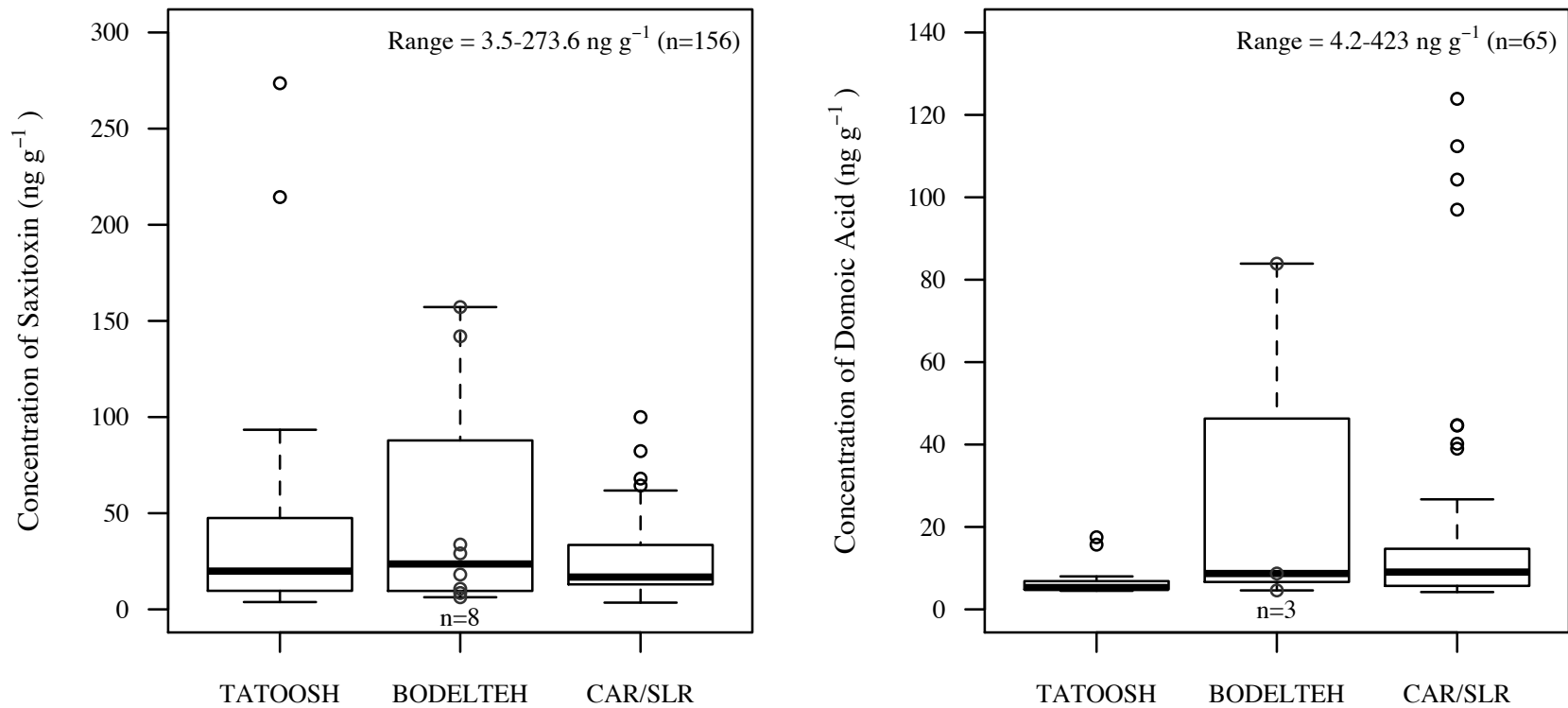
Figure 5. Concentrations of saxitoxin (left) and domoic acid (right) above detection limit in Steller sea lion *Eumetopias jubatus* scats by haulout complex. Points shown inside the boxes and whiskers represent the actual concentrations measured and are presented in the case of sample sizes <10, as denoted by *n* values. The maximum domoic acid concentration (423 ng g^{-1}), measured at the Carroll/Sea Lion Rock complex (CAR/SLR), is not displayed in the figure.

Toxin Prevalence by Season, Year, and Location

Logistic regression analysis identified several explanatory models for the presence of toxins in Steller sea lions, but for California sea lions the regression models were not successful. In the case of California sea lions, the intercept model had the lowest AIC among all models tested (season, year, and season+year) for both domoic acid and saxitoxin.

In Steller sea lions, predicting presence of saxitoxin by season and year, the most parsimonious model was the full model including season, year, and their interaction (Table 2). Saxitoxin exposure in year 1 was higher in spring, fall, and winter compared to year 2, while year 2 had greatest saxitoxin exposure in the summer (Figure 6A). Considering domoic acid, the best model included the additive effect of season and year (Table 3). Domoic acid exposure peaked in the summer of both years, but appeared higher in the spring and summer of year 2 compared to year 1 (Figure 6C).

Considering haulout complex (Tatoosh and Carroll/Sea Lion Rock), season (spring and fall), and year, the most parsimonious model for predicting saxitoxin was the full model including complex, year, and their interaction (Table 3). In year 1, saxitoxin exposure was higher at the Tatoosh Island complex compared to Carroll/Sea Lion Rock, while in year 2 the exposure rates appeared similar (Figure 6B). For domoic acid, the best model included haulout complex only (Table 3). Domoic acid exposure was higher at the Carroll/Sea Lion Rock complex compared to the Tatoosh Island complex (Figure 6D).

Table 2. Presence/absence of saxitoxin (n = 373 scats) and domoic acid (n = 383 scats) in Steller sea lion *Eumetopias jubatus* scats compared to season and year. Models displaying an interaction (a*b) represent the full model including the main effects and their interaction (a+b+a*b). The selected models are shown in bold.

Saxitoxin			Domoic Acid		
Formula	df	AIC	Formula	df	AIC
~intercept only	1	509.07	~intercept only	1	350.86
~season	4	511.67	~season	4	336.77
~year	2	484.84	~year	2	346.66
~season+year	5	488.15	**~season+year**	**5**	**331.99**
~season*year	**8**	**482.46**	~season*year	8	333.87

Table 3. Presence/absence of saxitoxin (n = 190 scats) and domoic acid (n = 197 scats) in Steller sea lion *Eumetopias jubatus* scats by season, year, and haulout complex. Models displaying an interaction (a*b) represent the full model including the main effects and their interaction (a+b+a*b). The selected models are shown in bold.

Saxitoxin			Domoic Acid		
Formula	df	AIC	Formula	df	AIC
~1	1	259.98	~1	1	166.63
~season	2	259.66	~season	2	167.56
~complex	2	260.09	**~complex**	**2**	**149.81**
~year	2	226.37	~year	2	167.43
~season+complex	3	260.69	~season+complex	3	151.75
~season+year	3	227.36	~season+year	3	168.50
~complex+year	3	225.62	~complex+year	3	150.00
~season*complex	4	262.69	~season*complex	4	153.32
~season*year	4	227.90	~season*year	4	167.76
~complex*year	**4**	**224.75**	~complex*year	4	151.76
~season+complex+year	4	227.36	~season+complex+year	4	151.97
~season*complex+year	5	228.02	~season*complex+year	5	153.77
~season+complex*year	5	226.71	~season+complex*year	5	153.70
~season*year+complex	5	228.43	~season*complex+site	5	153.12
~season*complex*year	8	226.68	~season*complex*year	8	157.29
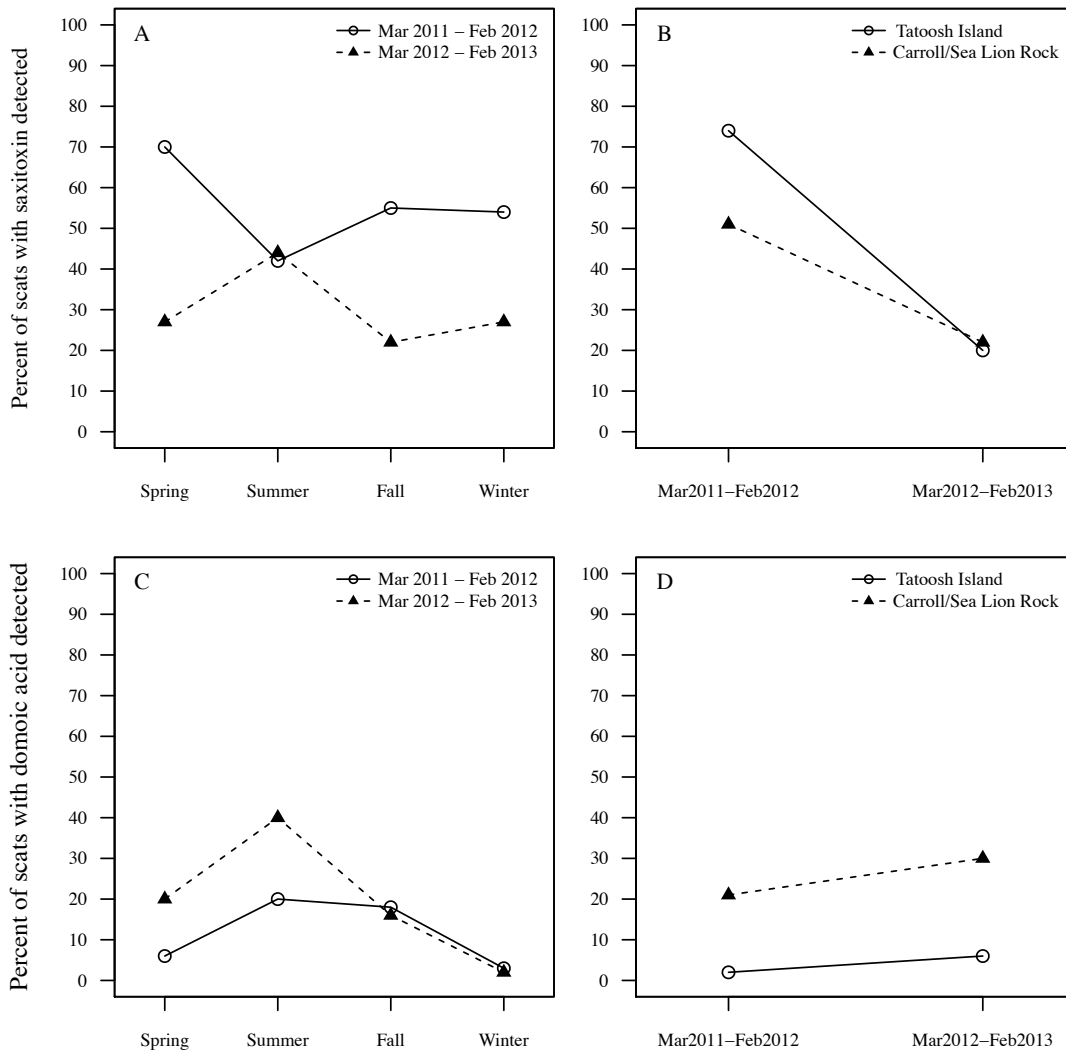
Figure 6. Percent of scats with saxitoxin (top) and domoic acid (bottom) detected for Steller sea lions *Eumetopias jubatus* by season and year (A and C) and by year and haulout complex (B and D).

Sea Lion Diet

A total of 39 prey taxa (lowest taxonomic group) was identified in Steller sea lion scats (Table 4) and 30 prey taxa were identified in California sea lion scats (Table 5). Eight scats from Steller sea lions contained no identifiable prey remains (unidentified bony fish) and were removed from diet analyses. The most common prey species (>20 % FO in the diet) were similar between sea lion species. For Steller sea lions, the most common prey items were clupeids (family Clupeidae, 56 %), salmonids (*Onchorynchus* sp., 40 %), skates (family Rajidae, 30 %), rockfish *Sebastes* spp. (36 %), Pacific spiny dogfish *Squalus suckleyi* (28 %), and flatfish (order Pleuronectiformes, 21 %). For California sea lions, the most common prey items were clupeids (79 %), salmon (38 %), Pacific hake *Merluccius productus* (32 %), and dogfish (30 %). The two sea lion species had several major differences in diet including skate consumption (40 % in Steller compared to 5 % in California), flatfishes (21 % in Steller compared to 8 % in California), and codfishes (family Gadidae) (18 % in Steller and 6 % in California).

Sea lion diet varied by season, collection year, and haulout complex. Steller sea lions had greatest consumption of clupeids, flatfishes, and Pacific hake in the summer and fall, while salmon, rockfish, and codfishes decreased dramatically during these seasons (Table 4). Flatfishes were almost completely absent in winter samples (1% FO). For California sea lions, due to small sample sizes in spring (n = 20, year 1 only) and summer (n = 19, year 2 only), I compared diet between years during fall season only. Most noticeably, hake consumption dropped dramatically from fall of year 1 to year 2, reduced from FO of 53% to only 8% (Table 5).

For Steller sea lions, comparing seasons between years revealed similar annual fluctuations in diet. Both skates and rockfish were noticeably higher in spring of year 2 (68 % and 60 % FO, respectively) compared to year 1 (34 % and 30 %), whereas dogfish was more common in year 2 (42 %) compared to year 1 (22 %). Juvenile salmon were present in 38 % of winter samples in year 2, but completely absent in winter of year 1. Similarly, walleye pollock *Theragra chalcogramma* had a spike in occurrence in spring of year 1 (30 % FO), but was present in <5 % of samples in all other seasons of both collection years. Other differences include greater Pacific herring *Clupea pallasii* in spring of year 2 (22 %) compared to spring of year 1 (6 %) and greater Pacific sand lance *Ammodytes hexapterus* in winter of year 2 (13 %) compared to year 1 (3 %). Northern anchovy *Engraulis mordax* was present in spring, summer, and fall of year 1 (14, 13, and 15 % FO), but virtually absent in year 2 (0, 2, and 4 %).

For Steller sea lions, I compared diet between the haulout complexes with the greatest sample size, Tatoosh Island (n = 97) and Carroll/Sea Lion Rock (n = 100), for the spring and fall seasons when both sites were sampled. Clupeids and salmonids were more common in samples from Tatoosh Island (67% and 57%, respectively) compared to Carroll/Sea Lion Rock (45% and 22%). Skates and flatfishes were higher at Carroll/Sea Lion Rock (56% and 40%) compared to Tatoosh (40% and 7%). Codfishes were more common at Tatoosh (29%) compared to Carroll/Sea Lion Rock (12%), primarily driven by the high occurrence of walleye pollock in the spring of year 1 (Table 4).

Table 4. Sample information and percent frequency of occurrence (FO) of Steller sea lion *Eumetopias jubatus* prey items by total FO and by season with years combined. Prey items are listed in decreasing order of FO within and between taxonomic groupings (i.e. family, order, or class).

	Total	Spring	Summer	Fall	Winter
Samples					
Total scat samples collected	383	100	96	105	82
Scat containing >0 identifiable prey	375	99	92	103	81
Scat containing no identifiable prey	8	1	4	2	1
Prey identified to lowest taxonomic group	39	29	25	35	23
Prey item					
Herring, shad, sardine: family Clupeidae	56	51	66	61	45
Clupeid, unidentified	31	30	34	32	26
Pacific herring (*Clupea pallasii*)	15	14	8.3	20	17
Pacific sardine (*Sadinops sagax*)	9.4	0.0	24	11	1.2
American shad (*Alosa sapidissima*)	4.7	8.0	1.0	3.8	6.1
Skate: family Rajidae					
Skate, unidentified	40	51	28	37	46
Salmon: family Salmonidae	40	45	18	34	67
Non-juvenile salmon	36	43	15	31	57
Juvenile salmon	8.9	7.0	3.1	6.7	21
Rockfish: family Sebastidae					
Rockfish (*Sebastes* spp.)	36	49	7.3	30	60
Dogfish sharks: family Squalidae					
Spiny dogfish (*Squalus accanthias*)	28	32	18	36	26
Flatfishes: order Pleuronectiformes	21	19	32	28	1.2
Starry flounder (*Platichthys stellatus*)	9.4	7.0	17	12	0.0
Flatfishes, unidentified	7.3	8.0	8.3	10	1.2
Arrowtooth flounder *(Atheresthes stomias)*	2.9	4.0	5.2	1.9	0.0
Butter sole (*Isopsetta exilis*)	2.4	4.0	4.2	1.0	0.0
Rex sole (*Glyptocephalus zachirus*)	1.0	0.0	1.0	2.9	0.0
Dover sole *(Microstomus pacificus)*	0.8	3.0	0.0	0.0	0.0
Sanddabs *(Citharichthys spp.)*	0.8	1.0	0.0	1.9	0.0
Sand sole (*Psettichthys melanostictus*)	0.5	2.0	0.0	0.0	0.0

	Total	Spring	Summer	Fall	Winter
Codfishes: family Gadidae	18	32	5.2	12	22
Codfishes, unidentified	7.6	14	1.0	2.9	13
Pacific cod *(Gadus macrocephalus)*	5.5	2.0	4.2	7.6	8.5
Walleye pollock *(Theragra chalcogramma)*	4.7	16	0.0	1.9	0.0
Bony fish: class Osteoichthyes					
Bony fish, unidentified	14	10	25	14	4.9
Hakes: family Merlucciidae					
Pacific hake *(Merluccius productus)*	12	3.0	18	25	0.0
Anchovies: family Engraulidae					
Northern anchovy *(Engraulis mordax)*	7.3	7.0	7.3	10	3.7
Greenlings: family Hexagrammidae	6.8	7.0	2.1	10	7.3
Lingcod *(Ophiodon elongatus)*	4.2	7.0	2.1	4.8	2.4
Hexagrammids, unidentified	1.8	0.0	0.0	2.9	4.9
Atka mackerel *(Pleurogrammus monopterygius)*	0.8	0.0	0.0	2.9	0.0
Sand lances: family Ammodytidae					
Pacific sand lance *(Ammodytes hexapterus)*	4.4	3.0	4.2	2.9	8.5
Squids and octopus: class Cephlapoda	4.2	3.0	1.0	7.6	4.9
Cephalopods, unidentified	2.9	2.0	1.0	4.8	3.7
Squid, unidentified	0.8	0.0	0.0	1.9	1.2
Octopus, unidentified	0.5	1.0	0.0	1.0	0.0
Cartilaginous fish: subclass Elasmobranchii					
Sharks and rays, unidentified	3.1	4.0	1.0	3.8	3.7
Smelts: family Osmeridae					
Smelts, unidentified	2.9	5.0	0.0	1.0	6.1
Lamprey: family Petromyzontidae	2.6	0.0	1.0	4.8	4.9
Pacific lamprey *(Lampetra tridentata)*	1.6	0.0	0.0	1.9	4.9
Lampreys, unidentified	1.0	0.0	1.0	2.9	0.0
Mackerel and tuna: family Scrombidae					
Pacific mackerel *(Scomber japonicus)*	1.6	0.0	4.2	1.9	0.0

	Total	Spring	Summer	Fall	Winter
Poachers: family Agonidae					
Poacher, unidentified	1.6	2.0	2.1	1.0	1.2
Sticklebacks: family Gasterosteidae					
Threespine stickleback *(Gasterosteus aculeatus)*	1.6	5.0	0.0	1.0	0.0
Sculpins: family Cottidae					
Sculpins, unidentified	1.3	0.0	0.0	2.9	2.4
Hagfishes: family Myxinidae					
Pacific hagfish *(Eptatretus stoutii)*	0.8	1.0	0.0	1.9	0.0
Gunnel: Family Pholidae					
Gunnels, unidentified	0.8	2.0	1.0	0.0	0.0
Snailfishes: family Liparidae					
Snailfishes, unidentified	0.5	0.0	0.0	0.0	2.4

Table 5. Sample information and percent frequency of occurrence (FO) of California sea lion *Zalophus californianus* prey items by total FO and by season for each collection period. Prey items are listed in decreasing order of FO within and between taxonomic groupings (i.e. family, order, or class).

	Total	2011 Spring	2011 Fall	2012 Summer	2012 Fall
Samples					
Total scat samples collected	123	20	45	19	39
Scat containing >0 identifiable prey	123	20	45	19	39
Scat containing no identifiable prey	0	0	0	0	0
Prey identified to lowest taxonomic group	30	15	21	16	26
Prey item					
Herring, shad, sardine: family Clupeidae	79	60	87	63	87
Pacific herring (Clupea pallasii)	31	35	22	32	38
Clupeid, unidentified	25	15	29	26	26
American shad (Alosa sapidissima)	23	25	36	5.3	15
Pacific sardine (Sadinops sagax)	19	0.0	33	0.0	20.5
Salmon: family Salmonidae	38	40	40	42	33
Non-juvenile salmon	33	25	36	42	31
Juvenile salmon	6.5	15	6.7	0.0	5.1
Hakes: family Merlucciidae					
Pacific hake (*Merluccius productus*)	32	40	53	21	7.7
Dogfish sharks: family Squalidae					
Spiny dogfish (*Squalus accanthias*)	30	45	29	58	10
Rockfish: family Sebastidae					
Rockfish (*Sebastes* spp.)	19	65	8.9	21	5.1
Squids and octopus: class Cephlapoda	12	30	4.4	16	10
Cephalopods, unidentified	4.1	15	0.0	5.3	2.6
Octopus, unidentified	6.5	15	2.2	11	5.1
Squid, unidentified	1.6	0.0	2.2	0.0	2.6
Anchovies: family Engraulidae					
Northern anchovy (*Engraulis mordax*)	11	5.0	18	5.3	10

| | | 2011 | | 2012 | |
	Total	Spring	Fal1	Summer	Fall
Smelts: family Osmeridae	8.9	15	6.7	0.0	13
Smelts, unidentified	8.9	15	6.7	0.0	13
Eulachon (*Thaleichthys pacificus*)	0.8	0.0	2.2	0.0	0.0
Bony fishes: class Osteoichthyes					
Bony fishes, unidentified	8.9	5.0	4.4	16	13
Flatfishes: order Pleuronectiformes	8.1	0.0	11	16	5.1
Arrowtooth flounder *(Atheresthes stomias)*	4.9	0.0	6.7	11	2.6
Flatfishes, unidentified	2.4	0.0	4.4	0.0	2.6
Dover sole *(Microstomus pacificus)*	0.8	0.0	2.2	0.0	0.0
Starry flounder *(Platichthys stellatus)*	0.8	0.0	0.0	5.3	0.0
Cartilaginous fishes: subclass Elasmobranchii					
Sharks and rays, unidentified	6.5	5.0	6.7	11	5.1
Codfishes: family Gadidae	5.7	10	0.0	5.3	10.3
Codfishes, unidentified	3.3	10	0.0	0.0	5.1
Pacific cod *(Gadus macrocephalus)*	1.6	0.0	0.0	5.3	2.6
Walleye pollock *(Theragra chalcogramma)*	0.8	0.0	0.0	0.0	2.6
Skate: family Rajidae					
Skate, unidentified	4.9	10	2.2	11	2.6
Greenlings: family Hexagrammidae					
Hexagrammids, unidentified	0.8	0.0	0.0	0.0	2.6
Jack macherels: family Carangidae					
Jackmackerel (*Trachurus symmetricus*)	0.8	0.0	0.0	0.0	2.6
Lamprey: family Petromyzontidae					
Pacific lamprey (*Lampetra tridentata*)	0.8	0.0	0.0	0.0	2.6
Sculpins: family Cottidae					
Sculpins, unidentified	0.8	0.0	0.0	0.0	2.6
Snailfishes: family Liparidae					
Snailfishes, unidentified	0.8	0.0	2.2	0.0	0.0

Diet Influences on Toxin Prevalence

There were no convergent solutions using nonmetric multidimensional scaling

(NMDS) to examine the diet of either Steller or California sea lions (Figures 7 and 8). I

present the lowest stress values calculated after 20 NMDS iterations, which between 0.1-

0.2 would typically represent good ordination (Kruskal 1964), however due to non-

convergence do not represent a true overall minimum stress or fit to he data. Analysis of

similarity (ANOSIM) found a significant difference in Steller sea lion diet between scats

with and without saxitoxin (R = 0.03, p = 0.001) and between scats with toxins grouped

by concentration level (saxitoxin: R = 0.05, p = 0.005; domoic acid: R = 0.05, p = 0.002).

However, based on the low R-values, these findings likely do not represent a meaningful

difference in diet. There were no significant differences in diet for California sea lions

based on ANOSIM.

Steller Sea Lions − Saxitoxin (n = 358 scats)

California Sea Lion − Saxitoxin (n = 113 scats)

Steller Sea Lions − Domoic Acid (n = 368 scats)

California Sea Lions − Domoic Acid (n = 117 scats)
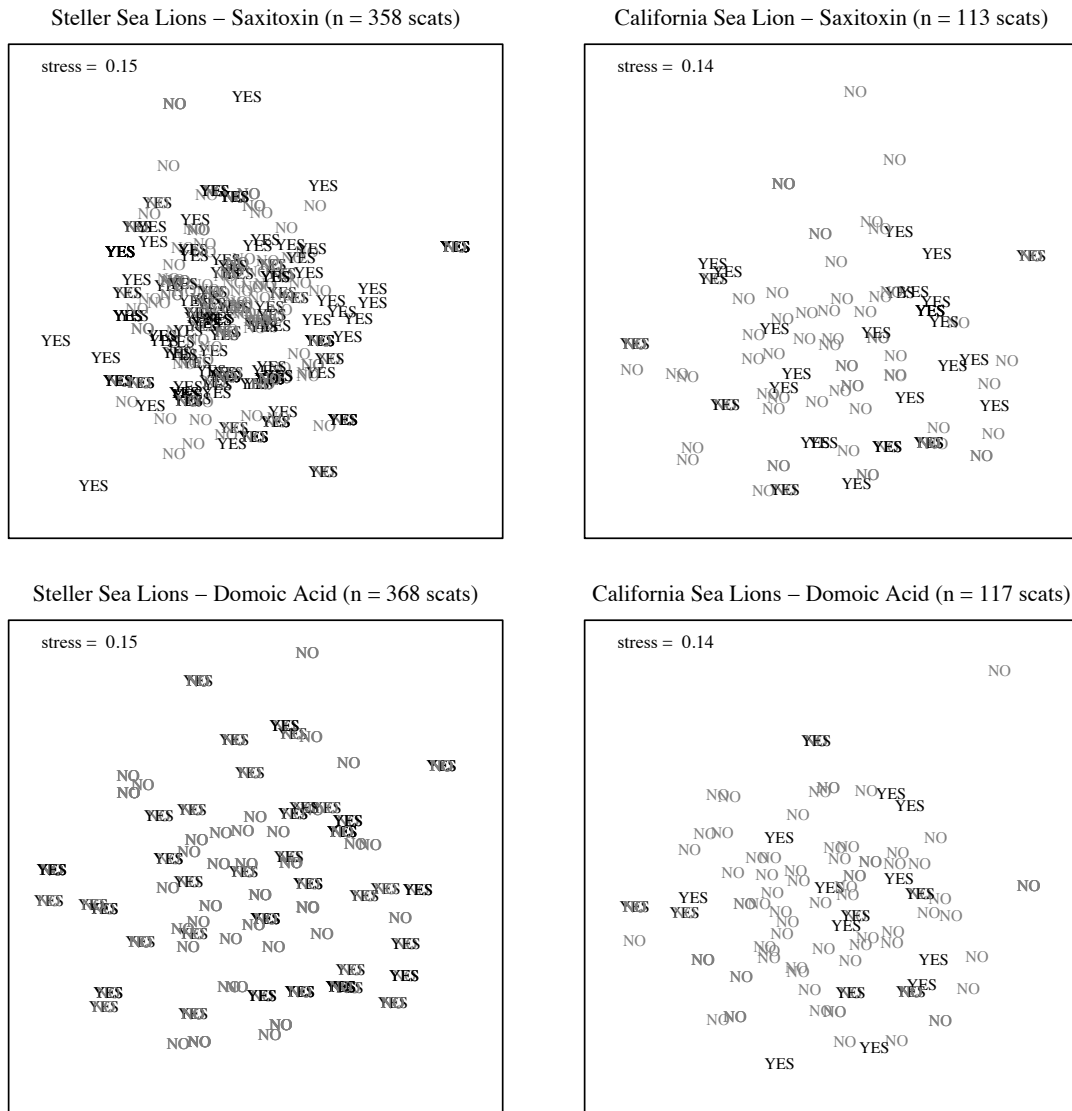
Figure 7. Nonmetric Multidimensional Scaling plot of Steller sea lion *Eumetopias jubatus* (left) and California sea lion *Zalophus californianus* (right) food habits compared to presence (YES) or absence (NO) of saxitoxin (top) and domoic acid (bottom). Because the NMDS analyses did not converge, configurations and stress values represent the best solution after 20 iterations. No ordination is apparent.
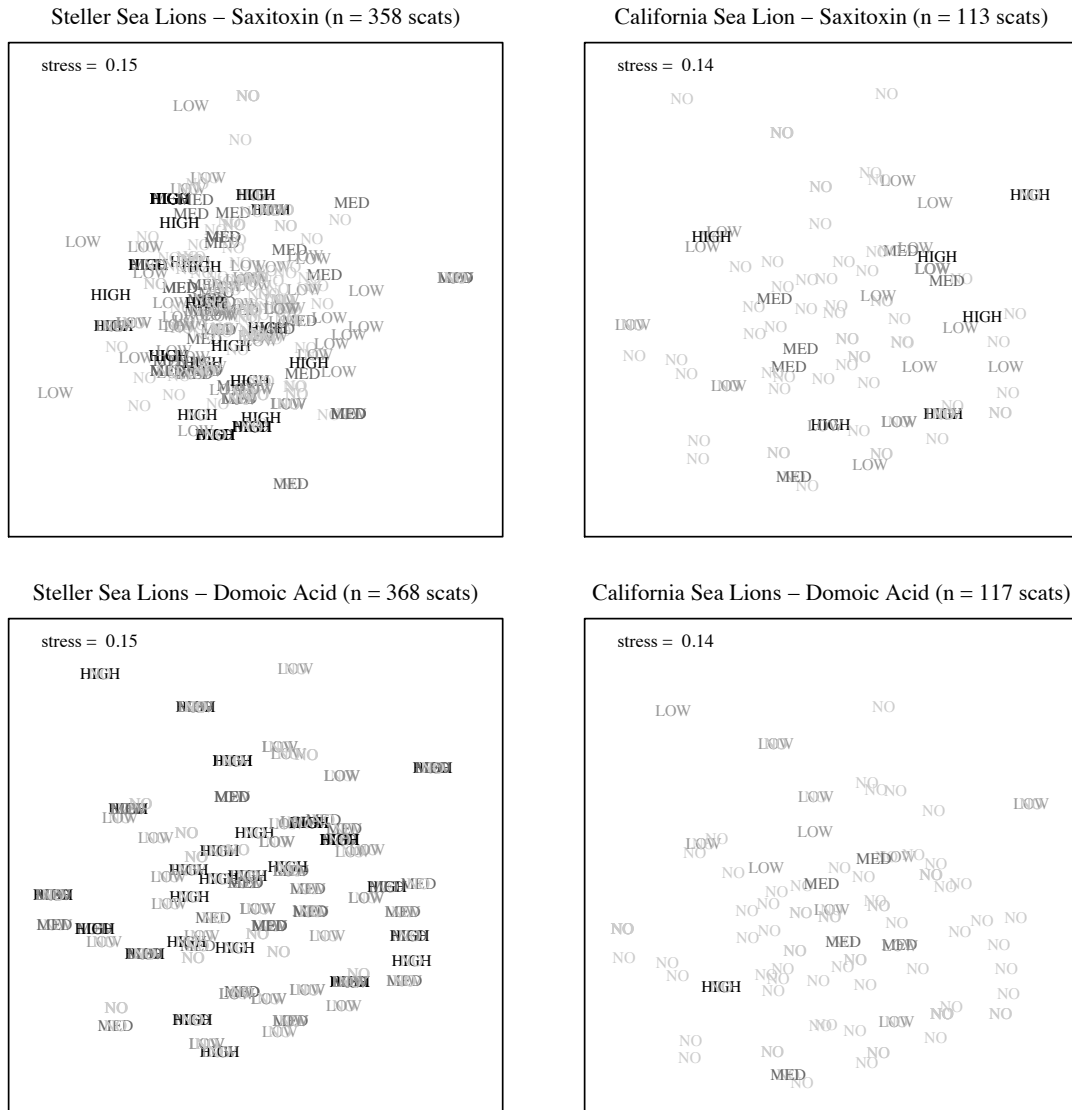
Figure 8. Nonmetric Multidimensional Scaling plot of Steller sea lion *Eumetopias jubatus* (left) and California sea lion *Zalophus californianus* (right) food habits compared to "no" (below detection limit), low (0-25 ng g^{-1}), medium (25-60 ng g^{-1}), or high (>60 ng g^{-1}) concentrations of saxitoxin (top) and domoic acid (bottom). Because the NMDS analyses did not converge, configurations and stress values represent the best solution after 20 iterations. No ordination is apparent.

61

Chi-squared and Fisher's Exact analyses identified several prey items significantly associated with the presence or concentration level of toxins found in the scat. In Steller sea lions, presence of saxitoxin was significantly different than expected the presence of non-juvenile salmonids, American shad *Alosa sapidissima*, walleye pollock, and skates (Table 6). When shad and pollock were present in the scat, saxitoxin presence was significantly higher than expected (Table 6). Saxitoxin was detected in every scat with pollock prey remains (n=18 scats). Presence of domoic acid was negatively associated with non-juvenile salmonids, but positively associated with spiny dogfish, Pacific sardine, and starry flounder *Platichthys stellatus* (Table 6). When sardine and starry flounder were present, domoic acid presence was significantly higher than expected (Table 6). In California sea lions, saxitoxin was positively associated with presence of non-juvenile salmonids, whereas scats with Northern anchovy were about five times more likely to have domoic acid compared to those without anchovy (Table 6).

When comparing presence of prey to specific toxin levels, the patterns were more complex. In Steller sea lions, if saxitoxin was detected in scats with rockfish, it was significantly more likely to be in low concentrations compared to other concentrations (Table 7). Saxitoxin appeared more frequently than expected in all concentration levels when non-juvenile salmonids were present, whereas skates were less frequent than expected in all concentration levels (Table 7). Spiny dogfish appeared most frequently associated with medium and high levels of saxitoxin (Table 7). Only walleye pollock was significantly different between saxitoxin concentration levels following Bonferroni adjustment (Table 8). Scats with pollock were significantly more likely to have medium and high levels of saxitoxin compared to low concentrations (Table 8). In spite of wide

confidence intervals, scats with sardine were significantly more likely to have high

concentrations of domoic acid (Table 8). In California sea lions, Northern anchovy was

detected with only low or medium concentrations of domoic acid (Table 8).

Table 6. Significant Pearson's Chi-squared or Fisher's Exact tests (p<0.05) for 2 x 2 contingency tables comparing presence/absence of prey to presence/absence of toxin in the scat. Chi-squared statistics (χ^2, representing $\sum \chi^2_{i-j}$) and p-values for each 2 x 2 table are presented. Pearson's residuals are shown for presence of prey item:presence of toxin, with residuals >|1.96| being significant (in bold). Positive residual values indicate higher than expected presence of toxin and negative residuals indicate lower than expected presence of toxin given the presence of each prey item. Fisher's exact tests are reported as a significant p-value (<0.05) with odds ratio and 95% confidence intervals (CI).

Toxin/Species	χ^2	p	Pearson's residual presence:presence
Saxitoxin			
Steller sea lion			
Non-juvenile salmonid	6.77	0.010	1.66
Skate spp.	9.54	0.002	-1.89
American shad	5.93	0.015	**1.99**
Walleye pollock	23.86	<0.001	**3.82**
California sea lion			
Non-juvenile salmonid	4.87	0.027	1.70
Domoic acid			
Steller sea lion			
Non-juvenile salmonid	4.84	0.028	-1.71
Dogfish	4.71	0.030	1.79
Pacific sardine	23.49	<0.001	**4.41**
Starry flounder	8.89	0.003	**2.79**
California sea lion			
Northern anchovy	-	*Fisher's p*: 0.011	*Odds:* 4.99 *CI*: 1.24-19.44

Table 7. Significant Pearson's Chi-squared tests (p<0.05) and p-values for 2 x 4 contingency tables comparing presence/absence of prey to levels of no, low (<20 ng g^{-1}), med (20-50 ng g^{-1}), and high (>50 ng g^{-1}) concentrations of toxin in the scat. Chi-squared statistics (χ^2, representing $\sum \chi^2_{i-j}$) and p-values for each 2 x 4 comparison are presented. Pearson's residuals are shown for presence of prey item:level of toxin, with residuals >|1.96| being significant (in bold). Positive residual values indicate higher than expected level of toxin and negative residuals indicate lower than expected level of toxin given the presence of each prey item.

Toxin/Species	χ^2	p	presence:no toxin	presence:low	presence:med	presence:high
Saxitoxin						
Steller sea lion						
Rockfish spp.	20.11	<0.001	-0.07	**2.35**	-1.03	**-2.55**
Salmonid	8.15	0.043	-1.41	0.74	1.19	1.14
Skate spp.	12.20	0.007	1.61	-1.44	-0.26	-1.62
Spiny dogfish	12.37	0.006	-0.56	-1.45	1.82	1.80

Table 8. Significant Fisher's Exact tests (p<0.05) for 2 x 4 contingency, comparing presence/absence of prey to levels of no, low (<20 ng g^{-1}), med (20-50 ng g^{-1}), and high (>50 ng g^{-1}) concentrations of toxin in the scat. Fisher's Exact p-values for the overall 2 x 4 contingency tables are presented. Post-hoc testing of all pairwise comparisons used a Bonferroni-adjusted alpha value to assess significance, where alpha was calculated as 0.05 divided by the number of pairwise comparisons. Significant 2 x 2 pairwise comparisons of presence of prey items at each toxin concentration level (e.g. presence of fish when low toxin levels in scat compared to presence of fish when no toxins in scat = Low:No) are presented by p-values with odds ratio and 95% confidence interval (CI). Bolded values indicate significance after Bonferroni-adjustment.

Toxin/Species	Fisher's p-value	Low:No	Medium:No	High:No	Med:Low	High:Low	High:Med	Bonferroni p-adjust
Saxitoxin								
Steller sea lion								
American shad	0.025	0.040	–	–	–	–	–	0.008
Walleye pollock	<0.001	NA	NA	NA	–	**<0.001** Odds: 10.23 CI: 2.6-49.69	**0.014** Odds: 5.01 CI: 1.24-24.8	0.0167
California sea lion								
Northern anchovy	0.040	–	–	0.021 Odds: 9.42 CI: 1.08-83.02	–	–	–	0.008
Octopus spp.	0.044	–	0.032 Odds: 13.03 CI: 0.866-155	NA	–	NA	NA	0.0167

Toxin/Species	Fisher's p-value	Low:No	Medium:No	High:No	Med:Low	High:Low	High:Med	Bonferroni p-adjust
Domoic acid								
Steller sea lion								
Salmonid	0.007	**0.005** _Odds_: 0.366 _CI_: 0.16-0.77	–	–	–	0.023 Odds: 0.120 CI: 0.009-0.96	–	0.008
Spiny dogfish	0.002	**0.002** _Odds_: 2.66 _CI:_ 1.41-5.02	NA	NA	NA	NA	NA	NA
Pacific sardine	<0.001	**0.001** _Odds_: 4.00 _CI_: 1.61-9.59	–	**<0.001** _Odds_: 75.61 _CI_: 7.9-3636	–	**0.004** _Odds_: 18.3 _CI_: 1.8-939	–	0.008
Starry flounder	<0.001	**0.016** _Odds_: 2.90 _CI_: 1.15-6.87	**0.004** _Odds_: 18.8 _CI_: 2.05-235	NA	–	NA	NA	0.0167
California sea lion								
American shad	0.024	–	–	NA	–	NA	NA	0.0167
Northern anchovy	0.006	**0.016** _Odds_: 6.59 _CI_: 1.17-33.62	–	NA	–	NA	NA	0.0167

*NA refer to cells where no comparison could be made due to the prey not being present at that toxin level

Comparison to Nearshore Bivalves

Between January 2011 and March 2013, saxitoxin was detected in concentrations >380 ng g^{-1} in 58 samples of California mussels *Mytilus californianus* and 4 samples of razor clams *Siliqua patula* (Figure 9). Saxitoxin concentrations appeared to peak in the summer of 2011 and the summer/fall of 2012, with the highest concentrations of saxitoxin recorded in the western Strait of Juan de Fuca at Clallam Bay, Sekiu, and Neah Bay (Figure 10). Domoic acid was almost exclusively detected in razor clams, which were only present at two southern beaches, Second Beach and Kalaloch Beach (Figure 10). Domoic acid peaked in the fall of both 2011 and 2012, detected in concentrations above 1000 ng g^{-1} in 24 samples and detected in a single sample of California mussels from Makah Bay at a concentration <1000 ng g^{-1} (Figure 11).

To compare saxitoxin in sea lion scat to concentrations in bivalves, I overlaid plots on a map of the study area showing scat concentrations grouped by concentration level and toxin concentration in bivalve samples taken three weeks before and after the scat sample date (Appendix 1). In general, sea lion scat concentrations did not follow the same concentration trend as the bivalves. For example, in periods of very high toxins in bivalves, such as in July and August 2012 with levels measured as high as 6,000-12,000 ng g^{-1} of saxitoxin, the majority of sea lion scats did not have detectable levels (Appendix 1, Figure O). However, in September of that year, higher concentrations were observed in California sea lion scats (Appendix 1, Figure Q). Conversely, in April and May 2011, when most Steller sea lion scats had detectable levels of toxins and many in the ranges of 50-100 ng g^{-1} or >200 ng g^{-1}, bivalves had no to very low (<380 ng g^{-1}) concentrations of

toxin (Appendix 1, Figures B and C). In several periods (e.g. February and March 2012), sea lions had detectable levels of saxitoxin (as high as 50-100 ng g^{-1}), while no toxin was detected in nearshore bivalves (Appendix 1, Figures J and K).
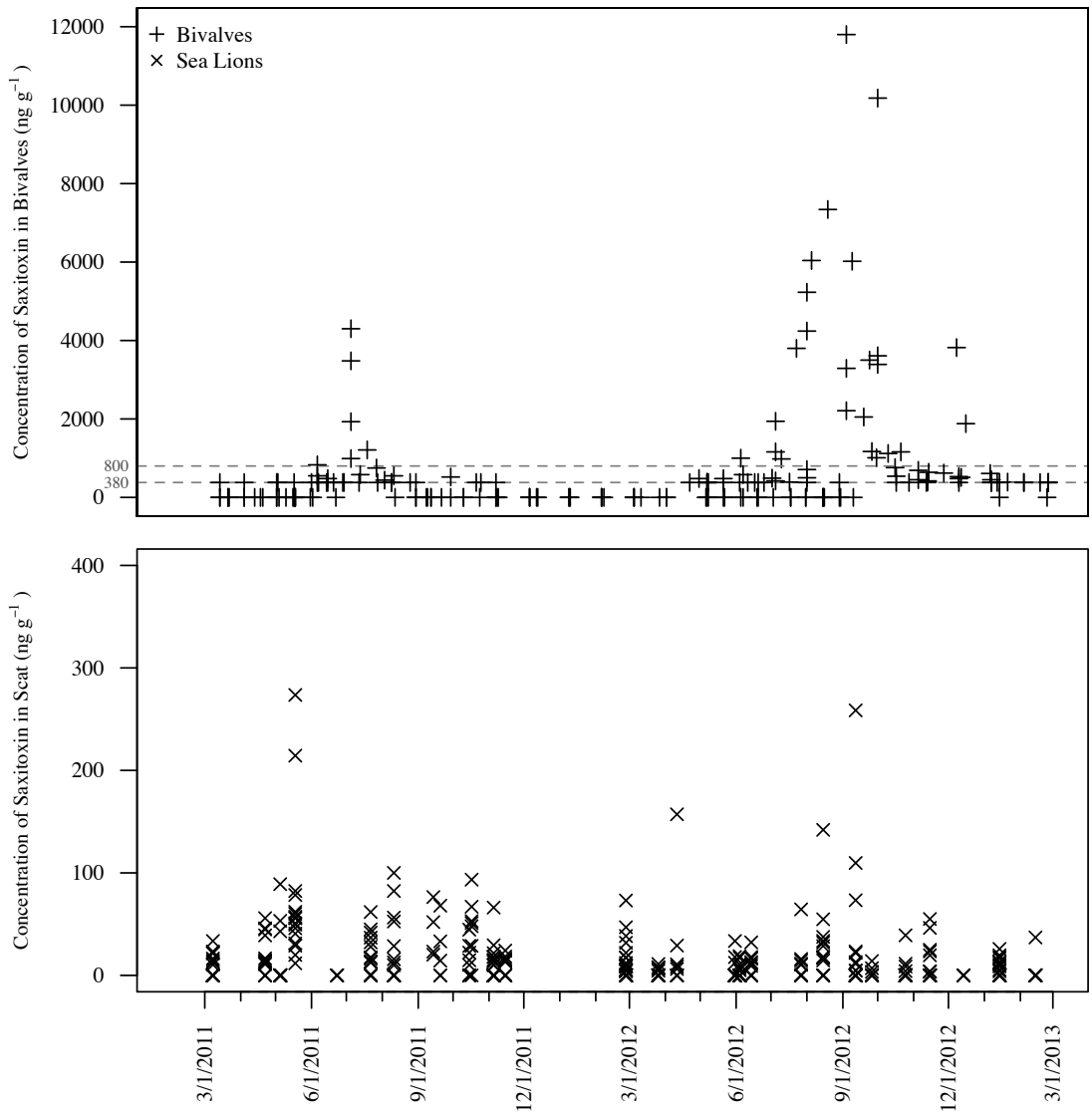
Figure 9. Concentrations of saxitoxin measured in bivalves and both Steller sea lions *Eumetopias jubatus* and California sea lions *Zalophus californianus* between March 2011 and March 2013. The dotted line indicates samples reported here as 380 ng g^{-1} that represent concentrations reported by WDOH of <380 ng g^{-1}. Samples >800 ng g^{-1} indicate concentrations above the regulatory limit.

70
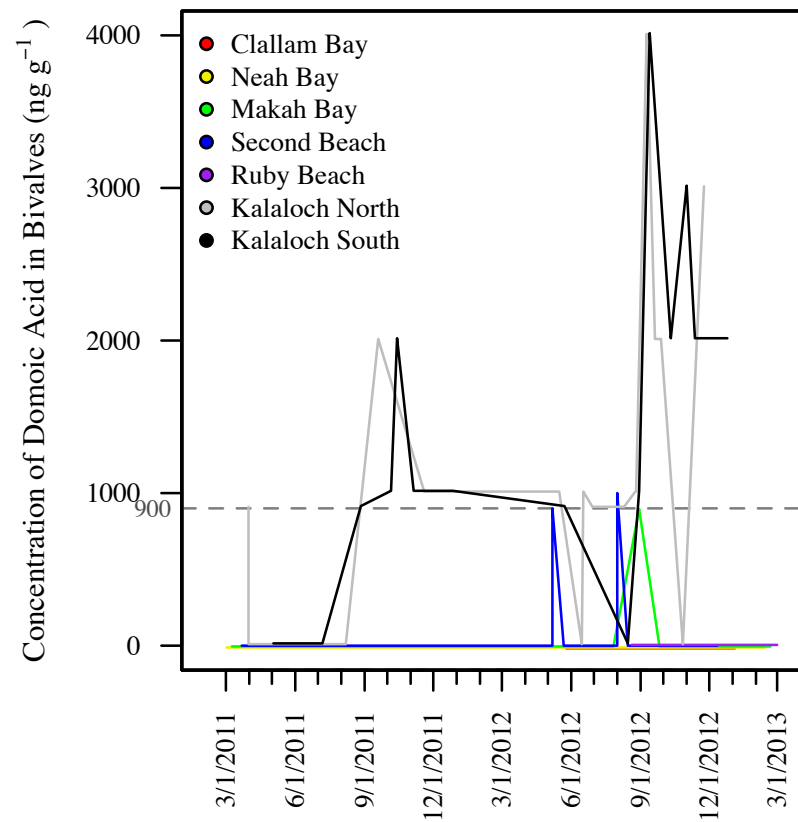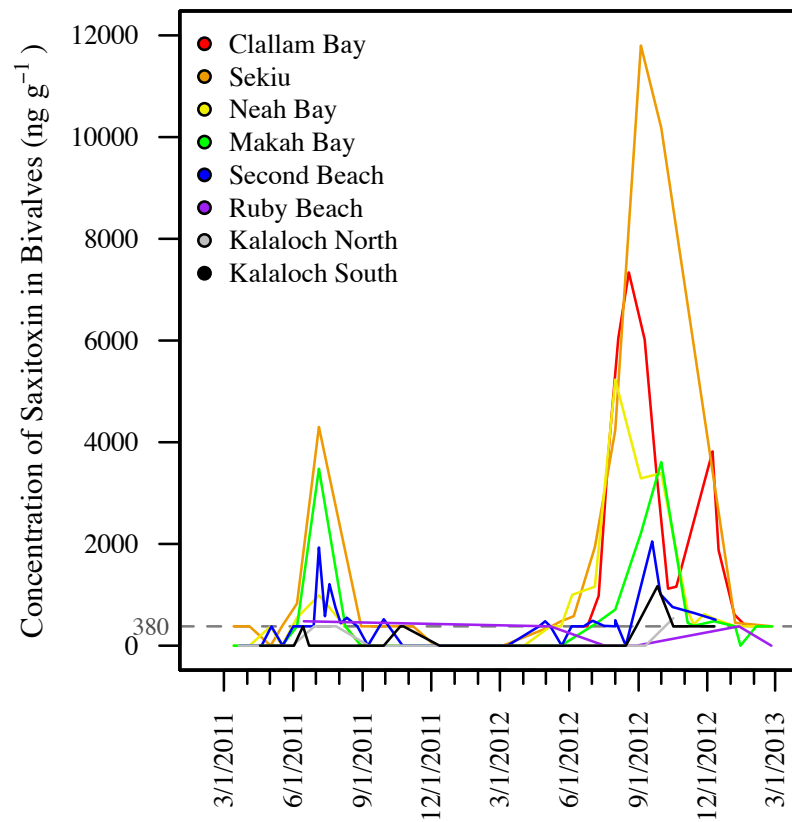
Figure 10. Concentrations of saxitoxin (left) and domoic acid (right) measured in bivalves between March 2011 and March 2013 by site. Sites are listed from the northeast to the south (Figure 2). The dotted lines indicate concentrations reported by WDOH of saxitoxin <380 ng g^{-1} (represented as 380 ng g^{-1}) and domoic acid <1000 ng g^{-1} (represented as 900 ng g-1).
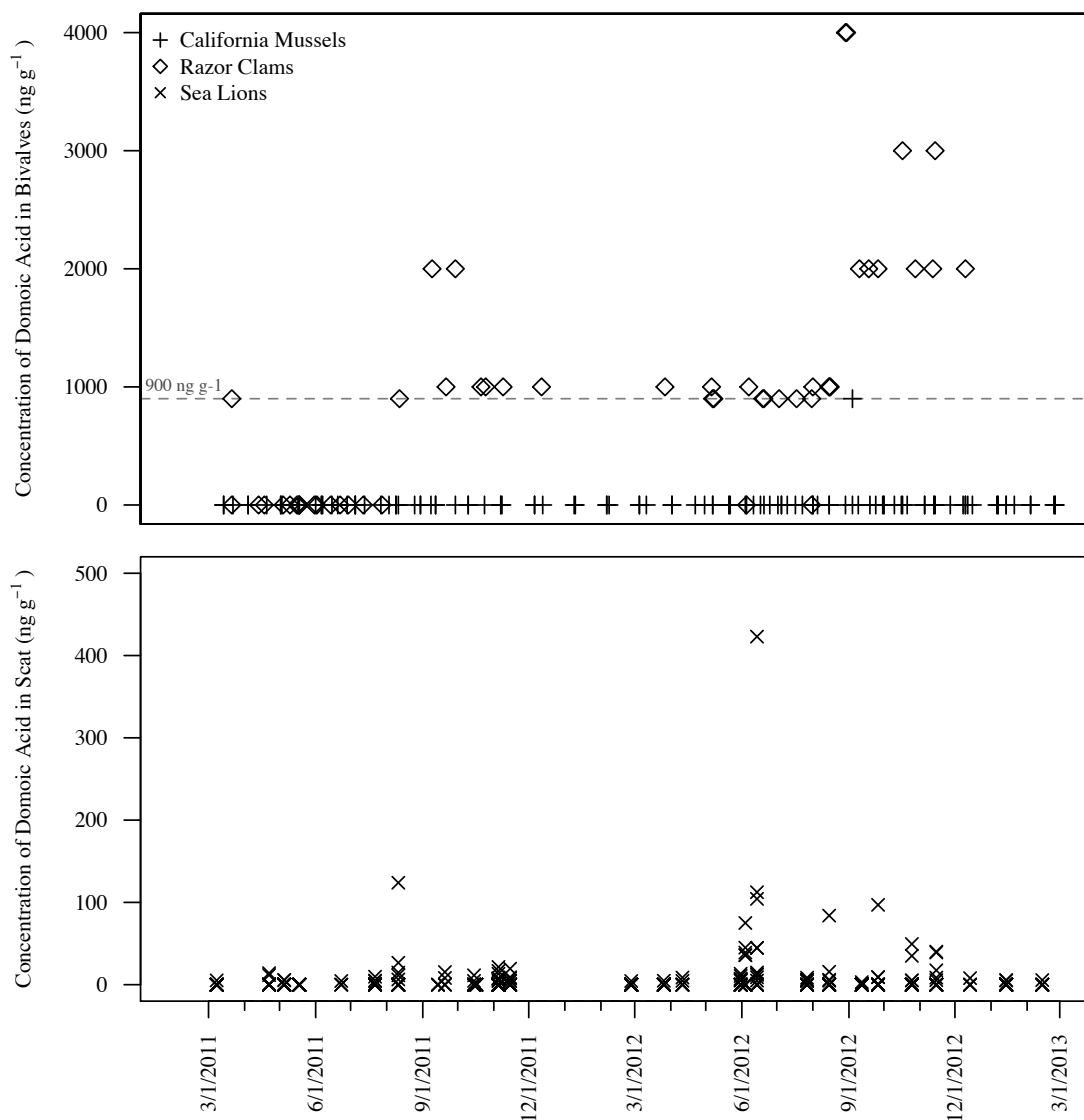
Figure 11. Concentrations of domoic acid measured in bivalves and Steller sea lions *Eumetopias jubatus* and California sea lions *Zalophus californianus* between March 2011 and March 2013. The dotted line indicates samples reported here as 900 ng g^{-1} that represent concentrations reported by WDOH of <1000 ng g^{-1}. All samples during this time period were below the regulatory limit of 20,000 ng g^{-1}.

DISCUSSION

Toxin Prevalence by Season, Year, and Location

This is the first study to document that marine mammals can be exposed to algal toxins year-round. Although Steller sea lions *Eumetopias jubatus* and California sea lions *Zalophus californianus* on the outer coast of Washington were more often exposed to saxitoxin than to domoic acid, both toxins were detected in scats in every season and month of the year. This last finding suggests that both saxitoxin and domoic acid were either retained in the food web for long periods after blooms (Jensen et al. 2015) or that the toxins were transferred from the benthic or pelagic food webs through sediments or dormant cysts (Vigilant & Silver 2007, Jester et al. 2009, Sekula-Wood et al. 2011).

Given the bloom dynamics of *Pseudo-nitzschia* and *Alexandrium* in Washington State, it is unlikely that there were active algal blooms in the winter when toxins were detected in sea lion scat. Harmful levels of domoic acid and saxitoxin may occur in Washington shellfish in late fall (e.g. November, Trainer 2002), while blooms in other areas may occur as late as December (Bates et al. 1998). On the Washington coast, *Pseudo-nitzschia* blooms are associated with the seasonal Juan de Fuca Eddy, which is formed in spring and dissipates in the fall (Hickey & Banas 2003). *Alexandrium* blooms require warming water temperatures typically beginning in the spring (around 13˚ C, Nishitani & Chew 1984). Thus, active blooms are not likely to occur in the winter.

Based on studies on domoic acid and saxitoxin retention in fish and mammals, it is unlikely that either the fish or the sea lions themselves were retaining the toxins for

long periods after the fall algal blooms. Domoic acid administered through oral gavage to northern anchovy *Engraulis mordax* and coho salmon *Oncorhynchus kisutch* was cleared from the digestive tracts within several days of exposure, but remained in some tissues for up to one week (Lefebvre et al. 2001, 2007). Paralytic shellfish toxins (specifically N-sulfocarbamoyl-11-hydroxysulfate toxins) were retained in fish as long as two weeks after exposure (Kwong et al. 2006). Conversely, some species of cephalopod appear to hold domoic acid for several months after algal blooms (Lage et al. 2012, Lopes et al. 2013); however, in my study, only two winter scat samples containing cephalopod remains also contained toxin (saxitoxin only). Although clearance rates of domoic acid and saxitoxin have not been studied directly in sea lions, in other mammal species clearance of these toxins typically occurs within 24 – 48 hours (Truelove et al. 1997, Andrinolo et al. 1999, Wittmaack et al. 2015). Therefore, it is unlikely that the toxins were retained in the prey or sea lion tissues for extended periods.

It is more likely that detection of domoic acid and saxitoxin in scat year-round was due to transfer from the benthic to the pelagic food web. Both domoic acid (Lefebvre et al. 2002, Vigilant & Silver 2007, Kvitek et al. 2008) and saxitoxin (Jensen et al. 2015) have been detected in several benthic flatfish species documented in pinniped diet (Riemer et al. 2011, Scordino et al. 2013, Jensen et al. 2015). Several pathways for transfer of these toxins to the benthos have been proposed. *Pseudo-nitzschia* cells and particulate domoic acid (domoic acid within the cells) sink to the bottom where they accumulate in sediments or degrade and release domoic acid (Vigilant & Silver 2007, Sekula-Wood et al. 2011). Particulate domoic acid may also reach the benthos via fish or invertebrate fecal pellets (Vigilant & Silver 2007). Fish may then ingest domoic acid

from feeding on benthic or epibenthic invertebrates (Vigilant & Silver 2007). Similarly, it is likely that resuspended *Alexandrium* cysts are re-incorporated into the marine food web leading to trophic transfer to top predators (Jensen et al. 2015). Winter saxitoxin toxicity in shellfish is primarily attributed to cysts, known to contain high concentrations of saxitoxin (Schwinghamer et al. 1994, Wyatt & Jenkinson 1997, Bricelj & Shumway 1998). Cysts can be resuspended into the water column by wave action of winter storms (Kirn et al. 2005, Butman et al. 2014, Feifel et al. 2015). The overlap of deposited cells or cyst hotspots with the prey and foraging areas of marine mammals likely leads to winter saxitoxin exposure.

The exact pathways of benthic to pelagic food web transfer of toxins, whether from sediments or from resuspended cysts and plankton, are unknown in this study. Consumption of flatfishes (order Pleuronectiformes) dropped sharply in the winter (Table 7), ruling out a strictly benthic feeding pathway. The most common prey items in winter scats containing saxitoxin were rockfish *Sebastes* spp. (22/31 scats) and non-juvenile salmon *Oncorhynchus* spp. (19/31 scats). Although about half of scats (16/31 scats) also contained clupeids (primarily unidentified or Pacific herring *Clupea pallasii*), herring and other clupeids typically fast in the winter (Paul et al. 1998, Foy & Paul 2011). The most common prey items in winter scats containing domoic acid (n = 5) were salmon, skates (Rajidae family), Pacific spiny dogfish *Squalus suckleyi*, and Pacific herring. Given that many of these species feed in the pelagic zone (Eschmeyer et al. 1996, Love 1996) and that the primary toxin detected in winter in this study was saxitoxin, it is likely that toxin exposure occurred through the resuspension of *Alexandrium* cysts into the water column (and thus into the marine food web) due to winter storms and wave action.

Lefebvre et al. (2002) propose that salmon and rockfish feeding on krill at the surface or at depth may transport domoic acid up the food chain. Rockfish and salmon recovered in sea lion scats cannot be easily identified to species without otoliths (which are uncommonly recovered) or through molecular genetic techniques (Tollit et al. 2003, Purcell et al. 2004). However, it is most likely that the salmon in these scats were chinook *Oncorhynchus tshawytscha* or coho *Oncorhynchus kisutch*, which are the primary species caught in winter fisheries (Pressey 1953, Erickson & Pikitch 1994). Based on the presence of other schooling fish (i.e. salmon, dogfish, and herring; Love 1996), the rockfish in these scats may also represent pelagic schooling species such as yellowtail rockfish *Sebastes flavidus* or widow rockfish *Sebastes entomelas*, which are common on the Washington coast (Keller et al. 2007). While the diets of salmon and rockfish differ by species, age class, and season, both feed on a variety of copepods, amphipods, and euphausiids as well as on juvenile and adult planktivorous fishes (Prakash 1962, Manzer 1968, Brodeur & Pearcy 1984, Brodeur et al. 1987). Thus, it is not possible based on this study alone to confirm by which of many possible pathways these fish acquired and transferred the toxins up the food chain to the sea lions.

In Steller sea lions, domoic acid was more consistently found at high concentrations in the summer and at the Carroll/Sea Lion Rock complex (Figure 6). The latter finding was somewhat surprising given the close proximity of Tatoosh Island to the Juan de Fuca Eddy. Domoic acid is detected in the Juan de Fuca Eddy throughout the summer, with the highest concentrations both in the eddy and in nearshore shellfish typically occurring in the fall (Trainer 2002, Trainer et al. 2009). If Steller sea lions fed in the vicinity of the eddy during the summer, then domoic acid would be detected earlier in

scats than in nearshore shellfish. Although it is unknown to what extent sea lions at either haulout foraged in or near the eddy, it is possible that the release of eddy waters to the southeast (MacFadyen et al. 2005, 2008, Trainer et al. 2009) allowed for domoic acid to enter the food chain closer to Carroll/Sea Lion Rock.

In contrast, there was no consistent season when saxitoxin was present in scats and there was no evidence that either haulout complex had consistently greater saxitoxin exposure (Figure 6). This lack of pattern likely relates to differences in both bloom dynamics and sea lion diet. Based on concentrations in nearshore bivalves over the same time period, *Alexandrium* blooms appeared higher in the summer and fall in year 2 compared to year 1 (Figure 9), which could, in part, account for the differences in saxitoxin prevalence by season and location. However, in general there were more detections and higher concentrations of saxitoxin in the scats in year 1 compared to year 2 (Figures 3 and 4). Further, there was no bloom apparent in nearshore shellfish in spring of year 1 (Figure 9), suggesting the occurrence of a bloom outside of the sampled area or the existence of another factor, such as diet (discussed in detail below), may have been a greater contributor to exposure.

Diet Influences on Toxin Prevalence

Based on overall diet analyses using non-metric multidimensional scaling (NMDS), there was no apparent separation in diet when considering presence/absence of toxin (Figure 7) or toxin concentrations (Figure 8). Although the NMDS did not

differentiate between season, year, or location, all of which may impact diet (Sinclair & Zeppelin 2002, McKenzie & Wynne 2008, Riemer et al. 2011, Scordino et al. 2013), the lack of a convergent solution and lack of spatial groups in the NMDS plots (showing best solution after 20 iterations) is indicative of the diversity of sea lion diet (Tables 7 and 8).

Most compelling in this study is evidence that both walleye pollock (hereafter pollock) and American shad (hereafter shad) may be vectors of saxitoxin exposure in Steller sea lions (Tables 6 and 8). Saxitoxin was detected in all scats containing pollock (n = 18 scats) and in most scats containing shad (13/18 scats). Because both species had relatively low frequency of occurrence in sea lion diet overall (Table 7), this finding requires some scrutiny. The occurrence of pollock is primarily traced to a single collection day (18 May 2011) at Tatoosh Island complex, where 17/17 scats were positive for saxitoxin; 15 of these contained pollock. The two remaining scats without pollock contained unidentified codfish bones, which could have also been pollock bones that were either degraded by digestion or were structures that could not be identified to species. The majority of these scats also contained spiny dogfish (14/15), however only 46 % of all scats containing dogfish had detectable saxitoxin. Six of these 15 scats also contained Pacific herring (1 scat) or unidentified clupeids (5 scats). Pollock was also found in three other scat samples in this study and saxitoxin was detected in all three samples. Scats with pollock present were significantly more likely to have medium (20-50 ng g^{-1}) or high (>50 ng g^{-1}) concentrations of saxitoxin. Scats with pollock contained two of the highest concentrations of saxitoxin measured (214.4 ng g^{-1} and 273.6 ng g^{-1}) and were the driver of the high prevalence and concentrations of saxitoxin in spring 2011 (Figure 6).

If pollock are indeed a vector of saxitoxin (or other algal toxins) to Steller sea lions, this could be a significant problem for the western DPS sea lions that rely on this fish in their diet. Pollock are a mid-water to benthic species that eat a variety of zooplankton and other fishes (Eschmeyer et al. 1996, Love 1996, Brodeur 1998) and are a common prey species in Steller sea lion diet in the eastern Aleutian Islands, Gulf of Alaska, and southeast Alaska (Merrick et al. 1997, Sinclair & Zeppelin 2002, Bredesen et al. 2006, Sigler et al. 2009). The low occurrence of pollock in scats in this study likely relates to their overall distribution, which is highest in the Bering Sea and Gulf of Alaska and lower along the coast of Washington and Oregon (Bredesen et al. 2006). Pollock were caught in low numbers in bottom trawl surveys on the Washington coast (Keller et al. 2007) and are caught infrequently as bycatch in tribal bottom trawl fisheries primarily around Swiftsure Bank (pers. comm. Joe Petersen[1]), a high spot offshore the Strait of Juan de Fuca. The proximity of Tatoosh Island to this location provides a likely explanation for why the majority of scats with pollock were from the Tatoosh Island complex. Although only low concentrations of domoic acid and saxitoxin have been documented in stranded Steller sea lions in Alaska (Lefebvre et al. 2016), further assessment of algal toxins levels in free-ranging sea lions is warranted. Given that several of the animals tested by Lefebvre et al. (2016) were pups or aborted fetuses and that stranded animals may not be foraging normally (Jensen 2015), the concentrations reported in those stranded sea lions may not represent exposure to live animals.

---

[1] Joe Peterson, Groundfish Biologist, Makah Fisheries Management, Neah Bay, WA, March 11, 2016

It is possible that shad also acted as a vector of saxitoxin to Steller sea lions in this study. Scats with shad (n = 18) were collected on a total of 10 collection days, in spring, fall, and winter of both years. These scats contained one to four additional prey items from six different family groups, therefore it cannot be ruled out that the presence of toxin was associated with another prey item. American shad eat zooplankton and small fish (Love 1996) that may be directly ingesting toxic phytoplankton, but neither toxin has previously been documented in shad. Scats with shad were associated with low concentrations of the toxin (<20 ng g^{-1}), suggesting that although they may have been vectors of the toxin, they resulted in relatively low toxin exposure.

Possible vectors identified for domoic acid were Pacific sardine *Sardinops sagax* and starry flounder *Platichthys stellatus* in Steller sea lions and northern anchovy in California sea lions (Tables 6 and 8). Domoic acid was detected in 57 % of Steller sea lion scats containing sardine (17/36 scats). These scats were collected in summer and fall of both years and contained one to seven other prey items. Scats with sardine included several of the highest concentrations of domoic acid detected in this study, including one sample with 423 ng g^{-1}. Given that sardine is a known vector of domoic acid and cause of marine mammal mortalities (Scholin et al. 2000) and that sardine may feed directly on the toxin-containing diatoms (Costa & Garrido 2004), it is likely that this species was, at least in part, responsible for the detection of domoic acid in these scats.

Another possible vector for domoic acid was starry flounder, with 36% of scats containing starry flounder (13/36) also containing detectable levels of domoic acid. These scats contained one to seven other prey, most commonly skates and dogfish. Starry flounder are typically found nearshore in shallow waters in the spring and summer and

move offshore in the winter, primarily feeding on benthic mollusks, crustaceans, and fishes (Eschmeyer et al. 1983, Love 1996). These fish were found almost exclusively in scats from the Carroll/Sea Lion Rock complex, collected in spring, summer, and fall of both years. Given that starry flounder are not likely to be found in the offshore areas of the Juan de Fuca Eddy during the summer when domoic acid is most prevalent, this may be further evidence that waters escaping the eddy to the southeast influence the benthic marine food web in nearshore environments.

Northern anchovy was likely a vector of domoic acid in California sea lions, with domoic acid detected in 6 of 14 samples that contained anchovy. Domoic acid was detected in scats primarily in November 2011 (5/6 scats), but scats contained several other prey species including herring, sardine, and shad. Being a known vector of domoic acid (Lefebvre et al. 2002) it is possible that anchovy did expose sea lions to domoic acid in this case. However, the small sample size suggests that this species did not provide significant exposure, and the presence of other planktivorous prey species make it impossible to know whether one or more prey species contained the toxin.

Other prey species that were identified as possible vectors of toxin exposure, though less convincingly, include non-juvenile salmon and spiny dogfish (Tables 6 and 7). In Steller sea lions, about 53% of scats containing non-juvenile salmon also had detectable levels of saxitoxin, with the highest prevalence in summer and fall. In California sea lions, only 43% of scats containing non-juvenile salmon also contained saxitoxin. Similarly, domoic acid in Steller sea lion scats was positively associated with presence of spiny dogfish, although only 24% of scats (26/108 scat) containing dogfish were positive for the toxin. These scats were collected in all seasons of both years and

contained one to seven other prey items, most commonly skates (17/26 scats) and clupeids (19/26 scats). Although algal toxin accumulation has been documented in salmon (Lefebvre et al. 2007, Sephton et al. 2007), toxins in elasmobranch species have not been reported in the wild (Vigilant & Silver 2007). Further, leopard sharks *Triakis semifasciata* given oral doses of domoic acid showed low retention of the toxin (Schaffer et al. 2006). Given the variety of other prey species in these scats, it is difficult to confirm that either non-juvenile salmon or dogfish were specifically related to the presence of toxin. The diversity of prey associated with toxins in this study, including planktivorous forage fish, benthic flatfish, and higher-tropic pelagic species, suggests that a variety of pathways lead to HAB toxin exposure in these top-predators.

Comparison to Nearshore Bivalves

In this and previous studies (Goldstein et al. 2008, Torres de la Riva et al. 2009), sea lions appear to act as indicators of offshore blooms, such as those occurring in the Juan de Fuca Eddy, before they are apparent in the nearshore waters (Figures 9 and 11). Therefore, nearshore monitoring of phytoplankton and particulate toxins likely underrepresents toxin exposure in marine mammals. Other studies have found evidence for co-occurrence of the toxins in marine mammals and in bivalves (Fire et al. 2010) and for a lack of correlation between the two (Scholin et al. 2000, Bargu et al. 2010). One study found significant correlations between marine mammal strandings and toxic phytoplankton blooms at several time scales, both before and after *Pseudo-nitzschia*

blooms were otherwise detected (Torres de la Riva et al. 2009). In my study, the detection of both saxitoxin and domoic acid in nearshore shellfish peaked in summer and fall of both years (Figure 10). Although the comparison of domoic acid in scats to that in bivalves was limited, sea lions appeared to carry the toxin somewhat prior to the spikes seen in nearshore shellfish (Figure 11). This precedence likely indicates that the sea lions were either foraging in areas of blooms, such as the Juan de Fuca Eddy, prior to those waters making it nearshore in the fall, or could indicate that their prey species travelled through or foraged in these areas.

The comparison between saxitoxin in bivalves and sea lions was more complex. High concentrations of saxitoxin were detected in sea lion scat (>200 ng g^{-1}) prior to concentrations in bivalves reaching levels above the regulatory limit, such as in spring 2011 (Appendix 1, Figure C). Similarly, in periods of relatively high concentrations in bivalves, such as in the summer and fall (Appendix 1, Figures C and O), some scats had medium and high concentrations (up to 50-100 ng g^{-1}), but most scats were below detection. The exception was California sea lions, which experienced high levels of saxitoxin (>200 ng g^{-1}) at the same time as very high concentrations (~12,000 ng g^{-1}) were detected in bivalves (Appendix 1, Figure Q). Of particular interest is the occurrence of saxitoxin in sea lion scat when no to low (<380 ng g^{-1}) saxitoxin was detected in bivalves, as seen in winter and spring (e.g. Appendix 1, Figures J- L), perhaps further confirming that winter toxin exposure in sea lions is through benthic pathways rather than active blooms. If this exposure resulted from *Alexandrium* cysts being resuspended into the water column, it is possible that this occurred outside of areas that are presently

83

sampled for bivalves, and thus was not detected in the bivalves, or it is possible the cysts re-entered the food web through another pathway.

Study Limitations

Despite many compelling findings, there are several limitations of diet analyses including how prey are reported in the diet, how the different prey species are digested, and how the toxins are metabolized. Frequency of occurrence (FO) is a commonly used metric for describing diet in pinnipeds (Lance et al. 2001), but has several limitations. FO describes the presence or absence of a prey item without enumerating the number of fish eaten or estimating volume or biomass of eaten prey. Scat analysis in general has known biases related to identifying prey structures, degradation of bones in digestion, and different passage and recovery rates of hard parts of fish ingested (Orr & Harvey 2001, Cottrell & Trites 2002, Tollit et al. 2003, 2007). In general, smaller fish species may pass through digestion more quickly compared to larger prey that may take several days for bones to pass (Orr & Harvey 2001, Tollit et al. 2007).

Because both saxitoxin and domoic acid are typically flushed from the digestive system quickly, it is likely that toxin in scats are from a recent feeding event. Although this study incorporated the use of all prey structures, which improves the diet analysis result (Lance et al. 2001, Browne et al. 2002, Cottrell & Trites 2002), it is possible that the hard parts in a single scat do not represent all fish eaten or do not represent all fish from the most recent meal. Given that multiple fish species have been documented as

having domoic acid and saxitoxin and that sea lion diet is very diverse (McKenzie &
Wynne 2008, Riemer et al. 2011), it is difficult to infer which prey item or items actually
held the toxin. My study incorporated anonymous scats from mixed demographic haulout
sites and thus did not account for differences in diet and foraging behavior by age or sex
(Merrick & Loughlin 1997, Trites & Calkins 2008, Orr et al. 2011), which in turn can
affect exposure to HAB toxins (Silvagni et al. 2005, Bejarano et al. 2008). Lastly, there is
no literature on what effects weathering might have on the toxin content in scats
deposited on these rock and beach haulout sites. Therefore, the toxin concentrations in
this study are assumed to represent the full amount at the time of deposition.

Consequences of Toxin Exposure

Despite evidence of year-round exposure, toxin concentrations measured in this
study are not likely to have caused acute illness. Marine mammal strandings in
Washington State due to acute HAB toxicity have not been yet confirmed. Northern fur
seals *Callorhinus ursinus* stranded in Washington and Oregon in the 1980's exhibited
clinical signs of domoic acid toxicity, however these cases occurred before the toxin was
recognized to cause health effects in marine mammals and was not confirmed as the
cause of the strandings (Lefebvre et al. 2010). Stranded Steller sea lions in Washington
have been tested for domoic acid, but only low concentrations have been found (Wiles
2015). Low levels of saxitoxin were documented previously in Washington in three live
sea otters (White et al. 2013), but testing for saxitoxin has not been done in other species.

Domoic acid concentrations in this study were well below those measured in scat from California sea lions and harbor seals exhibiting acute domoic acid toxicity (Lefebvre et al. 1999, McHuron et al. 2013), but were comparable to the lower range of concentrations in scat of California sea lions with acute and chronic symptoms reported by Goldstein et al. (2008). The concentrations were higher than those measured in stranded, harvested, and live-captured pinnipeds in Alaska (Lefebvre et al. 2016), but significantly lower than maximum concentrations in scat reported in stranded northern fur seals in Alaska (Lefebvre et al. 2010) and free-ranging harbor seals in Scotland (Jensen et al. 2015).

It is not known what concentrations of saxitoxin cause mortality in marine mammals. In humpback whales and Mediterranean monk seals affected by saxitoxin, samples of feces and other body fluids were not available for comparison to this study. Humpback whales were estimated to have ingested about 3,200 ng saxitoxin kg^{-1} body weight (Anderson & White 1989), which is much higher than any concentration measured in this study. Concentrations of saxitoxin in my study were higher than any reported in 13 marine mammals species sampled in Alaska (Lefebvre et al. 2016), but within the range of saxitoxin measured in feces of free-ranging and stranded harbor seals in Scotland (Jensen et al. 2015) and in free-ranging North Atlantic right whales in the Bay of Fundy, Canada (Doucette et al. 2006).

Estimating the doses of toxin that cause toxicity in marine mammals is difficult and comparing scat concentrations to toxicity is complicated by the fact that feces does not necessarily represent the concentrations absorbed by the digestive tract. While concentrations in feces represent exposure, measuring toxins in urine or serum is more

indicative of the amounts metabolized (Fire et al. 2009, Lefebvre et al. 2010). In other

studies, concentrations of domoic acid measured in feces were frequently orders of

magnitude higher than those measured in the urine or serum (Scholin et al. 2000,

Goldstein et al. 2008, Fire et al. 2009, 2010, Lefebvre et al. 2010, McHuron et al. 2013).

Saxitoxin concentrations similar to those in this study were reported in the feces of harbor

seals in Scotland; however, no saxitoxin was detected in the urine (Jensen 2015).

Whether the lack of detection in urine could be related to low toxin exposure and thus

low absorption across the digestive tract was not discussed. Therefore, it is difficult to

infer what urine concentrations, and thus metabolized concentrations, might have been

present in the sea lions studied here.

The concentrations measured in feces in this study likely indicate very low

concentrations metabolized into tissues. Although sea lions have a high digestion

efficiency of fish (>90%, Rosen & Trites 2000), the assimilation efficiency of these

HABs toxins is not known. Absorption of toxins across the digestive tract differs greatly

between mammal species (Lefebvre et al. 2002). In terrestrial mammal species, only

about 4-7% of ingested domoic acid is absorbed across the digestive tract (Truelove et al.

1997). The amount of saxitoxin absorbed by the digestive tract has not been reported in

mammals, however there is evidence in both humans (García et al. 2004) and whales

(Doucette et al. 2006) of biotransformation of saxitoxin into other derivatives.

Although the concentrations measured in this study were low, massive coast-wide

algal blooms occurred throughout the northern California Current in the spring and

summer of 2015, leading to high concentrations of both domoic acid and saxitoxin and to

coast-wide shellfish closures (Peterson et al. 2013). The results of my study could have

been drastically different had they included a year with large algal blooms such as those observed in 2015 and could have lead to different detection rates of the toxins and potentially health-threatening levels. Future blooms of similar magnitude could have profound effects on the health of sea lions on the Washington coast.

Conclusions

This study presents evidence of chronic low-level exposure to saxitoxin and domoic acid in Steller and California sea lions. While chronic exposure to domoic acid has been well documented in California sea lions (Goldstein et al. 2008), it is unclear what concentrations induce chronic toxicity and how those concentrations compare to the concentrations to which sea lions in Washington may be exposed. In addition to epileptic-like symptoms, one consequence of chronic exposure to domoic acid is compromised navigational ability, which can affect foraging and migration behavior (Thomas et al. 2010, Cook et al. 2015). Chronic saxitoxin toxicity has not been documented, although several authors have proposed possible effects. Durbin et al. (2002) suggest that chronic exposure to saxitoxin may affect diving capability or result in greater susceptibility to disease. Bogomolni et al. (2016) suggest that saxitoxin may increase the risk of morbillivirus and phocine distemper virus in harbor seals.

What effects either chronic or acute algal toxin exposure may have at the population level remain unknown. While Jensen et al. (2015) explored the possibility that HAB toxins may be related to the decline in harbor seals in Scotland, they observed no

apparent toxicity in any of the animals tested. Despite the rising number of strandings of California sea lions due to domoic acid toxicity (Bejarano et al. 2008), the population has been steadily increasing (Carretta et al. 2014). Given that female California sea lions are disproportionally affected by acute domoic acid toxicity (Silvagni et al. 2005, Bejarano, Van Dolah, et al. 2008) and that domoic acid can cause reproductive failure in California sea lions (Brodie et al. 2006, Goldstein et al. 2009), this toxin puts a more sensitive part of the population at risk.

While the reasons for the decline in western DPS Steller sea lions remain unknown (NRC 2003), it is evident that both domoic acid and saxitoxin are present in the Arctic food web and could play a role (Lefebvre et al. 2016). Given the small sample size in this study, the implication that a dominant prey resource of the western DPS population (i.e. walleye pollock) may be a vector of saxitoxin exposure warrants further study. This study also found evidence that prey with relatively low occurrence in the diet may act as vectors of significant algal toxin transfer up the food chain. Therefore, generalist predators with a more diverse diet may not have any respite from exposure to, or the effects of, marine algal toxins as compared to predators that specialize. Sampling at a finer timescale (e.g. weekly or monthly) may be necessary for understanding the role of infrequent prey species in transfer of algal toxins to top predators. This study confirms for the first time that marine mammals can be exposed to algal toxins through their prey outside of active algal bloom time periods and that benthic to pelagic food web transfer of precipitated cells and dormant cysts is the most likely cause.

LITERATURE CITED

Agresti, A (2007) An introduction to categorical data analysis, second edition. John Wiley & Sons, Inc, Hoboken, NJ

American Public Health Association (APHA) (1980) Recommended procedures for the examination of water and shellfish, fourth edition. APHA, New York, NY

Anderson DM (1998) Physiology and bloom dynamics of toxic Alexandrium species, with emphasis on life cycle transitions. NATO ASI Ser G Ecol Sci 41:29–48

Anderson CR, Brzezinski MA, Washburn L, Kudela R (2006) Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. Mar Ecol Prog Ser 327:119–133

Anderson DM, Burkholder JM, Cochlan WP, Gilbert PM, Gobler CJ, Heil CA, Kudela R, Parsons ML, Rensel JEJ, Townsend DW, Trainer VL, Vargo GA (2008) Harmful algal blooms and eutrophication: Examining linkages from selected coastal regions of the United States. Harmful Algae 8:39–53

Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: nutrient sources, compositions, and consequences. Estuaries 25:704–726

Anderson DM, White A (1989) Toxic dinoflagellates and marine mammal mortalities. Proceedings of an expert consultation held at the Woods Hole Oceanographic Institution. Woods Hole Oceanographic Institute Tech Rep WHOI-89-36

Andrinolo D, Michea LF, Lagos N (1999) Toxic effects, pharmacokinetics and clearance of saxitoxin, a component of paralytic shellfish poison (PSP), in cats. Toxicon 37: 447-464

Antonelis GA, Fiscus CH (1980) The pinnipeds of the California Current. CalCOFI Rep XXI:68–78

Association of Official Agricultual Chemists (AOAC) (1965) Biological Method (18) In: Horowitz W (eds) Official methods of analysis of the association of official agricultural chemists, tenth edition. AOAC, Washington, DC, p 282-284

Backer LC, McGillicuddy DJ (2006) Harmful algal blooms at the interface between coastal oceanography and human health. Oceanography 19:94–106

Bargu S, Powell C, Coale S, Busman M, Doucette G, Silver M (2002) Domoic acid detection in krill: a potential vector in marine food webs. Mar Ecol Prog Ser 237:209–219

Bargu S, Silver M, Goldstein T, Roberts K, Gulland F (2010) Complexity of domoic acid-related sea lion strandings in Monterey Bay, California: Foraging patterns, climate events, and toxic blooms. Mar Ecol Prog Ser 418:213–222

Bates S, Garrison D, Horner R (1998) Bloom dynamics and physiology producing *Pseudo-nitzschia* species. NATO ASI Ser G Ecol Sci 41:267–292

Bejarano AC, Van Dolah FM, Gulland FM, Rowles TK, Schwacke LH (2008) Production and toxicity of the marine biotoxin domoic acid and its effects on wildlife: A review. Hum Ecol Risk Assess 14:544–567

Bejarano AC, Van Dolah FM, Gulland FM, Schwacke L (2007) Exposure assessment of the biotoxin domoic acid in California sea lions: Application of a bioenergetic model. Mar Ecol Prog Ser 345:293–304

Bejarano AC, Gulland FM, Goldstein T, St Leger J, Hunter M, Schwacke LH, Van Dolah FM, Rowles TK (2008) Demographics and spatio-temporal signature of the biotoxin domoic acid in California sea lion (*Zalophus californianus*) stranding records. Mar Mammal Sci 24:899–912

Bogomolni AL, Bass AL, Fire S, Jasperse L, Levin M, Nielsen O, Waring G, De Guise S (2016) Saxitoxin increases phocine distemper virus replication upon in-vitro infection in harbor seal immune cells. Harmful Algae 51:89–96

Bossart G (2011) Marine mammals as sentinel species for oceans and human health. Vet Pathol 48:676–690

Bredesen EL, Coombs AP, Trites AW (2006) Relationship between Steller sea lion diets and fish distributions in the eastern North Pacific. In: Trites A, Atkinson S, DeMaster D, Fritz L, Gelatt T, Rea L, Wynne K (eds) Sea Lions of the World. Alaska Sea Grant College Program, University of Alaska Fairbanks, Fairbanks, p 131–140

Bricelj VM, Shumway SE (1998) Paralytic shellfish toxins in bivalve molluscs: Occurrence, transfer kinetics, and biotransformation. Rev Fish Sci 6:315–383

Brodeur R (1998) Prey selection by age-0 walleye pollock, *Theragra chalcogramma*, in nearshore waters of the Gulf of Alaska. Environ Biol Fishes 51:175–186

Brodeur RD, Lorz H V, Pearcy WG (1987) Food habits and dietary variability of pelagic nekton off Oregon. NOAA Tech Rep NMFS 57

Brodeur RD, Pearcy WG (1984) Food habits and dietary overlap of some shelf rockfishes (genus *Sebastes*) from the northeastern Pacific Ocean. Fish Bull 82:269–293

Brodie EC, Gulland FMD, Greig DJ, Hunter M, Jaakola J, St. Leger J, Leighfield TA, Van Dolah FM (2006) Domoic acid causes reproductive failure in California sea lions (*Zalophus californianus*). Mar Mammal Sci 22:700–707

Browne P, Laake JL, DeLong RL (2002) Improving pinniped diet analyses through identification of multiple skeletal structures in fecal samples. Fish Bull 100:423–433

Buckmaster PS, Wen X, Toyoda I, Gulland FMD, Van Bonn W (2014) Hippocampal neuropathology of domoic acid-induced epilepsy in California sea lions (*Zalophus californianus*). J Comp Neurol 522:1691–1706

Butman B, Aretxabaleta AL, Dickhudt PJ, Dalyander PS, Sherwood CR, Anderson DM, Keafer BA, Signell RP (2014) Investigating the importance of sediment resuspension in *Alexandrium fundyense* cyst population dynamics in the Gulf of Maine. Deep Res Part II Top Stud Oceanogr 103:79–95

Carretta J, Oleson E, Weller D, Lang A, Forney K, Baker J, Muta M, Hanson B, Orr A, Huber H, Lowry M, Barlow J, Moore J, Lynch D, Carswell L, Brownell Jr R (2014) U.S. Pacific Marine Mammal Stock Assessments: 2014 NOAA Tech Memo NMFS SWFSC-549

Cook PF, Reichmuth C, Rouse AA, Libby LA, Dennison SE, Carmichael OT, Kruse-Elliott KT, Bloom J, Singh B, Fravel VA, Barbosa L, Stuppino JJ, Van Bonn WG, Gulland FMD, Ranganath C (2015) Algal toxin impairs sea lion memory and hippocampal connectivity, with implications for strandings. Ecotoxicology 350:1545-1547

Costa PR, Garrido S (2004) Domoic acid accumulation in the sardine. Mar Ecol Prog Ser 284:261–268

Cottrell P, Trites AW (2002) Classifying prey hard part structures recovered from fecal remains of captive Steller sea lions (*Eumetopias jubatus*). Mar Mammal Sci 18:525–539

Doucette GJ, Cembella AD, Martin JL, Michaud J, Cole TVN, Rolland RM (2006) Paralytic shellfish poisoning (PSP) toxins in North Atlantic right whales *Eubalaena glacialis* and their zooplankton prey in the Bay of Fundy, Canada. Mar Ecol Prog Ser 306:303–313

Durbin E, Teegarden G, Campbell R, Cembella A, Baumgartner MF, Mate BR (2002) North Atlantic right whales, *Eubalaena glacialis*, exposed to paralytic shellfish poisoning (PSP) toxins via a zooplankton vector, *Calanus finmarchicus*. Harmful Algae 1:243–251

Dyson K, Huppert DD (2010) Regional economic impacts of razor clam beach closures due to harmful algal blooms (HABs) on the Pacific coast of Washington. Harmful Algae 9:264–271

Edgell TC, Demarchi MW (2012) California and Steller sea lion use of a major winter haulout in the Salish Sea over 45 years. Mar Ecol Prog Ser 467:253–262

Erickson DL, Pikitch EK (1994) Incidental catch of chinook salmon in commercial bottom trawls off the US West Coast. North Am J Fish Manag 14:550–563

Eschmeyer WN, Herald ES, Hammann H (1983) Peterson Field Guide Series: A field guide to Pacific coast fishes of North America. Houghton Mifflin Company, Boston, MA

Feifel KM, Fletcher SJ, Watson LR, Moore SK, Lessard EJ (2015) *Alexandrium* and *Scrippsiella* cyst viability and cytoplasmic fullness in a 60-cm sediment core from Sequim Bay, WA. Harmful Algae 47:56–65

Fire SE, Van Dolah FM (2012) Marine biotoxins: emergence of harmful algal blooms as health threats to marine wildlife In: Aguirre AA, Ostfeld R, Daszak P (eds) New directions in conservation medicine: applied cases of ecological health. Oxford University Press, New York, p 374-389

Fire S, Flewelling L, Stolen M, Noke Durden W, Wit M de, Spellman A, Wang Z (2015) Brevetoxin-associated mass mortality event of bottlenose dolphins and manatees along the east coast of Florida, USA. Mar Ecol Prog Ser 526:241–251

Fire SE, Wang Z, Berman M, Langlois GW, Morton SL, Sekula-Wood E, Benitez-Nelson CR (2010) Trophic transfer of the harmful algal toxin domoic acid as a cause of death in a minke whale (*Balaenoptera acutorostrata*) stranding in southern California. Aquat Mamm 36:342–350

Fire SE, Wang Z, Leighfield TA, Morton SL, McFee WE, McLellan WA, Litaker RW, Tester PA, Hohn AA, Lovewell G, Harms C, Rotstein DS, Barco SG, Costidis A, Sheppard B, Bossart GD, Stolen M, Durden WN, Van Dolah FM (2009) Domoic acid exposure in pygmy and dwarf sperm whales (*Kogia* spp.) from southeastern and mid-Atlantic U.S. waters. Harmful Algae 8:658–664

Forcada J, Hammond PS, Aguilar A (1999) Status of the Mediterranean monk seal *Monachus monachus* in the western Sahara and the implications of a mass mortality event. Mar Ecol Prog Ser 188:249–261

Foy RJ, Paul AJ (2011) Winter feeding and changes in somatic energy content of Age-0 Pacific herring in Prince William Sound, Alaska. Trans Am Fish Soc 128:1193–1200

García C, del Bravo MC, Lagos M, Néstor L (2004) Paralytic shellfish poisoning: Post-mortem analysis of tissue and body fluid samples from human victims in the Patagonia fjords. Toxicon 43:149–158

Geraci J, Anderson D, Timperi R, St. Aubin DJ, Early G, Prescott J, Mayo C (1989) Humpback whales (*Megaptera novaeangliae*) fatally poisoned by dinoflagellate toxin. Can J Fish Aquat Sci 46:1895–1898

Goldstein T, Calkins D, Atkinson S (2005) Workshop to assess the effects of disease on Steller sea lion populations. In: Loughlin TR, Calkins DG, Atkinson S (eds) Synopsis of research on Steller sea lions: 2001-2005. Alaska Sea Life Center and Sea Script Company, Seattle, p302-308

Goldstein T, Mazet JAK, Zabka TS, Langlois G, Colegrove KM, Silver M, Bargu S, Van Dolah F, Leighfield T, Conrad PA, Barakos J, Williams DC, Dennison S, Haulena M, Gulland FMD (2008) Novel symptomatology and changing epidemiology of domoic acid toxicosis in California sea lions (*Zalophus californianus*): an increasing risk to marine mammal health. Proc Biol Sci 275:267–276

Goldstein T, Zabka TS, Delong RL, Wheeler EA, Ylitalo G, Bargu S, Silver M, Leighfield T, Dolah F Van, Langlois G, Sidor I, Dunn JL, Gulland FMD (2009) The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus Californianus*) on San Miguel Island, California. J Wildl Dis 45:91–108

Gulland F (2000) Domoic acid toxicity in California sea lions (*Zalophus californianus*) stranded along the central California coast, May-October 1998. Report to the National Marine Fisheries Service Working Group on Unusual Marine Mammal Mortality Events. NOAA Tech Memo NMFS-OPR- 17

Hernández M, Robinson I, Aguilar A, González LM, López-Jurado LF, Reyero MI, Cacho E, Franco J, López-Rodas V, Costas E (1998) Did algal toxins cause monk seal mortality? Nature 393:28–29

Hickey BM, Banas NS (2003) Oceanography of the U.S. Pacific Northwest Coastal Ocean and estuaries with application to coastal ecology. Estuaries 26:1010–1031

Holtermann KE, Bates SS, Trainer VL, Odell A, Armbrust EV (2010) Mass sexual reproduction in the toxigenic diatoms *Pseudo-nitzschia australis* and *P. pungens* (bacillariophyceae) on the Washington coast, USA. J Phycol 46:41–52

Horner RA, Garrison DL, Plumley FG (1997) Harmful algal blooms and red tide problems on the U.S. west coast. Limnol Ocean 42:1076–1088

Islam MS, Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Mar Pollut Bull 48:624–649

James KJ, Carey B, O'Halloran J, van Pelt FNAM, Skrabáková Z (2010) Shellfish toxicity: human health implications of marine algal toxins. Epidemiol Infect 138:927–40

Jeffries SJ, Gearin PJ, Huber HR, Saul D, Pruett D (2000) Atlas of seal and sea lion haulout sites in Washington. Washington Department of Fish and Wild life, Wildlife Science Division, 600 Capitol Way North, Olympia WA pp 150

Jensen S-K (2015) Are toxins from harmful algae a factor involved in the decline of harbour seal populations in Scotland? PhD dissertation, University of St. Andrews, St. Andrews, Scotland

Jensen S-K, Lacaze J-P, Hermann G, Kershaw J, Brownlow A, Turner A, Hall A (2015) Detection and effects of harmful algal toxins in Scottish harbour seals and potential links to population decline. Toxicon 97:1–14

Jester RJ, Baugh KA, Lefebvre KA (2009) Presence of *Alexandrium catenella* and paralytic shellfish toxins in finfish, shellfish and rock crabs in Monterey Bay, California, USA. Mar Biol 156:493–504

Keller AA, Horness BH, Simon VH, Tuttle V., Wallace JR, Fruh EL, Bosley KL, Kamikawa DJ, Buchanan JC (2007) The 2004 U.S. West Coast bottom trawl survey of groundfish resources off Washington, Oregon, and California: estimates of distribution, abundance, and length composition. NOAA Tech Memo NMFS-NWFSC-87

Kirn SL, Townsend DW, Pettigrew NR (2005) Suspended *Alexandrium* spp. hypnozygote cysts in the Gulf of Maine. Deep Res Part II 52:2543–2559

Kreuder C, Miller MA, Jessup DA, Lowenstine LJ, Harris MD, Ames JA, Carpenter TE, Conrad PA, Mazet JAK (2003) Patterns of mortality in southern sea otters (*Enhydra lutris nereis*) From 1998 – 2001. J Wildl Dis 39:495–509

Kruskal JB (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. Psychometrika 29:1–27

Kvitek RG, Goldberg JD, Smith GJ, Doucette GJ, Silver MW (2008) Domoic acid contamination within eight representative species from the benthic food web of Monterey Bay, California, USA. Mar Ecol Prog Ser 367:35–47

Kwong RWM, Wang WX, Lam PKS, Yu PKN (2006) The uptake, distribution and elimination of paralytic shellfish toxins in mussels and fish exposed to toxic dinoflagellates. Aquat Toxicol 80:82–91

Lage S, Raimundo J, Brotas V, Costa PR (2012) Detection and sub-cellular distribution of the amnesic shellfish toxin, domoic acid, in the digestive gland of *Octopus vulgaris* during periods of toxin absence. Mar Biol Res 8:784-789

Lance MM, Orr AJ, Riemer SD, Weise MJ, Laake JL (2001) AFSC Processed Rep 2001–04: Pinniped food habits and prey identification techniques protocol. Alaska Fisheries Science Center, National Marine Fisheries Service, Seattle, WA

Landsberg JH (2002) The effects of harmful algal blooms on aquatic organisms. Rev Fish Sci 10:113–390

Law RJ, Alaee M, Allchin CR, Boon JP, Lebeuf M, Lepom P, Stern GA (2003) Levels and trends of polybrominated diphenylethers and other brominated flame retardants in wildlife. Environ Int 29:757–770

Lefebvre, KA, Powell CL, Busman M, Doucette GJ, Moeller PDR, Silver JB, Miller PE, Hughes MP, Singaram S, Silver MW, Tjeerdema RS (1999) Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. Nat Toxins 7:85–92

Lefebvre KA, Bargu S, Kieckhefer T, Silver MW (2002) From sandabs to blue whales: the pervasiveness of domoic acid. Toxicon 40:971–977

Lefebvre KA, Dovel SL, Silver MW (2001) Tissue distribution and neurotoxic effects of domoic acid in a prominent vector species, the northern anchovy *Engraulis mordax*. Mar Biol 138:693–700

Lefebvre KA, Noren DP, Schultz IR, Bogard SM, Wilson J, Eberhart BL (2007) Uptake, tissue distribution and excretion of domoic acid after oral exposure in coho salmon (*Oncorhynchus kisutch*). Aquat Toxicol 81:266–274

Lefebvre KA, Quakenbush L, Frame E, Huntington KB, Sheffield G, Stimmelmayr R, Bryan A, Kendrick P, Ziel H, Goldstein T, Snyder JA, Gelatt T, Gulland F, Dickerson B, Gill V (2016) Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment. Harmful Algae 55:13–24

Lefebvre KA, Robertson A, Frame ER, Colegrove KM, Nance S, Baugh KA, Wiedenhoft H, Gulland FMD (2010) Clinical signs and histopathology associated with domoic acid poisoning in northern fur seals (*Callorhinus ursinus*) and comparison of toxin detection methods. Harmful Algae 9:374–383

Lefebvre KA, Silver MW, Coale SL, Tjeerdema RS (2002) Domoic acid in planktivorous fish in relation to toxic Pseudo-nitzschia cell densities. Mar Biol 140:625–631

Lopes VM, Lopes AR, Costa P, Rosa R (2013) Cephalopods as vectors of harmful algal bloom toxins in marine food webs. Mar Drugs 11:3381-3409

Love MS (1996) Probably more than you want to know about the fishes of the Pacific coast. Really Big Press, Santa Barbara, CA

Lowry MS, Stewart BS, Heath CB, Yochem PK, Francis JM (1991) Seasonal and annual variability in the diet of California sea lions Zalophus californianus at San Nicolas Island, California, 1981-86. Fish Bull 89:331–336

MacFadyen A, Hickey BM, Cochlan WP (2008) Influences of the Juan de Fuca Eddy on circulation, nutrients, and phytoplankton production in the northern California Current System. J Geophys Res Ocean 113:1–19

MacFadyen A, Hickey BM, Foreman MGG (2005) Transport of surface waters from the Juan de Fuca eddy region to the Washington coast. Cont Shelf Res 25:2008–2021

Maniscalco JM, Wynne K, Pitcher KW, Hanson MB, Melin SR, Atkinson S (2004) The occurrence of California sea lions (*Zalophus californianus*) in Alaska. Aquat Mamm 30:427–433

Manzer JI (1968) Food of Pacific salmon and steelhead trout in the Northeast Pacific Ocean. J Fish Res Board Canada 25:1085–1089

Marchetti A, Trainer VL, Harrison PJ (2004) Environmental conditions and phytoplankton dynamics associated with *Pseudo-nitzschia* abundance and domoic acid in the Juan de Fuca eddy. Mar Ecol Prog Ser 281:1–12

McHugh ML (2012) The Chi-square test of independence. Biochem Medica 23:143–149

McHuron E, Greig DJ, Colegrove KM, Fleetwood M, Spraker TR, Gulland FMD, Harvey JT, Lefebvre KA, Frame ER (2013) Domoic acid exposure and associate clinical signs and histopathology in Pacific harbor seals (*Phoca vitulina richardii*). Harmful Algae 23:28–33

McKenzie J, Wynne KM (2008) Spatial and temporal variation in the diet of Steller sea lions in the Kodiak Archipelago, 1999 to 2005. Mar Ecol Prog Ser 360:265–283

Merrick RL, Chumbley MK, Byrd GV (1997) Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Can J Fish Aquat Sci 54:1342–1348

Merrick RL, Loughlin TR (1997) Foraging behavior of adult female and young-of-the-year Steller sea lions in Alaskan waters. Can J Zool 75:776–786

Merrick RL, Loughlin TR, Antonelis GA, Hill R (1994) Use of satellite-linked telemetry to study Steller sea lion and northern fur seal foraging. Polar Res 13:105–114

Miller A, Trites AW, Maschner DG (2005) Ocean climate changes and the Steller sea lion decline. Arct Res United States 19:54-63

Moore SK, Trainer VL, Mantua NJ, Parker MS, Laws EA, Backer LC, Fleming LE (2008) Impacts of climate variability and future climate change on harmful algal blooms and human health. Environ Health 7 Suppl 2:S4

Mos L (2001) Domoic acid: a fascinating marine toxin. Environ Toxicol Pharmacol 9:79–85

National Research Council (NRC) (2003) Decline of the Steller sea lion in Alaskan waters: untangling food webs and fishing nets. National Academies Press, Washington, DC.

Nishitani L, Chew KK (1984) Recent developments in paralytic shellfish poisoning research. Aquaculture 39:317–329

NMFS (2008) Recovery plan for the Steller sea lion (*Eumetopias jubatus*): eastern and western distinct population segments (*Eumetopias jubatus*), revision. National Marine Fisheries Service, Silver Spring, MD

NMFS (2013) Status review of the eastern distinct population segment of Steller sea lion (*Eumetopias jubatus*). Protected Resources Division, Alaska Region, National Marine Fisheries Service, 709 West 9th St, Juneau, AK

Novaczek I, Madhyastha MS, Ablett RF, Donald A, Johnson G, Nijjar MS, Sims DE (1992) Depuration of domoic acid from live blue mussels (*Mytilus edulis*). Can J Fish Aquat Sci 49:312–318

National Shellfish Sanitation Program (NSSP) (2013) Guide for the control of molluscan shellfish: 2013 revision. Public Health Service, U.S. Food and Drug Administration, Silver Spring, MD

Oksanen AJ, Blanchet FG, Kindt R, Legendre P, Minchin PR, Hara RBO, Simpson GL, Solymos P, Stevens MHH, Wagner H (2015) Package " vegan ": Community Ecology Package 2.2-1. http://CRAN.R-project.org/package=vegan

Oleson EM, Calambokidis J, Falcone E, Schorr G, Hildebrand, JA (2009) Acoustic and visual monitoring for cetaceans along the outer Washington coast- Technical Report, July 2004-September 2008. U.S. Navy, Chief of Naval Operations, Energy and Environmental Readiness Division (CNO N45), Washington, DC

Orr AJ, Harvey JT (2001) Quantifying errors associated with using fecal samples to determine the diet of the California sea lion (*Zalophus californianus*). Can J Zool 79:1080–1087

Orr AJ, Van Blaricom GR, DeLong RL, Cruz-Escalona VH, Newsome SD (2011) Intraspecific comparison of diet of California sea lions (*Zalophus californianus*) assessed using fecal and stable isotope analyses. Can J Zool 89:109–122

Paul AJ, Paul JM, Brown ED (1998) Fall and spring somatic energy content for Alaskan Pacific herring (*Clupea pallasi* Valenciennes 1847) relative to age, size and sex. J Exp Mar Bio Ecol 223:133–142

Peterson W, Robert M, Bond N (2013) The 2013 inter-sessional science board meeting: a note from the science board chairman. PICES Press 21:44–46

Pitcher KW, Olesiuk PF, Brown RF, Lowry MS, Jeffries SJ, Sease JL, Perryman WL, Stinchcomb CE, Lowry LF (2007) Abundance and distribution of the eastern North Pacific Steller sea lion (*Eumetopias jubatus*) population. Fish Bull 107:102–115

Prakash A (1962) Seasonal changes in feeding of coho and chinook (spring) salmon in southern British Columbia waters. J Fish Res Board Canada 19:851-866

Pressey RT (1953) The sport fishery for salmon on Puget Sound. Washington State Department of Fisheries, Fisheries Research Papers 1:33-48

Purcell M, Mackey G, LaHood E, Huber H, Park L (2004) Molecular methods for the genetic identification of salmonids prey from Pacific harbor seal (*Phoca vitulina richardsi*) scat. Fish Bull 102:213–220

Quilliam MA, Xie M, Hardstaff WR (1995) Rapid extraction and cleanup for liquid chromatography determination of domoic acid in unsalted seafood. J AOAC Int 78:543–554

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reyero M, Cacho E, Martínez A, Vázquez J, Marina A, Santiago F, Franco JM (1999) Evidence of saxitoxin derivates as causative agents in the 1997 mass mortality of monk seals in the Cape Blanc Peninsula. Nat Toxins 7:311–315

Riemer S, Wright BE, Brown RF (2011) Food habits of Steller sea lions (*Eumetopias jubatus*) off Oregon and northern California, 1986-2007. Fish Bull 108:369–381

Rosen DAS, Trites AW (2000) Digestive efficiency and dry-matter digestibility in Steller sea lions fed herring, pollock, squid, and salmon. Can J Zool 78:234–239

Schaffer P, Reeves C, Casper DR, Davis CR (2006) Absence of neurotoxic effects in leopard sharks, *Triakis semifasciata*, following domoic acid exposure. Toxicon 47:747–752

Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW (2007) Effects of environmental methylmercury on the health of wild birds, mammals, and fish. Ambio 36:12–18

Scholin CA, Gulland F, Doucette GJ, Benson S, Busman M, Chavez FP, Cordaro J, DeLong R, De Vogelaere A, Harvey J, Haulena M, Lefebvre K, Lipscomb T, Loscutoff S, Lowenstine LJ, Marin R, Miller PE, McLellan WA, Moeller PD, Powell CL, Rowles T, Silvagni P, Silver M, Spraker T, Trainer V, Van Dolah FM (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403:80–84

Schwinghamer P, Hawryluk M, Powell C, MacKenzie CH (1994). Resuspended hypnozygotes of Alexandrium fundyense associated with winter occurrence of PSP in inshore Newfoundland waters. Aquaculture 122:171-179

Scordino J (2010) West coast pinniped program investigations on California sea lion and Pacific harbor seal impacts on salmonids and other Fishery Resources. Pacific States Marine Fisheries Commission, Portland, OR

Scordino JJ, Akmajian AM (2013) California and Steller sea lion seasonal use patterns at haulouts in NW Washington. In: Scordino JJ, Akmajian AM (eds) Research and education/outreach to benefit ESA listed and recently delisted marine mammals of Northwest Washington: Final report for Species Recovery Grant award NA10NMF4720372, Makah Fisheries Management, 150 Resort Drive, Neah Bay, WA

Scordino JJ, Akmajian AM, Riemer SD (2013) California and Steller sea lion diets in northwest Washington, 2010-2013. In: Scordino JJ, Akmajian AM (eds) Research and education/outreach to benefit ESA listed and recently delisted marine mammals of Northwest Washington: Final report for Species Recovery Grant award NA10NMF4720372, Makah Fisheries Management, 150 Resort Drive, Neah Bay, WA

Sekula-Wood E, Benitez-Nelson C, Morton S, Anderson C, Burrell C, Thunell R (2011) *Pseudo-nitzschia* and domoic acid fluxes in Santa Barbara Basin (CA) from 1993 to 2008. Harmful Algae 10:567–575

Shearn-Bochsler V, Lance EW, Corcoran R, Piatt J, Bodenstein B, Frame E, Lawonn J (2014) Fatal paralytic shellfish poisoning in Kittlitz's murrelet nestlings, Alaska, USA. J Wildl Dis 50:933–937

Shumway SE (1990) A review of the effects of algal blooms on shellfish and aquaculture. J World Aquac Soc 21:65–104

Shumway SE, Allen SM, Boersma PD (2003) Marine birds and harmful algal blooms: sporadic victims or under-reported events? Harmful Algae 2:1–17

Sierra Beltrán A, Palafox-Uribe M, Grajales-Montiel J, Cruz-Villacorta A, Ochoa JL (1997) Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. Toxicon 35:447–453

Sigler MF, Tollit DJ, Vollenweider JJ, Thedinga JF, Csepp DJ, Womble JN, Wong MA, Rehberg MJ, Trites AW (2009) Steller sea lion foraging response to seasonal changes in prey availability. Mar Ecol Prog Ser 388:243–261

Silvagni PA, Lowenstine LJ, Spraker T, Lipscomb TP, Gulland FMD (2005) Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). Vet Pathol 42:184–191

Sinclair EH, Zeppelin TK (2002) Seasonal and spatial differences in diet in the western stock of Steller sea lions (*Eumetopias jubatus*). J Mammal 83:973–990

Sydeman WJ and Jarman WM (1998) Trace metals in seabirds, Steller sea lion, and forage fish and zooplankton from Central California. Mar Pollut Bull 36:828–832

Tanabe S (2002) Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. Mar Pollut Bull 45:69–77

Thomas K, Harvey JT, Goldstein T, Barakos J, Gulland F (2010) Movement, dive behavior, and survival of California sea lions (*Zalophus californianus*) posttreatment for domoic acid toxicosis. Mar Mammal Sci 26:36–52

Tollit DJ, Heaslip SG, Barrick RL, Trites AW (2007) Impact of diet-index selection and the digestion of prey hard remains on determining the diet of the Steller sea lion (*Eumetopias jubatus*). Can J Zool 85:1–15

Tollit DJ, Wong M, Winship AJ, Rosen DAS, Trites AW (2003) Quantifying errors associated with using prey skeletal structures from fecal samples to determine the diet of Steller's sea lion (*Eumetopias Jubatus*). Mar Mammal S 19:724–744

Torres de la Riva G, Johnson CK, Gulland FMD, Langlois GW, Heyning JE, Rowles TK, Mazet JAK (2009) Association of an unusual marine mammal mortality event with *Pseudo-nitzschia* spp. blooms along the southern California coastline. J Wildl Dis 45:109–121

Townsend DW, Pettigrew NR, Thomas AC (2001) Offshore blooms of the red tide dinoflagellate, *Alexandrium* sp., in the Gulf of Maine. Cont Shelf Res 21:347–369

Trainer VL (2002) Harmful algal blooms on the U.S. west coast. In: Taylor FJ, Trainer VL (eds) Harmful algal blooms in the PICES region of the North Pacific. PICES Scientific Report No. 23, p 89–118

Trainer VL, Baden DG (1999) High affinity binding of red tide neurotoxins to marine mammal brain. Aquat Toxicol 46:139–148

Trainer VL, Bates SS, Lundholm N, Thessen AE, Cochlan WP, Adams NG, Trick CG (2012) *Pseudo-nitzschia* physiological ecology, phylogeny, toxicity, monitoring and impacts on ecosystem health. Harmful Algae 14:271–300

Trainer VL, Bill BD (2004) Characterization of a domoic acid binding site from Pacific razor clam. Aquat Toxicol 69:125–132

Trainer VL, Cochlan WP, Erickson A, Bill BD, Cox FH, Borchert JA, Lefebvre KA (2007) Recent domoic acid closures of shellfish harvest areas in Washington State inland waterways. Harmful Algae 6:449–459

Trainer VL, Eberhart BTL, Wekell JC, Adams NG, Hanson L, Cox F, Dowell J (2003) Paralytic shellfish toxins in Puget Sound, Washington State. J Shellfish Res 22:213–223

Trainer VL, Hickey BM, Horner RA (2002) Biological and physical dynamics of domoic acid production off the Washington coast. Limnol Oceanogr 47:1438–1446

Trainer VL, Hickey BM, Lessard EJ, Cochlan WP, Trick CG, Wells ML, MacFadyen A, Moore SK (2009) Variability of *Pseudo-nitzschia* and domoic acid in the Juan de Fuca eddy region and its adjacent shelves. Limnol Oceanogr 54:289–308

Trites AW, Calkins DG (2008) Diets of mature male and female Steller sea lions (*Eumetopias jubatus*) differ and cannot be used as proxies for each other. Aquat Mamm 34:25–34

Truelove J, Mueller R, Pulido O, Martin L, Fernie S, Iverson F (1997) 30-day oral toxicity study of domoic acid in cynomolgus monkeys: Lack of overt toxicity at doses approaching the acute toxic dose. Nat Toxins 5:111–114

Turner J, Tester P (1997) Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. Limnol Oceanogr 42:1203–1214

Tweddle JF, Strutton PG, Foley DG, Higgins L, Wood AM, Scott B, Everroad RC, Peterson WT, Cannon D, Hunter M, Forster Z (2010) Relationships among upwelling, phytoplankton blooms, and phycotoxins in coastal Oregon shellfish. Mar Ecol Prog Ser 405:131–145

Twiner MJ, Fire S, Schwacke L, Davidson L, Wang Z, Morton S, Roth S, Balmer B, Rowles TK, Wells RS (2011) Concurrent exposure of bottlenose dolphins (*Tursiops truncatus*) to multiple algal toxins in Sarasota Bay, Florida, USA. PLoS One 6

Van Dolah FM (2000) Marine algal toxins: origins, health effects, and their increased occurrence. Environ Heal Perspect Suppl 108:133–141

Van Dolah FM, Doucette GJ, Gulland FMD, Rowles TL, Bossart GD (2003) Impacts of algal toxins on marine mammals. In: Vos JG, Bossart GD, Fournier M, O'Shea TJ (eds) Toxicology of marine mammals. Taylor and Francis, New York, p 247-269

Van Dolah FM, Roelke D, Greene RM (2001) Health and ecological impacts of harmful algal blooms: risk assessment needs. Hum Ecol Risk Assess 7:1329–1345

Vigilant VL, Silver MW (2007) Domoic acid in benthic flatfish on the continental shelf of Monterey Bay, California, USA. Mar Biol 151:2053–2062

Wang DZ (2008) Neurotoxins from marine dinoflagellates: A brief review. Mar Drugs 6:349–371

Weise MJ (2000) Abundance, food habits, and annual fish consumption of California sea lion (*Zalophus californianus*) and its impact on salmonid fisheries in Monterey Bay, California. MSc Thesis, San Jose State University, San Jose, CA

Weise MJ, Costa DP, Kudela RM (2006) Movement and diving behavior of male California sea lion (*Zalophus californianus*) during anomalous oceanographic conditions of 2005 compared to those of 2004. Geophys Res Lett 33:1-6

Weise MJ, Harvey JT (1999) Food habits of California sea lions (*Zalophus californianus*) and their impact on salmonid fisheries in Monterey Bay, California. Moss Landing Marine Laboratories Tech Pub No. 99-1

Weise AM, Levasseur M, Saucier FJ, Senneville S, Bonneau E, Roy S, Sauvé G, Michaud S, Fauchot J (2002) The link between precipitation, river runoff, and blooms of the toxic dinoflagellate *Alexandrium tamarense* in the St. Lawrence. Can J Fish Aquat Sci 59:464–473

Wekell JC, Gauglitz Jr EJ, Barnett HJ, Hatfield CL, Simons D, Ayres D (1994) Occurrence of domoic acid in Washington state razor clams (*Siliqua patula*) during 1991-1993. Nat Toxins 2:197–205

White CL, Schuler KL, Thomas NJ, Webb JL, Saliki JT, Ip HS, Dubey JP, Frame ER (2013) Pathogen exposure and blood chemistry in the Washington, USA population of northern sea otters (*Enhydra lutris kenyoni*). J Wildl Dis 49:887–99

Wiles GJ (2015) Periodic status review for the Steller sea lion. Washington Department of Fish and Wildlife, Olympia, WA

Womble JN, Sigler MF, Willson MF (2009) Linking seasonal distribution patterns with prey availability in a central-place forager, the Steller sea lion. J Biogeogr 36:439–451

Womble JN, Willson MF, Sigler MF, Kelly BP, VanBlaricom GR (2005) Distribution of Steller sea lions *Eumetopias jubatus* in relation to spring-spawning fish in SE Alaska. Mar Ecol Prog Ser 294:271–282

Work TM, Barr B, Beale AM, Fritz L, Quilliam M, Wright JLC (1993) Epidemiology of domoic acid poisoning in brown pelicans (*Pelecanus occidentalis*) and Brandt's cormorants (*Phalacrocorax penicillatus*) in California. J Zoo Wildl Med 24:54–62

Wright BE, Tennis MJ, Brown RF (2010) Movements of male California sea lions captured in the Columbia River. Northwest Sci 84:60–72

Wyatt T, Jenkinson IR (1997) Notes on *Alexandrium* population dynamics. J Plankton Res 19:551–575

Zabka TS, Goldstein T, Cross C, Mueller RW, Kreuder-Johnson C, Gill S, Gulland FMD (2009) Characterization of a degenerative cardiomyopathy associated with domoic acid toxicity in California sea lions (*Zalophus californianus*). Vet Pathol 46:105–119
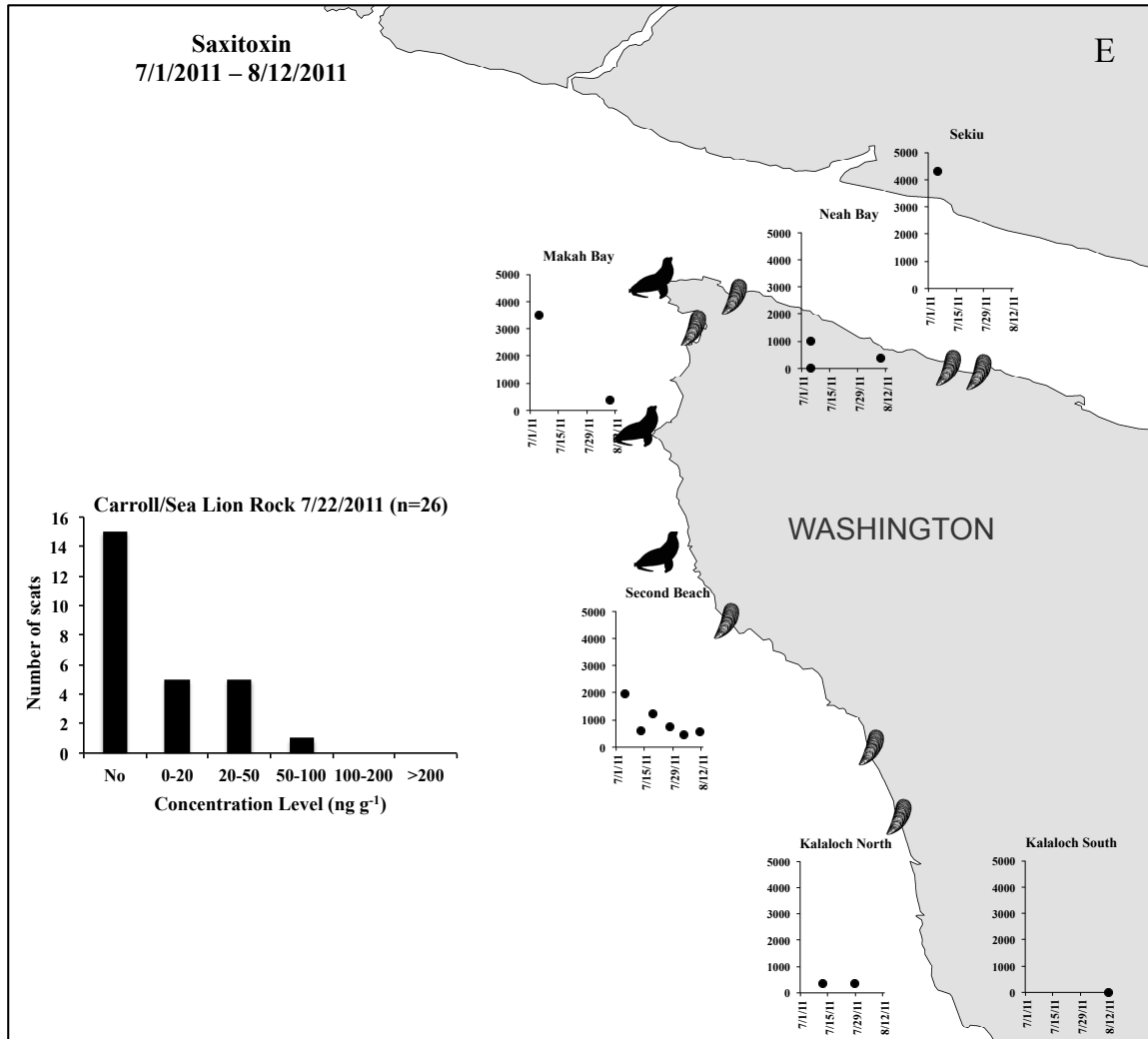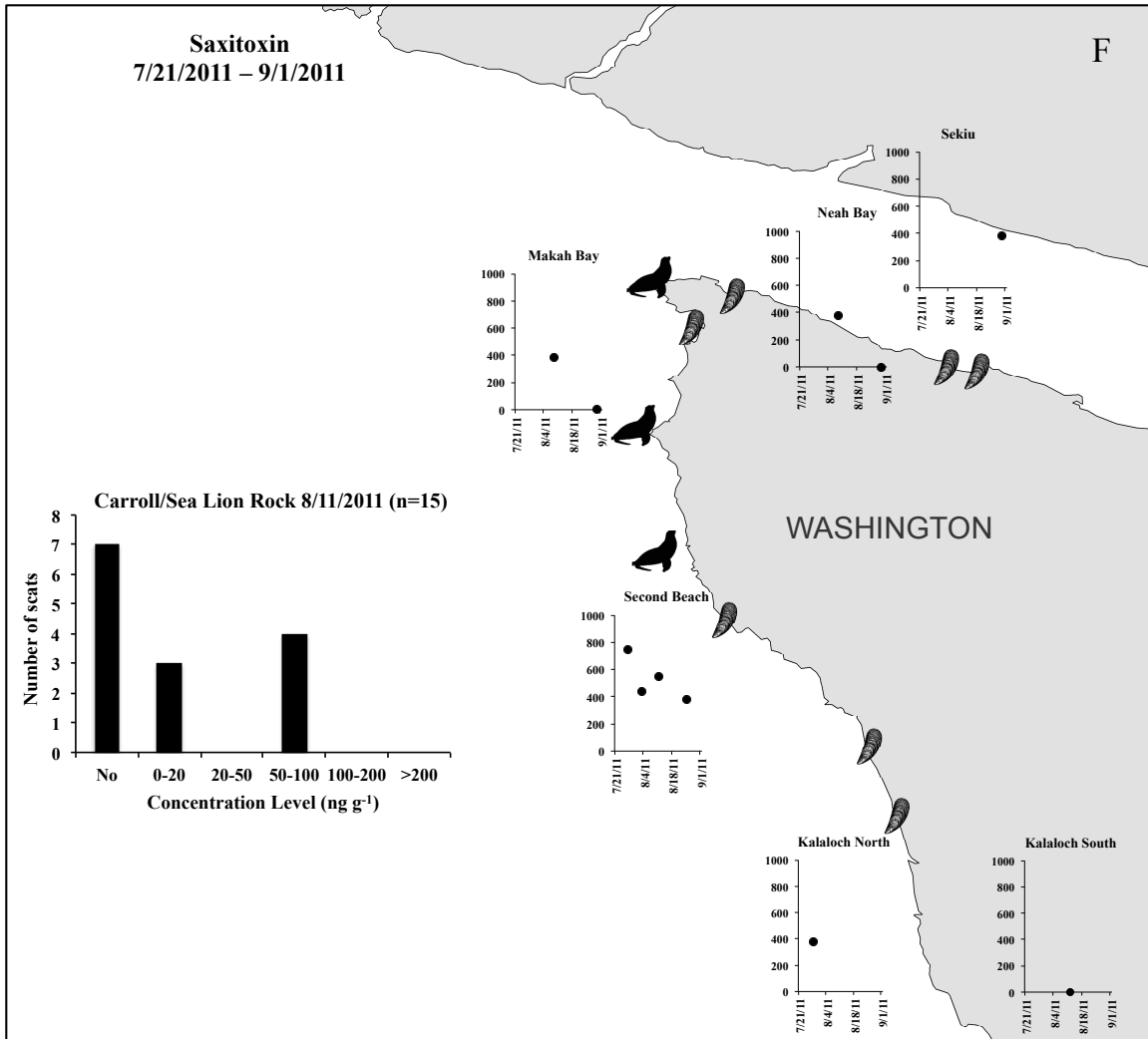
Appendix 1. Saxitoxin concentration (ng g^{-1}) by site in sea lions (bar plots) and nearshore bivalves (scatter plots). Scats from Steller sea lions *Eumetopias jubatus* (black) and California sea lions *Zalophus californianus* (gray) are summarized by concentration level (left) as having no toxin, 0-20 ng g^{-1}, 20-50 ng g^{-1}, 50-100 ng g^{-1}, 100-200 ng g^{-1} and > 200 ng g^{-1}. Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots represe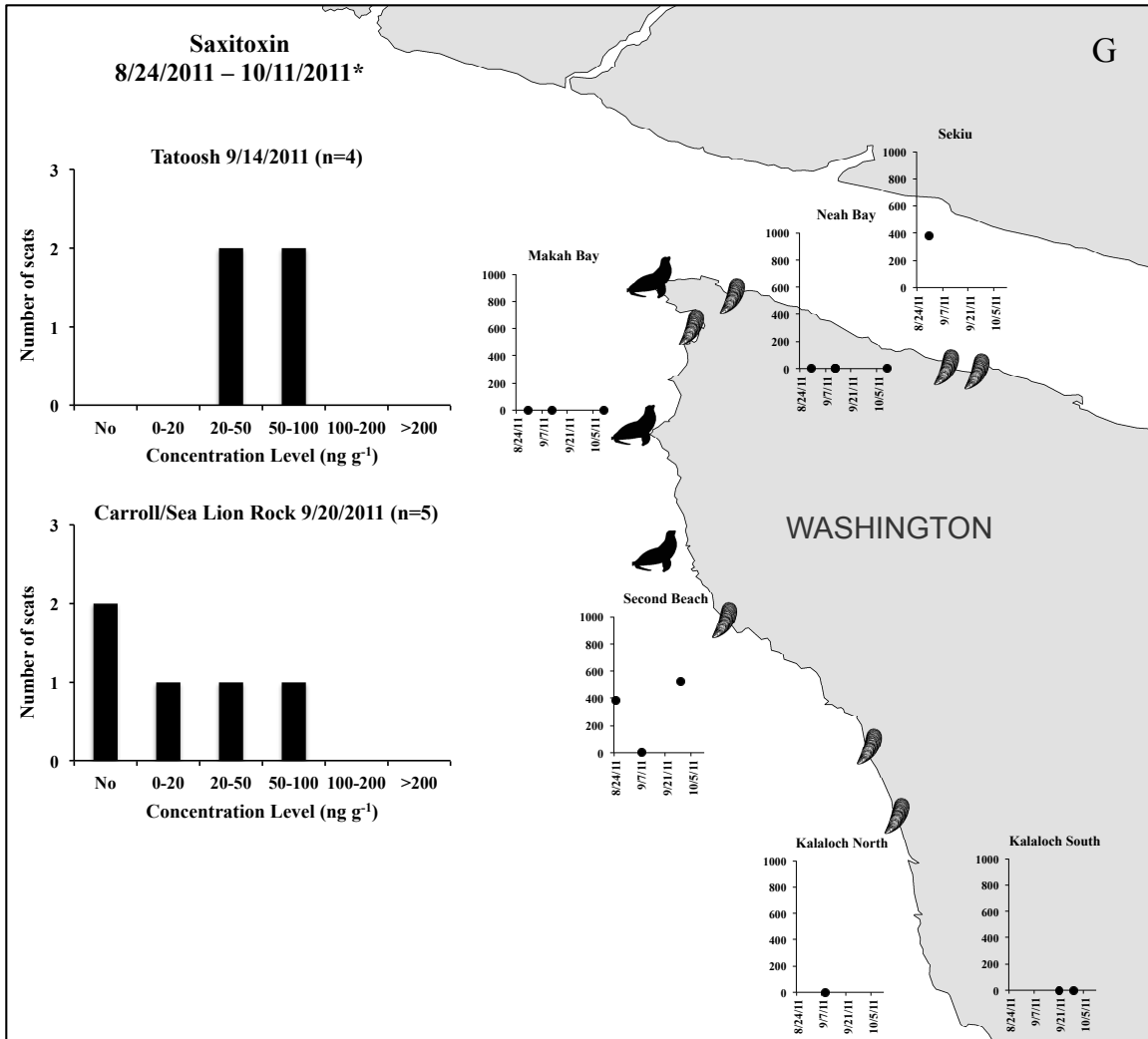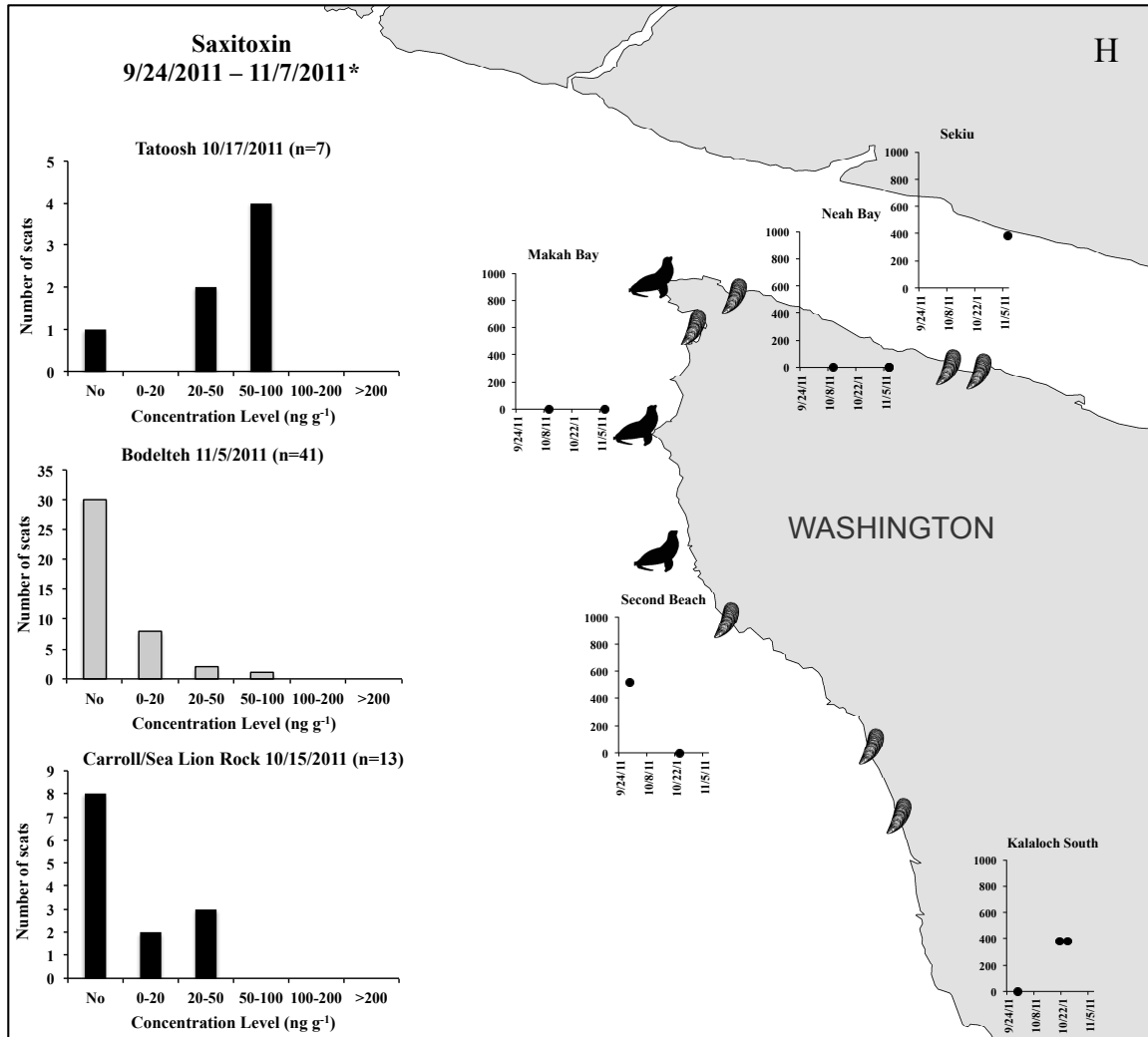nting the concentration measured in 100 g of bivalve tissue. Time periods are defined as three weeks before and three weeks after scat sampling dates. Dates with * indicate time periods where two scat collection occurred within one week and the time period displayed was lengthened. Note that some dates are repeated in subsequent graphs.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
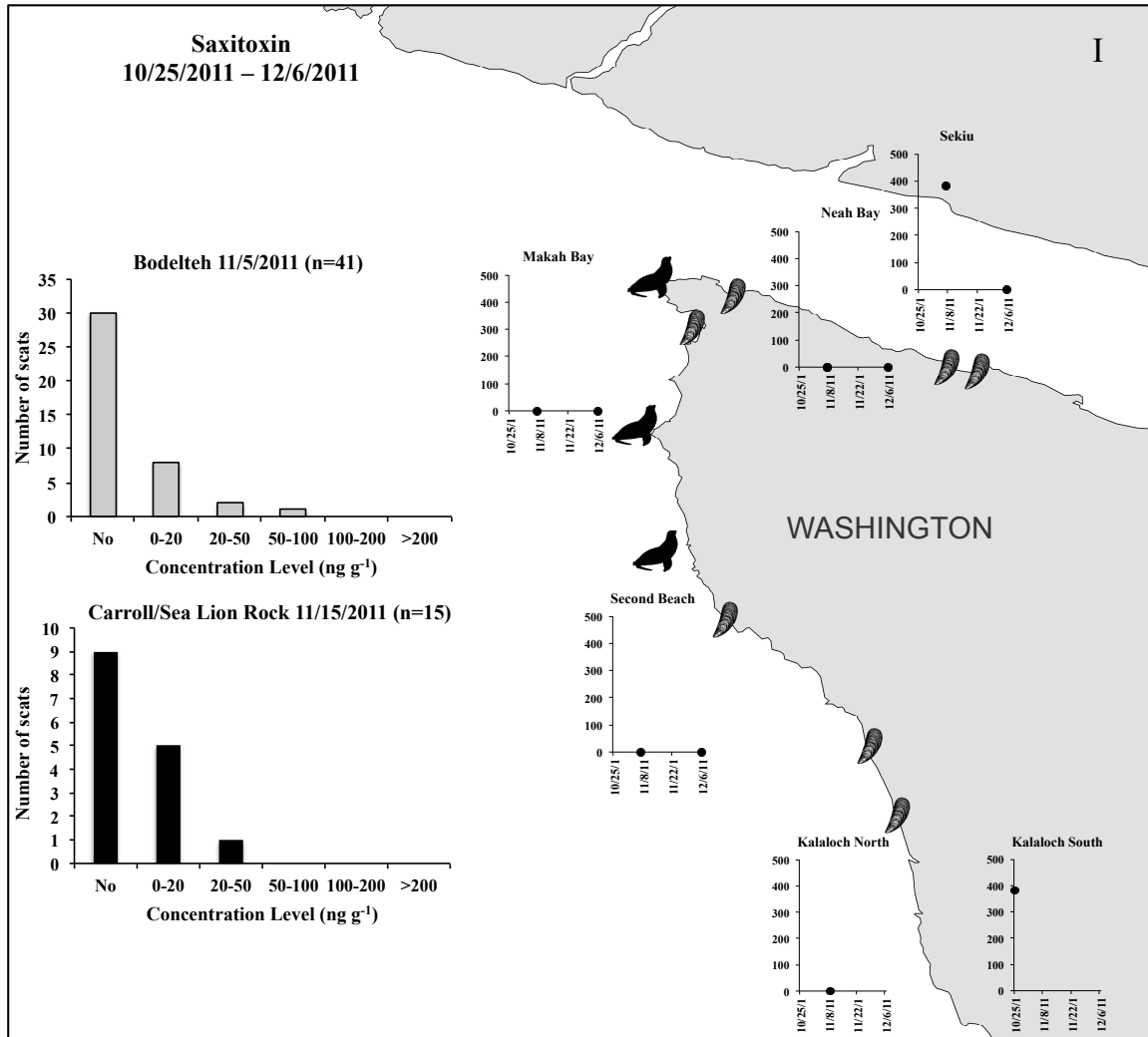
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
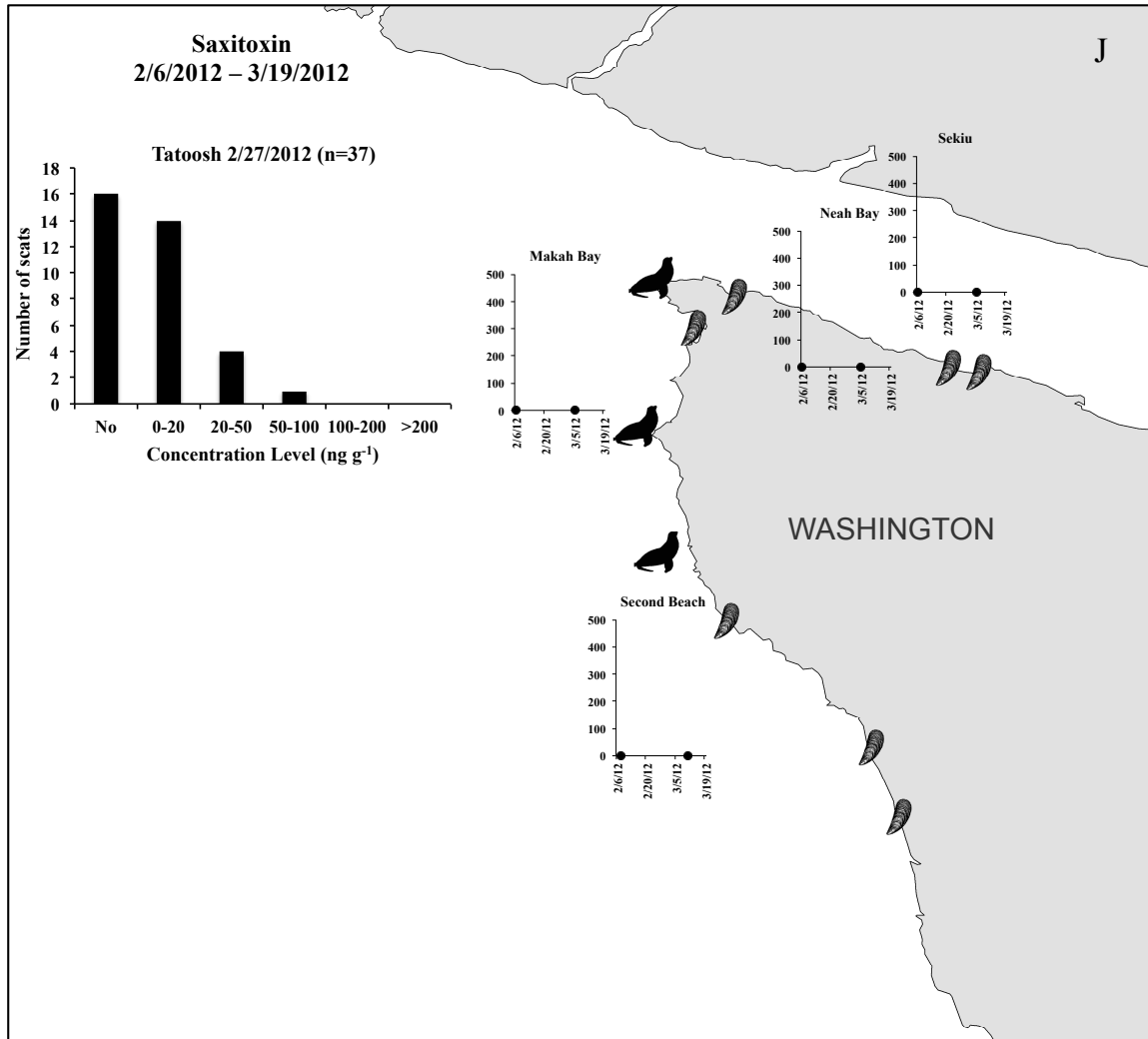
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
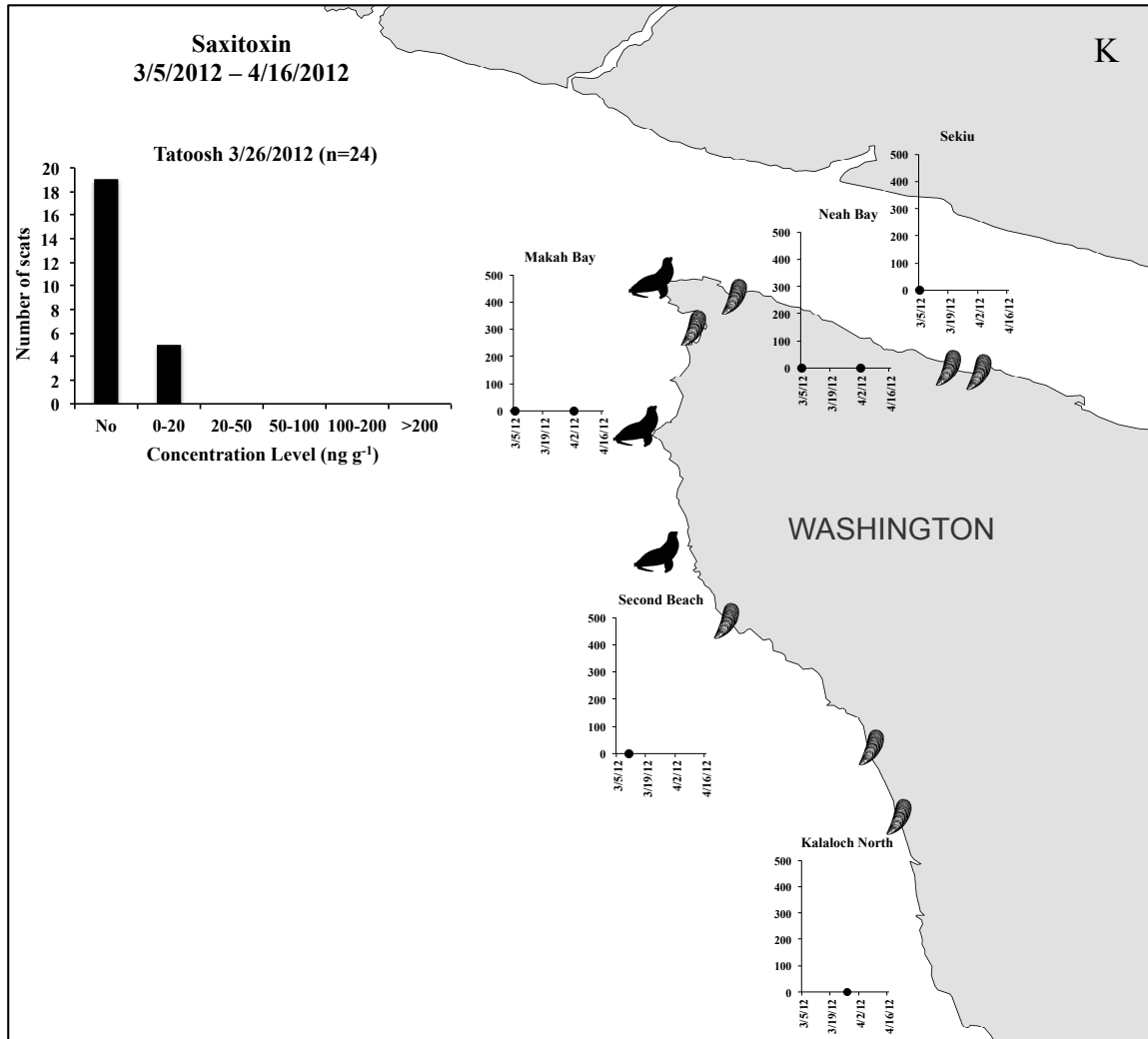
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
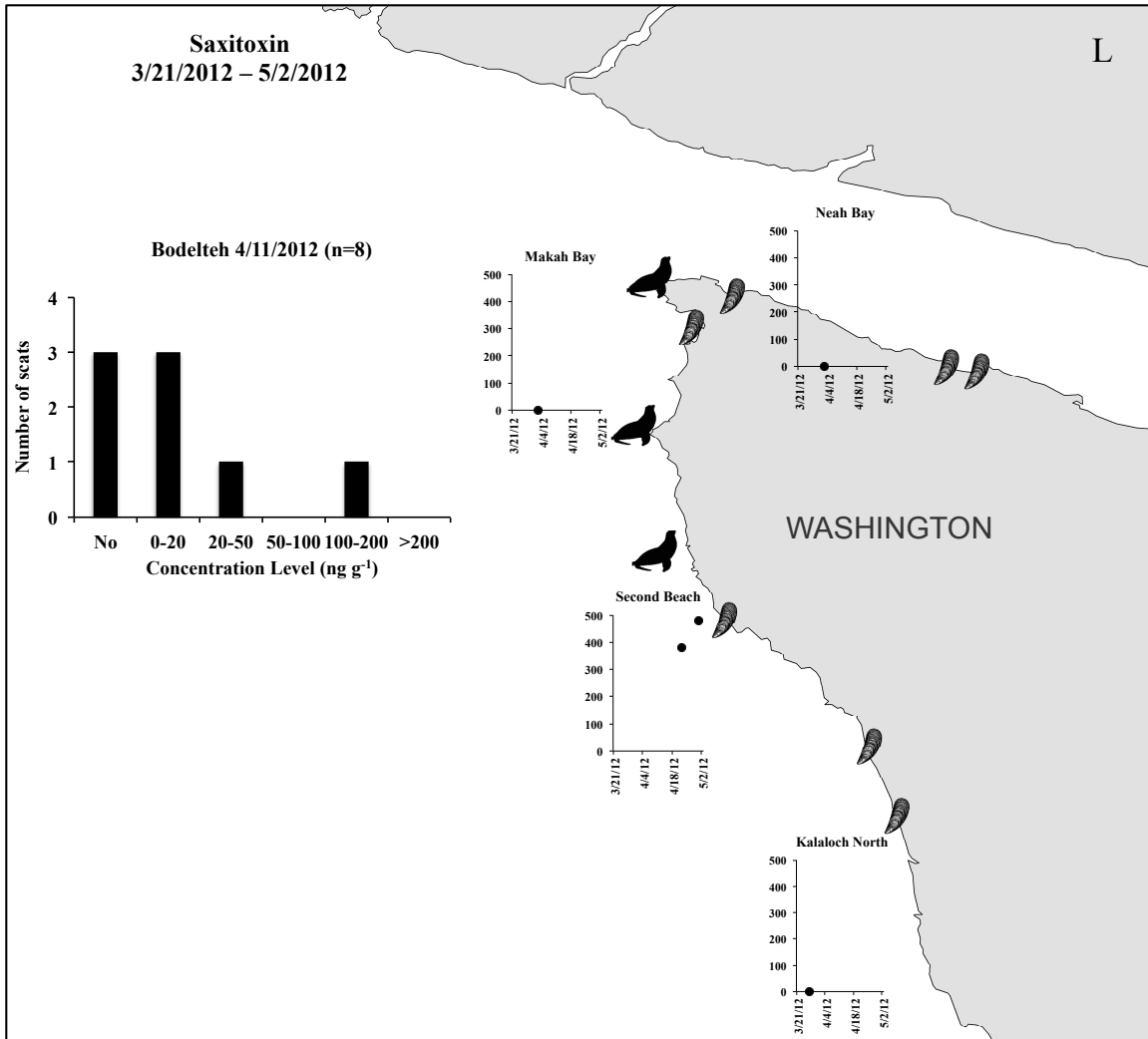
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
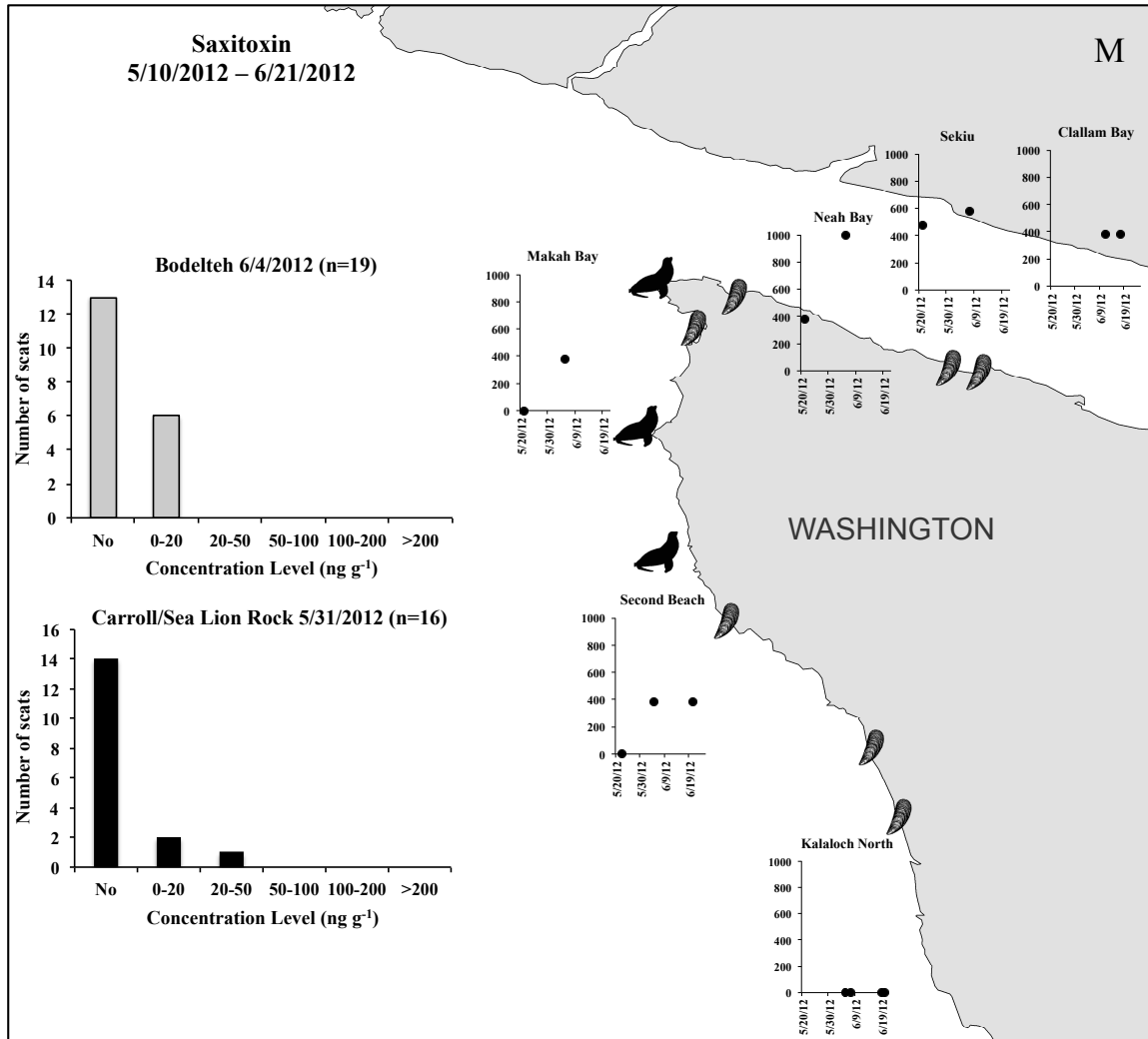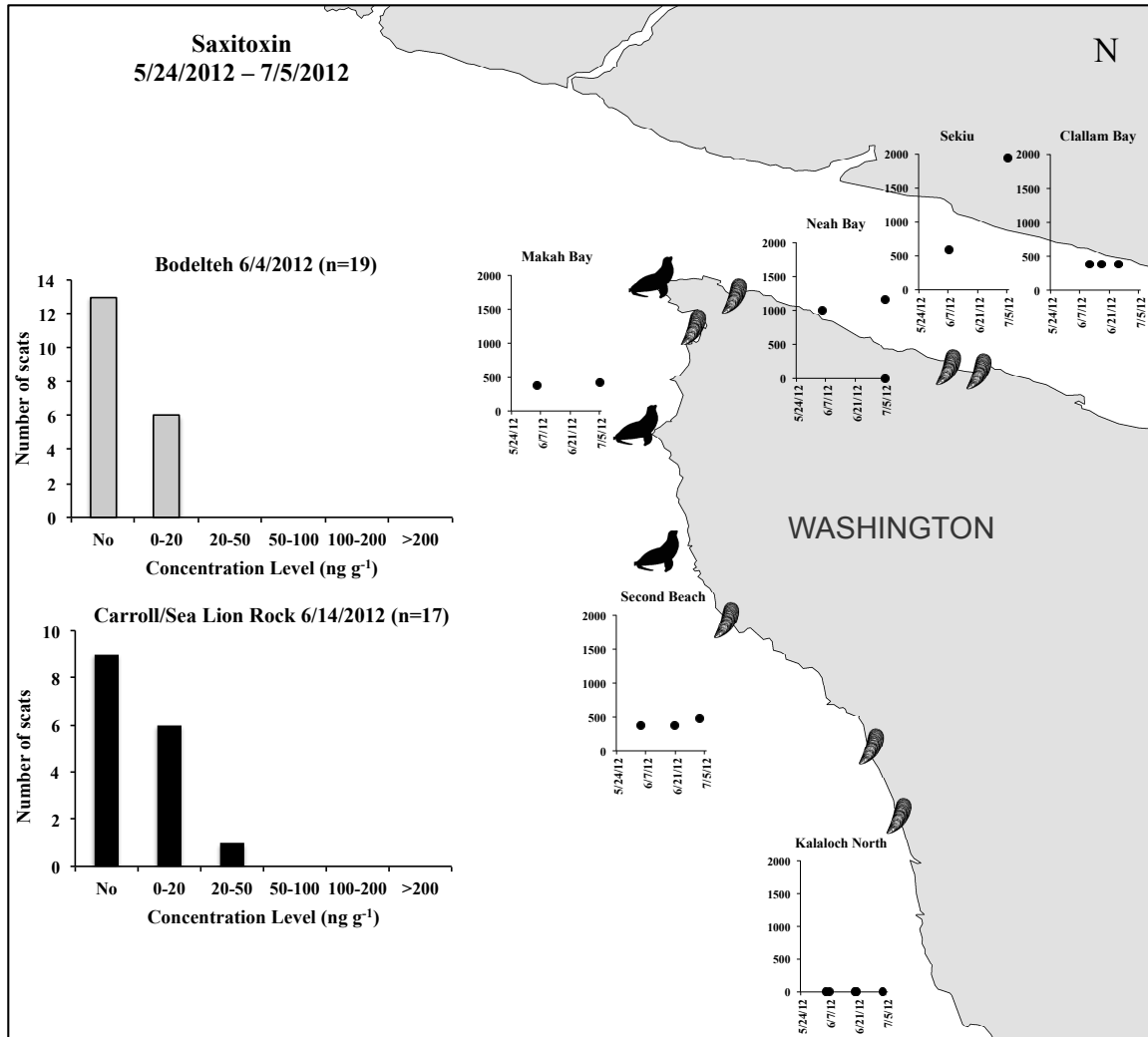
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
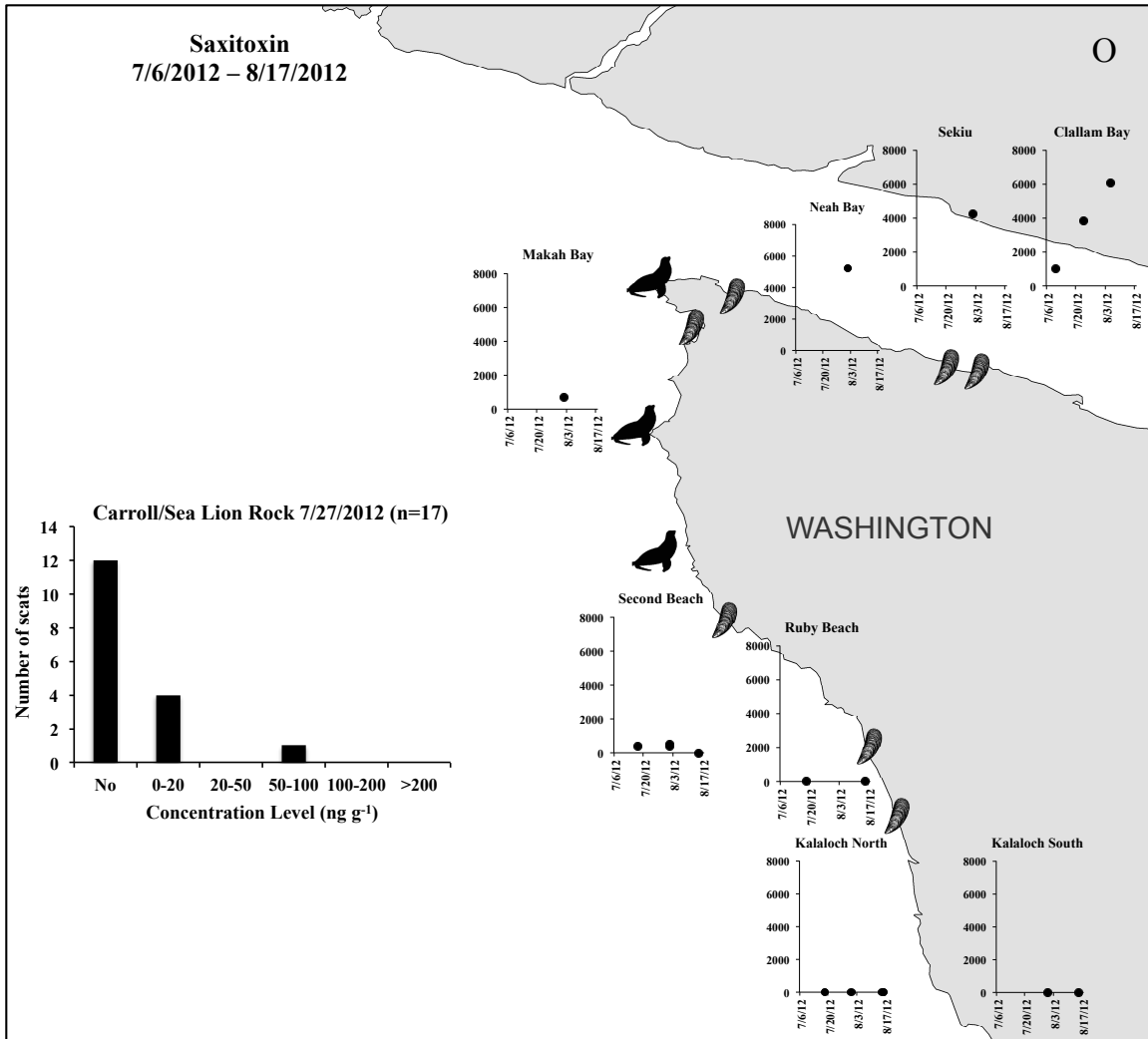
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
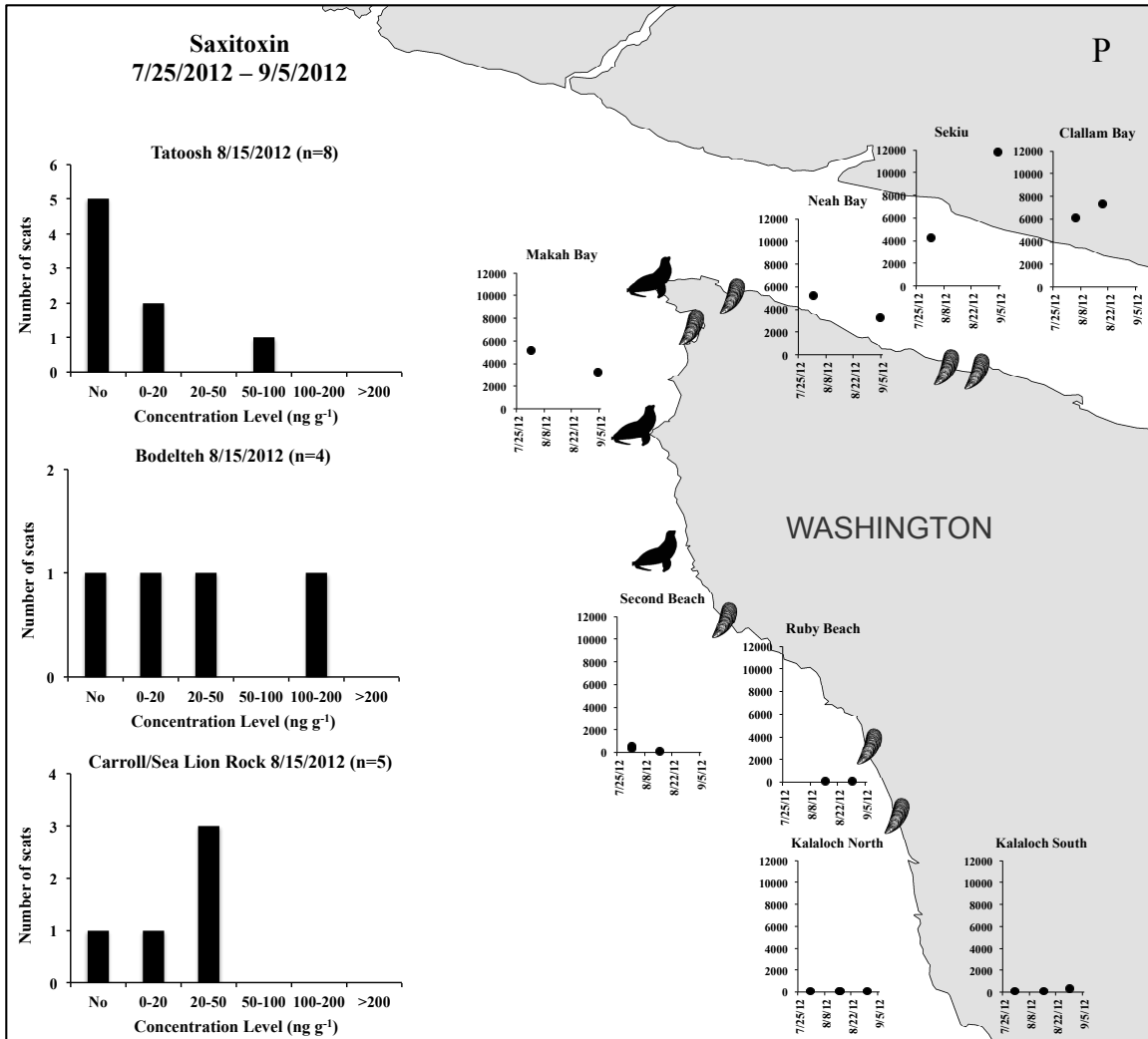
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
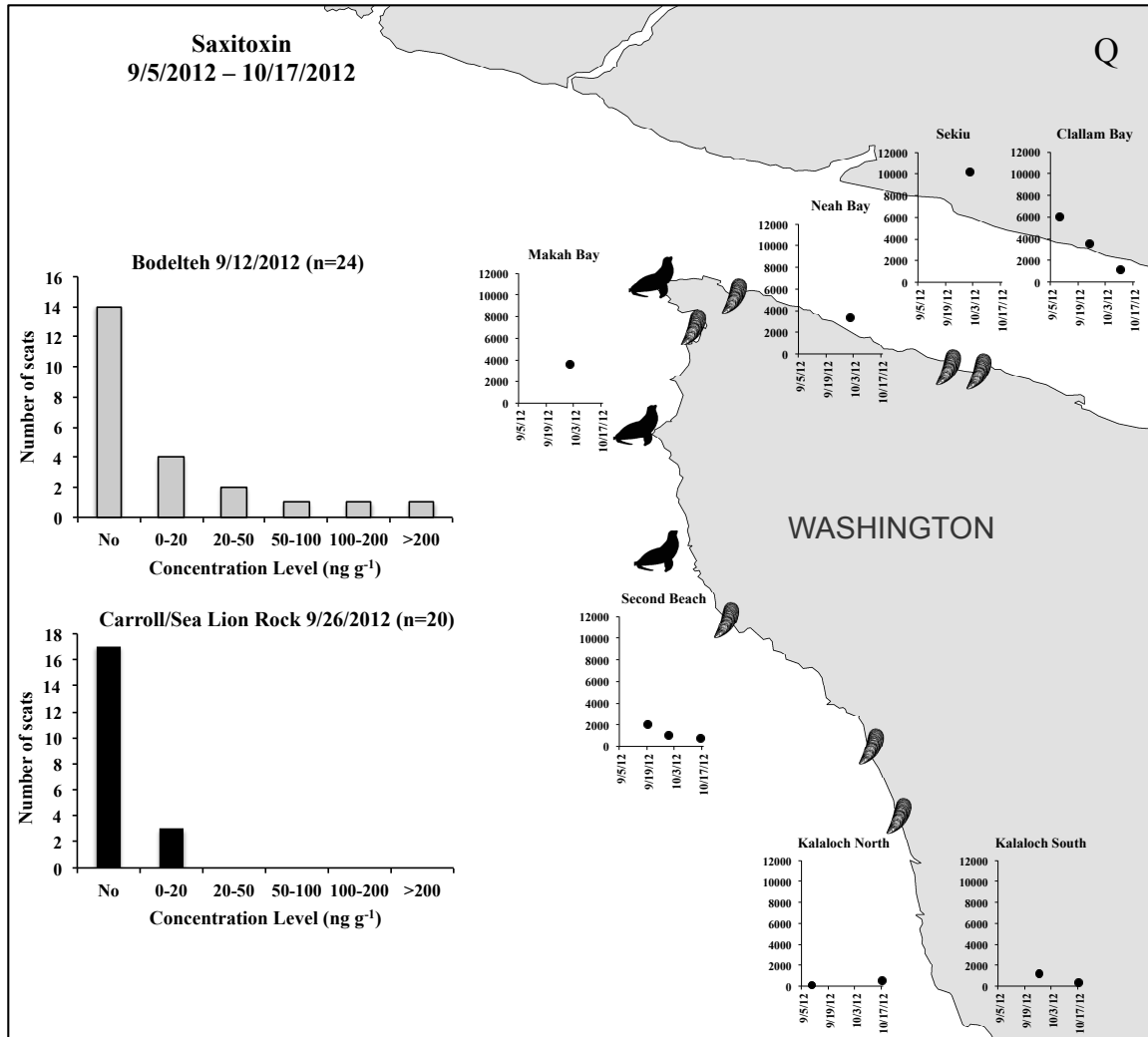
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
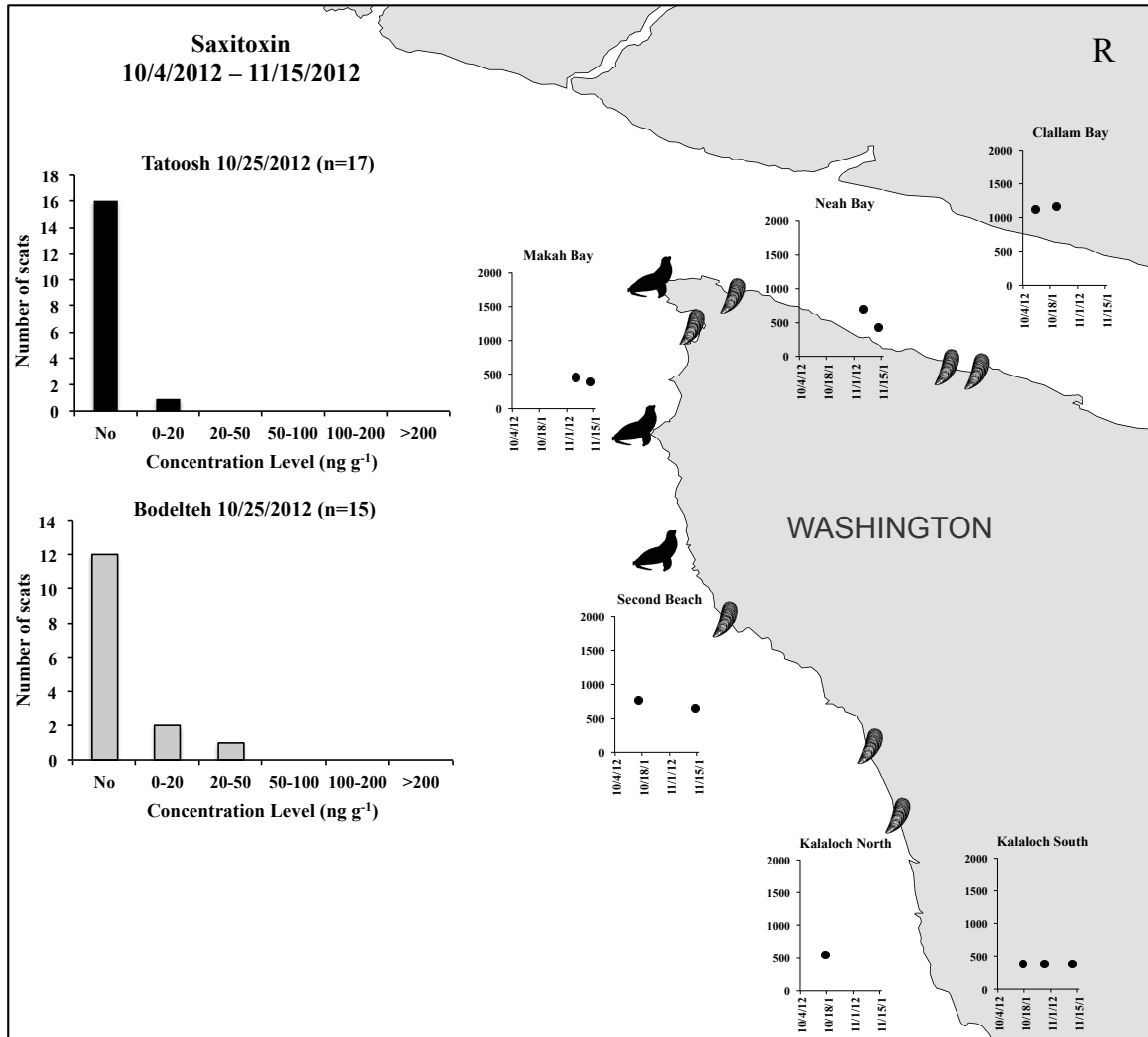
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
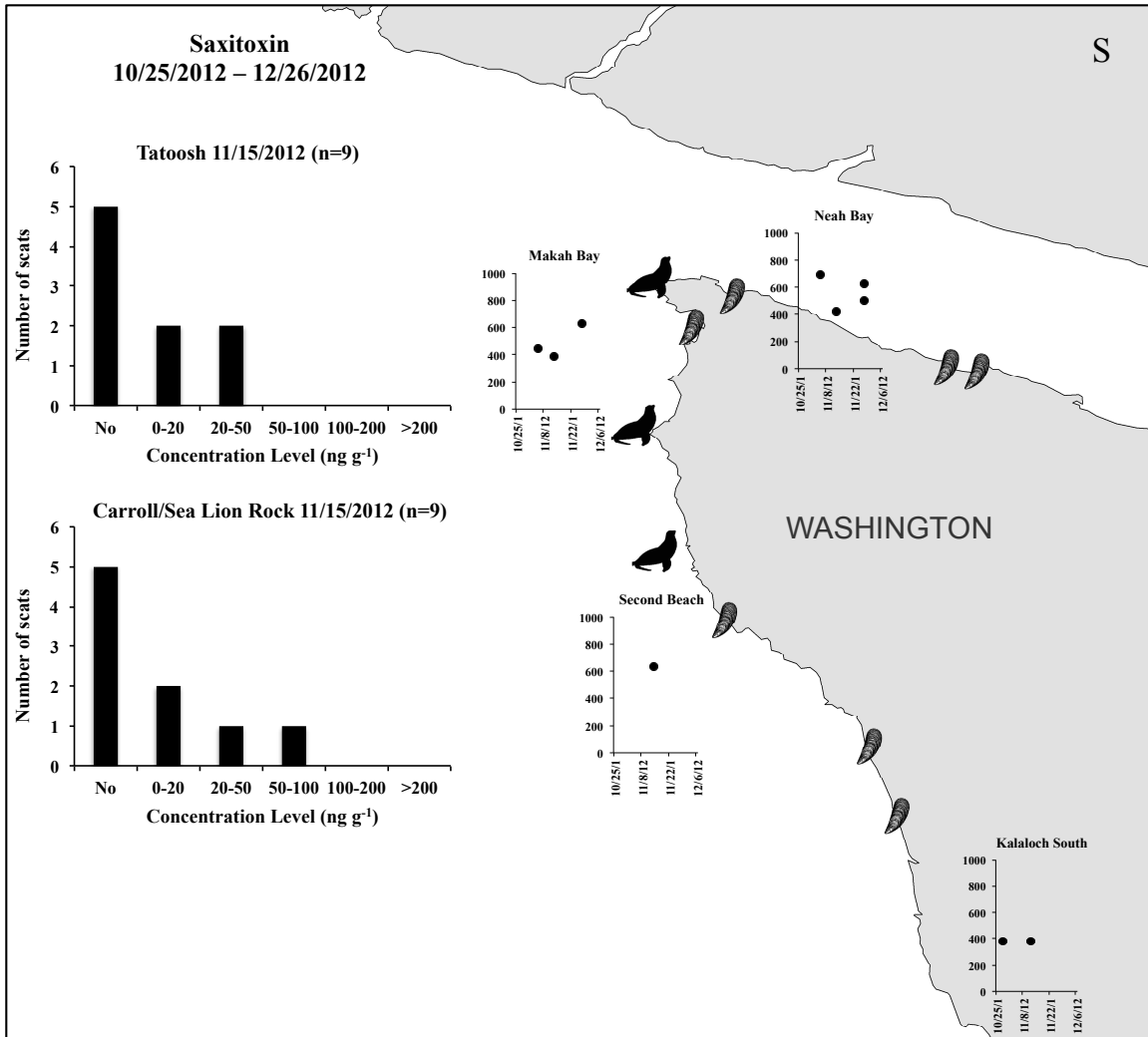
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
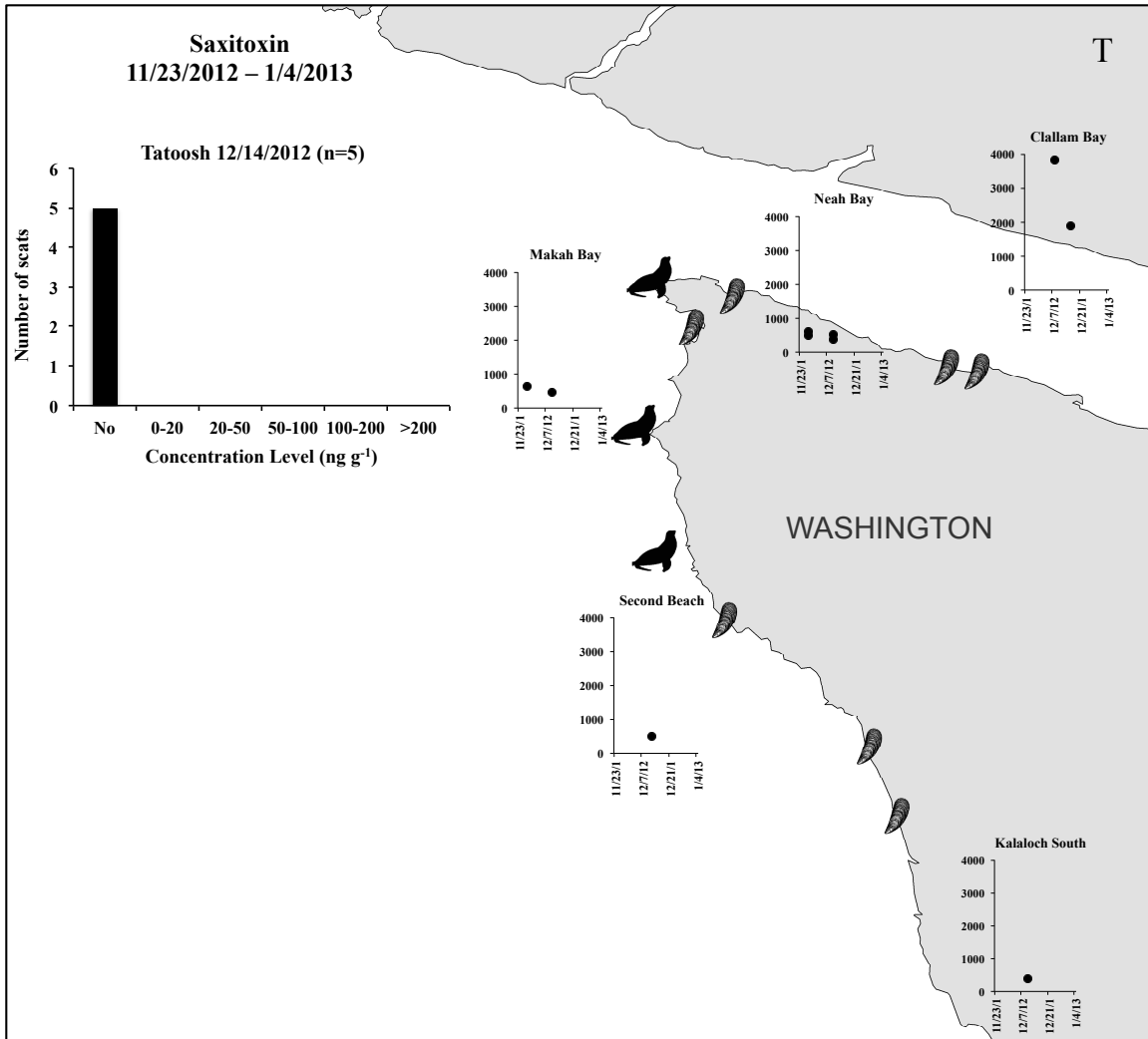
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.
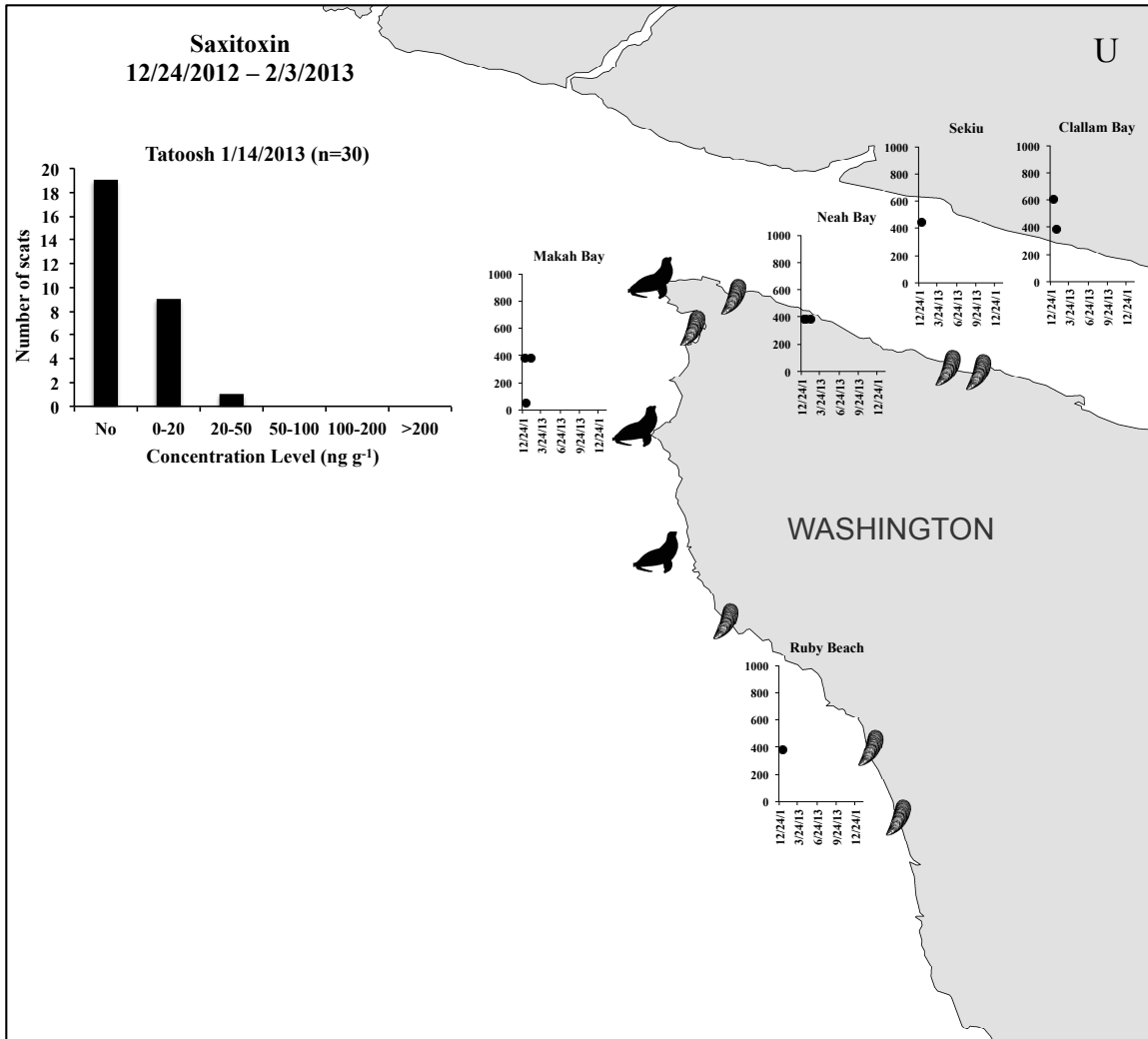
Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.

Appendix 1 cont. Saxitoxin concentration (ng g^{-1}) by site in sea lions (barplots) and nearshore bivalves (scatterplots). Scats from Steller sea lions (black) and California sea lions (gray) are summarized by concentration level (left). Scatterplots show concentrations of saxitoxin (ng g^{-1}) by sample date, with individual dots representing the concentration measured in 100 g of bivalve tissue.