

1 **Prevalence of Algal Toxins in Alaskan Marine Mammals Foraging in a Changing**
2 **Arctic and Subarctic Environment**

3 Kathi A. Lefebvre^{1*}, Lori Quakenbush², Elizabeth Frame^{1,3}, Kathy Burek Huntington⁴,
4 Gay Sheffield⁵, Raphaela Stimmelmayer⁶, Anna Bryan², Preston Kendrick¹, Heather Ziel⁷,
5 Tracey Goldstein⁸, Jonathan A. Snyder⁹, Tom Gelatt⁷, Frances Gulland¹⁰, Bobette
6 Dickerson⁷, Verena Gill^{9,11}

7 ¹Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA

8 ²Alaska Department of Fish and Game, Arctic Marine Mammal Program, 1300 College
9 Road, Fairbanks, AK

10 ³Current: Aquatic Toxicology Unit, King County Environmental Laboratory, Seattle, WA

11 ⁴Alaska Veterinary Pathology Services (AVPS), 23834 The Clearing Drive, Eagle River,
12 AK

13 ⁵University of Alaska Fairbanks, Alaska Sea Grant, Marine Advisory Program, PO Box
14 400, Nome, AK

15 ⁶North Slope Borough Department of Wildlife Management, PO Box 69, Barrow, AK

16 ⁷Marine Mammal Laboratory, Alaska Fisheries Science Center, NOAA, Seattle, WA

17 ⁸One Health Institute, School of Veterinary Medicine, University of California, Davis,
18 CA

19 ⁹U.S. Fish and Wildlife Service, Marine Mammals Management, 1011 East Tudor Rd.,
20 Anchorage, AK

21 ¹⁰The Marine Mammal Center, 2000 Bunker Road, Fort Cronkhite, Sausalito, CA

22 ¹¹Current: Bureau of Ocean Energy Management, 3801 Centerpoint Dr., Anchorage, AK

23 ***Corresponding Author: Kathi.Lefebvre@noaa.gov**

1 **Abstract:**

2 Current climate trends resulting in rapid declines in sea ice and increasing water
3 temperatures are likely to expand the northern geographic range and duration of favorable
4 conditions for harmful algal blooms (HABs), making algal toxins a growing concern in
5 Alaskan marine food webs. Two of the most common HAB toxins along the west coast of
6 North America are the neurotoxins domoic acid (DA) and saxitoxin (STX). Over the last
7 20 years, DA toxicosis has caused significant illness and mortality in marine mammals
8 along the west coast of the USA, but has not been reported to impact marine mammals
9 foraging in Alaskan waters. Saxitoxin, the most potent of the paralytic shellfish poisoning
10 toxins, has been well-documented in shellfish in the Aleutians and Gulf of Alaska for
11 decades and associated with human illnesses and deaths due to consumption of toxic
12 clams. There is little information regarding exposure of Alaskan marine mammals. Here,
13 the spatial patterns and prevalence of DA and STX exposure in Alaskan marine mammals
14 are documented in order to assess health risks to northern populations including those
15 species that are important to the nutritional, cultural, and economic well-being of Alaskan
16 coastal communities. In this study, 905 marine mammals from 13 species were sampled
17 including; humpback whales, bowhead whales, beluga whales, harbor porpoises,
18 Northern fur seals, Steller sea lions, harbor seals, ringed seals, bearded seals, spotted
19 seals, ribbon seals, Pacific walruses, and northern sea otters. Domoic acid was detected in
20 all 13 species examined and had the greatest prevalence in bowhead whales (68%) and
21 harbor seals (67%). Saxitoxin was detected in 10 of the 13 species, with the highest
22 prevalence in humpback whales (50%) and bowhead whales (32%). Pacific walruses
23 contained the highest concentrations of both STX and DA, with DA concentrations

1 similar to those detected in California sea lions exhibiting clinical signs of DA toxicosis
2 (seizures) off the coast of Central California, USA. Forty-six individual marine mammals
3 contained detectable concentrations of both toxins emphasizing the potential for
4 combined exposure risks. Additionally, fetuses from a beluga whale, a harbor porpoise
5 and a Steller sea lion contained detectable concentrations of DA documenting maternal
6 toxin transfer in these species. These results provide evidence that HAB toxins are
7 present throughout Alaska waters at levels high enough to be detected in top predators
8 and have the potential to impact marine mammal health in the Arctic marine
9 environment.

10

11 **1. Introduction**

12 Harmful algal blooms (HABs) most commonly occur in temperate and tropical
13 regions; however, current climate trends such as ocean warming and loss of seasonal sea
14 ice, are likely to expand the geographic distribution and the duration of conditions that
15 support blooms (Moore et al., 2008; Van Dolah, 2000), making HAB exposure
16 potentially more common among marine mammals in Alaskan waters (Burek et al.,
17 2008). Species of phytoplankton known to be toxic are not new to Alaskan waters.
18 Diatoms of the genus *Pseudo-nitzschia*, have been documented as far north as the eastern
19 Beaufort Sea (Bursa, 1963) and produce domoic acid (DA), the toxin responsible for
20 amnesic shellfish poisoning (ASP). Domoic acid was first detected in low levels in razor
21 clams in Kachemak Bay in July 1992 (RaLonde and Wright, 2011). Dinoflagellates of the
22 genus *Alexandrium* produce toxins that cause paralytic shellfish poisoning (PSP) and
23 have been well documented in Alaskan waters (Gessner and Middaugh, 1995; Gessner et

1 al., 1997; Gessner and Schloss, 1996; Lewitus et al., 2012; Trainer et al., 2014). Saxitoxin
2 (STX) is the most potent of the PSP causing toxins. Both DA and STX affect the central
3 nervous system of vertebrates. Saxitoxin acts as a sodium channel blocker and prevents
4 action potential activity in nerves causing paralysis primarily of the respiratory system
5 (Cusick and Sayler, 2013). Domoic acid is an excitotoxin that over-stimulates glutamate
6 receptors in the vertebrate central nervous system causing stimulation of nerves (Berman
7 et al., 2002; Berman and Murray, 1997; Todd, 1993). While PSP has been documented in
8 humans in Alaska since 1799, the only documented cases of ASP in humans occurred in
9 Southeastern Canada in 1989 (Perl et al., 1990). Unlike temperate regions, no incidences
10 of DA toxicosis and very few incidences of STX toxicosis have been documented in
11 Alaskan marine mammals.

12 Ocean temperatures around Alaska are warming; shelf waters of the eastern
13 Bering Sea have increased by almost 3°C during the past decade (Stabeno et al., 2007).
14 The lowest sea ice extent measurements since satellite monitoring began in 1979 were
15 recorded during 2007-2009 ((Stroeve et al., 2008) National Snow and Ice Data Center
16 press release, October 6, 2009), until 2012, which is the record Arctic sea ice minimum
17 documented to date. Loss of sea ice has allowed industrial maritime ship traffic across the
18 Arctic to increase substantially. Ships can transport HAB species to new areas through
19 ballast water discharge (Reeves et al., 2012), a process that is currently unregulated in the
20 Arctic (Hallegraeff, 1998). Filter-feeding benthic invertebrates, zooplankton, and finfish
21 can accumulate STX and DA and are well-known vectors of algal toxins to higher trophic
22 level predators (Bargu et al., 2002; Costa et al., 2009; Lefebvre et al., 2002b; Wekell et
23 al., 1994; White, 1986; Wohlgeschaffen et al., 1992).

1 The potential for health effects on Alaskan marine mammals may be high
2 considering more than 40% of marine mammal unusual mortality events (UMEs) in the
3 contiguous USA during the last 20 years have been attributed to algal toxin exposure
4 (Flewelling et al., 2005; Gulland and Hall, 2007; Landsberg et al., 2014; Scholin et al.,
5 2000; Torres De La Riva et al., 2009). The number of HAB-related strandings appears to
6 be increasing in the contiguous USA as these events were relatively rare even in
7 temperate regions only two decades ago (Landsberg et al., 2014). The negative impacts of
8 algal toxins on marine mammal health have been well documented along the west coast
9 of the USA. For example, the neurotoxic effects of DA were first reported in stranded
10 California sea lions (*Zalophus californianus*) in 1998 through exposure from toxic
11 planktivorous prey such as northern anchovies and Pacific sardines (Gulland, 2000;
12 Lefebvre et al., 1999; Scholin et al., 2000). Clinical signs of acute DA poisoning in
13 marine mammals include ataxia, head weaving, seizures or coma and/or death (Gulland et
14 al., 2002). The frequency of DA-associated California sea lion strandings has increased
15 since 1998 and strandings now occur annually, affecting hundreds of sea lions per year
16 (Bargu et al., 2010). Additionally, a chronic neurological syndrome associated with
17 repetitive sub-lethal exposure to the toxin is now recognized by behavioral changes,
18 seizures, and atrophy of the hippocampal formation (Cook et al., 2015; Goldstein et al.,
19 2008). Domoic acid has also been documented to cross the placenta of California sea
20 lions and be present in milk, thus, neonates may be exposed *in utero* and after birth until
21 weaning (Brodie et al., 2006; Rust et al., 2014). In addition to contributing to
22 reproductive failure, *in utero* and lactational exposure to DA can result in developmental
23 abnormalities leading to neurological and behavioral deficits in surviving offspring.

1 Given that California sea lions and humans share a common prey base, sea lions serve as
2 important food safety sentinels regarding the presence of HABs near California. The
3 findings associated with DA exposure in California sea lions demonstrate the potential
4 health effects for other marine mammal species, as well as the potential for marine
5 mammals in other regions to be sentinels for public health threats. The effects of STX on
6 marine mammals are not as well documented as they are for DA. The first reported STX-
7 related mortality event involved humpback whales in the late 1980s when 14 humpback
8 whales died near Cape Cod Bay after ingesting mackerel containing STX (Geraci et al.,
9 1989). Saxitoxin was suspected (although not substantiated) to be a factor in 60 sea otter
10 deaths in Alaska (Degange and Vacca, 1989) and in 117 Mediterranean monk seal
11 (*Monachus monachus*) deaths in Western Sahara, Africa (Costas and Lopez-Rodas,
12 1998).

13 The goal of this study was to document the presence and extent of two algal
14 toxins (DA and STX) in Alaskan marine mammals to identify emerging exposure risks in
15 northern-ranging marine mammal populations, including those species that are important
16 to people for subsistence purposes. Thirteen species were examined including: four
17 cetaceans (humpback whales, *Megaptera novaeangliae*; bowhead whales, *Balaena*
18 *mysticetus*; beluga whales, *Delphinapterus leucas*; and harbor porpoises, *Phocoena*
19 *phocoena*), two otariids (northern fur seals, *Callorhinus ursinus* and Steller sea lions,
20 *Eumetopias jubatus*), five phocids (harbor, *Phoca vitulina*; ringed *P. hispida*; bearded,
21 *Erignathus barbatus*; spotted, *P. largha* and ribbon seals, *Histiophoca fasciata*), Pacific
22 walruses (*Odobenus rosmarus*), and northern sea otters (*Enhydra lutra*).

23 **2. Methods**

1 2.1 *Marine mammal sample collection:*

2 A variety of samples (feces, stomach contents, intestinal contents, serum, milk,
3 urine, amniotic fluid, bile, aqueous humor, and pleural, peritoneal and pericardial fluid)
4 were collected from Alaskan marine mammals that were stranded, harvested for
5 subsistence purposes, or captured for research. Samples were also collected during the
6 Northern Alaska Pinniped Unusual Mortality Event (UME)
7 (<http://www.nmfs.noaa.gov/pr/health/mmume/events.html>). Not all sample types were
8 collected from each animal. The majority of the samples consisted of feces, urine, serum
9 and stomach and intestinal contents. Samples were frozen as soon as possible after
10 collection to prevent degradation, although some stranded animals had various levels of
11 degradation. Samples were stored frozen until shipped to the Northwest Fisheries Science
12 Center's Wildlife Algal-Toxin Research and Response Network (WARRN-West)
13 laboratory (Seattle, WA, USA) for algal toxin testing. All live and stranded animal
14 handling was consistent with approved humane practices under the following permits:
15 Marine Mammal Protection Act (MMPA) permit number MA041309-5, and National
16 Marine Fisheries (NMFS) research permit numbers 358-1787, 15324, and 10091. A
17 summary of the total number of animals, collection period, and locations is shown in
18 Table 1. Additional detailed information on sample collection is provided in the sections
19 below.

20

21 2.1.1 *Humpback whale fecal, stomach and intestinal contents, aqueous humor, pleural*
22 *fluid, and urine samples (n = 8 animals):* During 2007-2011, samples were collected
23 from stranded humpback whales from Southeast Alaska (n = 5), Kodiak (n = 2) and The

1 Alaska Peninsula (n = 1). Samples were stored frozen in Whirl-Pak® bags at -40 or -80
2 °C until analyzed for algal toxins.

3

4 *2.1.2 Bowhead whale fecal samples (n = 25 animals):* During 2006-2011, fecal samples
5 from bowhead whales harvested for subsistence purposes were collected during the
6 spring and fall in Barrow, Alaska. Sections of colon were cut and fecal matter was
7 removed using plastic spoons. Samples were stored frozen in Whirl-Pak® bags at -20 °C
8 until analyzed for algal toxins.

9

10 *2.1.3 Beluga whale fecal, stomach contents, amniotic fluid, pericardial fluid, and urine*
11 *samples (n = 15 animals):* During 2007-2012, samples were collected from stranded
12 Cook Inlet beluga whales. Three females were pregnant and samples were collected from
13 both the mother and the fetus in all three cases. Stomachs from two belugas harvested for
14 subsistence purposes at Hooper Bay were also collected. Samples were stored frozen in
15 Whirl-Pak® bags at -40 or -80 °C until analyzed for algal toxins.

16

17 *2.1.4 Harbor porpoise fecal, stomach and intestinal contents, aqueous humor, and*
18 *urine (n = 5 animals):* During 2010-2013, samples were collected from five stranded
19 harbor porpoises from Cook Inlet, with both mother and fetus analyzed in one case.
20 Samples included feces (n = 2), aqueous humor (n = 1), stomach (n = 1) and intestinal
21 contents (n = 1), and urine (n = 2) with some animals having multiple sample types
22 analyzed. Samples were stored in Whirl-Pak® bags at -40 or -80 °C until analyzed for
23 algal toxins.

1

2 2.1.5 *Northern fur seal fecal and serum samples (n = 179 animals)*: Between 7-15
3 October 2010, serum samples were collected from 131 live-captured adult female
4 northern fur seals with pups on Saint George Island (Pribilof Islands) and fecal samples
5 were collected from 48 northern fur seals harvested on Saint Paul Island (Pribilof Islands)
6 for subsistence purposes. Samples were frozen in cryovials or Whirl-Pak® bags and
7 stored at -20 °C until analyzed for algal toxins.

8

9 2.1.6 *Steller sea lion fecal, stomach and intestinal contents, amniotic, pleural,*
10 *peritoneal and pericardial fluid, bile, and urine samples (n = 42 animals)*: During 2004-
11 2013, samples were collected from 42 stranded Steller sea lions, some of which were
12 rookery pups and aborted fetuses on rookeries. Stranded animals were sampled across
13 Alaska from southeast Alaska through Prince William Sound and through the Aleutian
14 Islands. Samples were collected in amber vials (bile), Whirl-Pak® bags (feces, and
15 stomach and intestinal contents) and cryovials (urine, and amniotic, pleural, peritoneal
16 and pericardial fluid) and stored at -80 °C until analyzed for algal toxins.

17

18 2.1.7 *Harbor seal bile, feces, aqueous humor, placenta, and urine samples (n = 9*
19 *animals)*: During 2008-2012, samples were collected from nine stranded harbor seals;
20 three in Southeast Alaska (Bartlett Cove in Glacier Bay, Lynn Canal, and Sitka), four in
21 Southcentral Alaska (Kachemak Bay, Resurrection Bay, Kenai, and Cook Inlet), one in
22 Southwest Alaska (Izembek Lagoon), and one in Bristol Bay (Egegik). Samples were

1 collected in amber vials (bile), Whirl-Pak® bags (feces and placenta) or cryovials
2 (aqueous humor and urine) and stored at -60°C until analyzed.

3

4 *2.1.8 Ice seals (ringed (n = 113), bearded (n = 55), spotted (n = 158), and ribbon seals*
5 *(n = 21)) stomach/intestinal contents, fecal, and urine samples:* During 2006-2013,
6 samples from ice seals harvested during spring and fall for subsistence purposes were
7 collected from the coastal communities of Hooper Bay, Savoonga, Gambell, Little
8 Diomede, Shishmaref, Kotzebue, Point Hope, Wainwright, and Barrow in the Bering
9 Strait region and Chukchi Sea. Whole stomachs or a piece of intestine were collected in
10 Ziploc® bags. Urine was collected in a centrifuge tube. All samples were frozen and
11 shipped to the Alaska Department of Fish and Game (ADF&G) laboratory in Fairbanks
12 and stored at -20°C. Stomachs and intestines were thawed and 5 ml of content was
13 removed from each, placed in centrifuge tubes with screw caps, and refrozen. During
14 May of 2009-2010, live captures were conducted by the National Marine Mammal Lab
15 (Alaska Fisheries Science Center's Polar Ecosystems Program) and samples were
16 obtained from ice floes and collected using a metal shovel to scoop the urine soaked ice
17 which was placed in Whirl-Pak® bags and frozen at -80°C until analyzed for algal toxins.

18

19 *2.1.9 Pacific walrus stomach and intestinal content samples (n = 82 animals):* During
20 May 2012-2013, stomach and intestinal contents were collected from walrus harvested
21 for subsistence purposes from the coastal communities of Gambell and Savoonga on
22 Saint Lawrence Island. Hunters collected the samples in situ and brought them to shore
23 where they were frozen on site at -18°C and shipped to the ADF&G laboratory in

1 Fairbanks and subsequently stored at -20°C until algal toxin analysis. Stomachs and
2 intestines were thawed and 5 ml of content was removed and placed in centrifuge tubes
3 with screw tops and refrozen.

4

5 2.1.10 *Sea otter urine, pericardial fluid, and maternal milk samples (n = 193 animals):*

6 From 2004-2011, samples were collected from Northern sea otter carcasses (n=172)
7 recovered in the Gulf of Alaska and Aleutian Islands - notably Sitka, Juneau, Glacier
8 Bay, Yakutat Bay, Prince William Sound, lower Kenai Peninsula, lower Cook Inlet,
9 Kodiak, eastern Aleutians, and Cold Bay. Additionally, urine samples (n=21) were
10 collected during 2011 from live-captured otters from the northern end of Kuiu Island in
11 the southern Gulf of Alaska. Samples were collected and stored at -20°C until analyzed
12 for algal toxins.

13

14 2.2 *Sample extraction for toxin analysis:*

15 All samples were thawed at room temperature. Depending on the amount of
16 sample available, 1-4 g was weighed out or 1-4 ml was aliquoted into a 15 ml
17 polypropylene screw-cap tube (Falcon-BD). The initial extraction step was carried out by
18 adding 50% aqueous methanol (for DA extraction) or 80% ethanol (for STX extraction)
19 to the sample in a 1:4 wt/wt ratio (1 part sample, 3 parts solvent) and thoroughly
20 vortexing the sample. For fecal material, stomach contents and intestinal contents,
21 samples were homogenized for at least 60 seconds using an Omni ES homogenizer. The
22 homogenized sample was then centrifuged at 10,000 x g (Sorvall RC 5C Plus centrifuge)
23 for 20 minutes at 4°C. The supernatant was then filtered through a 0.22 µm membrane

1 microcentrifuge tube filter (Millipore Ultrafree-MC centrifugal concentration device,
2 Durapore membrane, 0.22 µm pore size) and spun in a desk-top microcentrifuge
3 (Eppendorf model 5415C) for at least 10 minutes at a setting of 14. For urine, serum and
4 other body fluids, samples were sonicated (Branson Sonifier 450) at 50% pulse for 45
5 seconds at a setting of 5. Samples were then centrifuged at 10,000 x g for 20 minutes at
6 4°C. The supernatant was then filtered through a 25 mm diameter, 0.45 µm pore size
7 syringe filter (Pall Gelman Acrodisc PSF G x F with GHP membrane). All sample
8 extracts were stored at 4°C until analysis by ELISA.

9

10 2.3 *Quantification of algal toxins in marine mammal extracts:*

11 Algal toxins were quantified using commercially available enzyme-linked
12 immunosorbent assay (ELISA) kits; Biosense[®] DA ELISA for DA and Abraxis saxitoxin
13 ELISA for STX, following the instruction protocol supplied by the manufacturer
14 (Biosense[®] Laboratories, Bergen, Norway and Abraxis LLC, Warminster, PA) with slight
15 modifications based on sample matrix. These kits were originally developed for testing
16 shellfish rather than marine mammal samples. Consequently, testing in order to
17 determine matrix effects for feces, stomach and intestinal contents, urine, bile, aqueous
18 humor, serum, and milk were performed in a previous study (Lefebvre et al., 2010). For
19 DA ELISAs, the minimum dilutions of the 1:4 50% MeOH extracts required to eliminate
20 all matrix effects were 1:100 for feces and bile, 1:50 for milk and stomach and intestinal
21 contents, and 1:10 for urine, aqueous humor and serum, using our extraction methods and
22 ELISA kits. For STX ELISAs, a 1:50 dilution of the 1:4 80% ethanol extracts was
23 sufficient to eliminate matrix effects in all sample types. Additionally, the Abraxis

1 ELISA kit is designed to measure only STX (with some limited cross-reactivity to several
2 other PSP toxins, as listed in the Abraxis product documents), consequently all PSP
3 levels are listed as STX equivalents and as such, may underestimate the presence of other
4 congeners. With these minimum dilutions, the detection limits for DA in sample material
5 were, 4 ng/g or ml for feces and bile, 2 ng/g or ml for stomach and intestinal contents and
6 milk, and 0.4 ng/ml urine, aqueous humor and serum. The detection limit for STX in all
7 sample matrices was 3 ng/ml.

8

9 **3. Results**

10 Algal toxins were detected in at least one animal from all 13 species of marine
11 mammals sampled (n = 905 total animals; Tables 2 and 3). In addition, 46 individuals
12 contained detectable concentrations of both DA and STX, including three of eight
13 humpbacks, six of 25 bowheads, five of 110 ringed seals, three of 44 bearded seals, and
14 20 of 82 walruses tested for both toxins (Table 4). Saxitoxin and DA were present in
15 marine mammals sampled throughout our study area, from the southeastern Gulf of
16 Alaska to the eastern Beaufort Sea (Figure 1). Domoic acid was detected in more animals
17 (Table 2) and species (all 13) than STX (10 species; Table 3).

18

19 3.1 *Domoic acid*

20 Bowhead whales had the greatest prevalence of DA (68%), followed by harbor
21 seals (67%), walruses (41%), harbor porpoises (40%), humpback whales (38%), Steller
22 sea lions (27%), bearded seals (25%), northern sea otters (25%), ribbon seals (24%),
23 ringed seals (17%), beluga whales (13%), northern fur seals (5%), and spotted seals (3%;

1 Table 2). The highest concentrations were found in feces, stomach contents, intestinal
2 contents, and urine (Table 2). The maximum DA concentration detected was from the
3 intestinal contents of a 15-year-old female walrus (6,457 ng/g) from the northern Bering
4 Sea. Domoic acid was also detected in three fetuses (one beluga, one harbor porpoise and
5 one Steller sea lion). Figure 2 shows all DA positive fecal, gastrointestinal, and urine
6 samples for all species examined. Additionally, DA concentrations in feces and urine
7 from ten California sea lions (CSLs) sampled from the central California coast that were
8 exhibiting clinical signs of DA toxicosis (seizures) are shown in red in Figure 2 for
9 comparison. These CSLs were selected from Appendix A, Table 1 of a report on toxin
10 detection methods for marine mammals where both urine and feces were analyzed for
11 comparison in multiple animals (Frame and Lefebvre, 2012). The identification numbers
12 were used to access the Marine Mammal Center Database to determine which animals
13 were observed to have seizures.

14

15 3.2 *Saxitoxin*

16 Humpback whales had the greatest prevalence of STX (50%), followed by
17 bowhead whales (32%), walruses (28%), northern sea otters (23%), ringed seals (14%),
18 bearded seals (14%), Steller sea lions (10%), beluga whales (8%), northern fur seals
19 (5%), and spotted seals (1%; Table 3). The highest STX concentrations were detected in
20 feces, stomach and intestinal contents, and urine (Table 3). The maximum STX
21 concentration was detected from the intestinal contents of a 21-year-old male walrus (240
22 ng STX equiv./g; Table 3) from the northern Bering Sea. Additionally, this walrus also
23 contained a high concentration of DA (991 ng/g) in the intestinal contents sample.

1

2 **4. Discussion**

3 The number of species and the extensive geographic range in which DA and STX
4 were detected demonstrates that HABs are present throughout Alaska's marine
5 environment and thus the potential for health effects due to exposure is present for all 13
6 Alaskan marine mammal species tested in this study. For adult marine mammals, DA and
7 STX are being ingested through prey, however, in the case of DA, fetuses and suckling
8 young can also be exposed through amniotic fluid and milk (Rust et al., 2014). Maternal
9 transfer of DA has been well documented by laboratory studies and natural
10 environmental exposures with California sea lions (Maucher and Ramsdell, 2005;
11 Maucher and Ramsdell, 2007; Ramsdell and Zabka, 2008). In the present study, one
12 beluga whale fetus, one harbor porpoise fetus, and one Steller sea lion fetus contained
13 detectable concentrations of DA in stomach contents and feces, further documenting the
14 risk of maternal transfer of toxins from pregnant females with environmental exposures
15 to these biotoxins. Additionally, several sea otter pups and harbor seal neonates contained
16 detectable concentrations of DA, however whether they were actively nursing is
17 unknown. The diets of the 13 species tested in this study are varied due to their diverse
18 marine mammal life histories and range from zooplankton, to benthic invertebrates, to
19 finfish.

20

21 *4.1 Cetaceans*

22 *4.1.1 Humpback whales:* In Alaska, humpback whales seasonally range from the
23 southern Gulf of Alaska to the Chukchi Sea during the summer months. During winter

1 they migrate south to Mexico, Baja California and the Hawaiian Islands to breed and
2 calve. Feeding in cooler Alaskan waters typically occurs during the spring, summer and
3 fall months (Baker et al., 1986). There may be resident populations of humpback whales
4 in the southeastern Gulf of Alaska. In Alaska, their diet consists of krill and many
5 different kinds of fish including herring (*Clupea pallasii*) and capelin (*Mallotus villosus*);
6 all of which are planktivorous and therefore likely vectors of DA and STX (Bargu et al.,
7 2002; Doucette et al., 2005; Lefebvre et al., 2002a). A lower percentage of humpbacks
8 tested positive for DA (38%, highest concentration = 51 ng/g feces) (Table 2) than STX
9 (50%, highest concentration = 62 ng/g) (Table 3). The highest DA and STX
10 concentrations were found in an individual that died from a ship strike, which may not be
11 a coincidence because STX and DA intoxication have been suggested to be a factor in the
12 loss of ability to avoid ships and to be a cause of stranding (Geraci et al., 1989).

13

14 *4.1.2 Bowhead whales:* The entire population of western Arctic bowhead whales
15 ranges through Arctic Alaskan waters from the central Bering Sea to the Canadian
16 Beaufort Sea during their annual migration cycle. Bowhead whales are an important
17 subsistence species for western and northern Alaska providing more than ten villages
18 with substantial meat and blubber each year. This stock of bowhead whales is listed as
19 endangered under the Endangered Species Act (ESA), however the population is
20 increasing (3% annually) and believed to have recovered substantially (George et al.,
21 2004; Gerber et al., 2007; Givens et al., 2013; Zeh and Punt, 2005), suggesting that the
22 current reduction in sea ice has had no detectable negative effects on population growth.
23 Bowheads feed on small zooplankton consisting mainly of calanoid copepods and

1 euphausiids, both of which consume phytoplankton (Moore et al., 2010) and are likely
2 the source of the high occurrence rates of DA (68%) and STX (32%) in fecal samples.

3

4 4.1.3 *Beluga whales*: In Alaska, there are four stocks of beluga whales, which
5 range from the Bering Sea to the Canadian Beaufort Sea. These stocks are abundant and
6 support subsistence harvests. In addition, there is a fifth stock in Cook Inlet, a tidal
7 estuary located in the northern Gulf of Alaska. All but two of the animals sampled were
8 part of the Cook Inlet stock. This stock is the most genetically isolated (O'Corry-Crowe et
9 al., 2002), was listed in 2008 as “endangered” under the ESA, and is not currently
10 showing signs of recovery; no harvest is currently allowed. Generally, beluga whales
11 prey on a wide variety of fish, crustaceans, and cephalopods. In Cook Inlet, primary prey
12 species consist of at least three species of Pacific salmon (Chinook, *Oncorhynchus*
13 *tshawytscha*; chum, *O. keta*; and coho, *O. kisutch*), which have been found in beluga
14 stomachs, however it is likely that sockeye (*O. nerka*) and pink salmon (*O. gorbuscha*)
15 are also eaten when they are available (Quakenbush et al., 2015). In addition, eulachon
16 (*Thaleichthys pacificus*), Pacific cod (*Gadus macrocephalus*), walleye pollock (*Theragra*
17 *chalcogramma*), saffron cod (*Eleginus gracilis*), starry flounder (*Platichthys stellatus*)
18 and yellowfin sole (*Limanda aspera*) have also been found in the stomachs of Cook Inlet
19 belugas. Seven types of invertebrates were found in Cook Inlet beluga stomachs, with the
20 frequency of occurrence in non-empty stomachs being highest for shrimp (39%),
21 followed by polychaetes and amphipods (Quakenbush et al., 2015). Invertebrates appear
22 to be much less important to Cook Inlet beluga diet compared to the other stocks.

1 Therefore, analysis of HABs in other beluga stocks would be of interest (Moore et al.,
2 2000).

3 The two belugas sampled from the Eastern Bering stock did not have detectable
4 levels of DA and were not tested for STX. A relatively low percentage of Cook Inlet
5 beluga whales we examined were positive for DA (13%) and fewer for STX (8%). In
6 addition, all concentrations of both toxins were low with the highest level of DA at 7 ng/g
7 from stomach contents of one animal and STX at 4 ng/g feces in another, the only STX
8 positive beluga (Tables 2 and 3). This may be because beluga prey consists of fewer
9 planktivorous fish and invertebrate species. Although DA has been shown to be widely
10 distributed in fish species in California, the non-planktivorous fish species contained
11 lower concentrations of toxin compared to planktivorous species such as anchovies and
12 sardines (Lefebvre et al., 2002a; Lefebvre et al., 2002b). This is similarly true for STX in
13 that PSP toxins can be found in several of the prey species including Pacific cod and
14 chum salmon, but at lower levels. Crabs and polychaetes are known to concentrate STX
15 (Deeds et al., 2008).

16

17 *4.1.4 Harbor porpoises:* Harbor porpoises are widespread in the Northern
18 Hemisphere, found in most cool temperate and subpolar waters (Jefferson et al., 1993),
19 including coastal and inland waters, and are seldom found in waters with an annual
20 average temperature above 17°C (Read, 1999). They generally forage on small, pelagic
21 schooling fish in waters less than 200 m deep (Shelden et al., 2014). Harbor porpoises are
22 not harvested for food in Alaska. In Alaskan waters, harbor porpoise stock structure is
23 unclear, but three stocks are currently recognized for management purposes: Southeast

1 Alaska, Gulf of Alaska, and Bering Sea (Shelden et al., 2014), all of which belong to the
2 subspecies *P.p. yomerina*. Harbor porpoises eat a wide variety of fish, cephalopods and
3 benthic invertebrates with the main prey items varying by region and season (Culik,
4 2004; Jefferson et al., 1993; Reyes, 1991). In Cook Inlet, harbor porpoises feed on
5 schooling planktivorous fish such as smelt (Family Osmeridae) and Pacific herring
6 (*Clupea pallasii pallasii*) (Shelden et al., 2014), which are known to accumulate DA.
7 Harbor porpoise samples were collected primarily from Cook Inlet with one animal each
8 from Prince William Sound and Kachemak Bay. The highest concentration of DA was
9 15.3 ng/g, which occurred in the feces of one animal and the intestinal contents of
10 another animal. One of these was pregnant and the fetus also contained DA at 8 ng/g in
11 its feces documenting maternal transfer of algal toxins. Saxitoxin was not detected in any
12 of the harbor porpoise samples. The higher prevalence of DA is likely a result of a
13 planktivorous fish diet.

14

15 4.2 *Otariids*

16 4.2.1 *Northern fur seals*: Northern fur seals breed and give birth on the Pribilof
17 Islands of Alaska in the southern Bering Sea during the summer (June-August) and pups
18 remain dependent until mid-November when the rookeries are abandoned for the winter
19 and weaning occurs abruptly. Immature males are harvested for food in summer. In
20 Alaska, the majority of northern fur seals winter in the North Pacific and return to
21 Alaskan waters the following spring. Movements from seals tagged with satellite-linked
22 transmitters indicate that all sex and age classes can migrate thousands of kilometers as
23 far west as Kamchatka and east to the west coast of the USA (Baker, 2007; Lea et al.,

1 2009; Pelland et al., 2014; Ream et al., 2005; Sterling et al., 2014). Newly weaned pups
2 may remain south of the Aleutian chain for two or more years before returning to the
3 Pribilofs to breed. Northern fur seals in Alaska predominantly forage on schooling fish
4 and gonatid squid with walleye pollock representing approximately 40-75% of the prey
5 observed in scat collections (Ream et al., 2005; Sinclair et al., 1994). Little is known
6 about the recent winter diet since pelagic sealing and the collection of seals for scientific
7 purposes has ceased. Collections made during 1958-1974 in the eastern Bering Sea,
8 however, indicated that in addition to pollock, northern fur seals consumed capelin,
9 Pacific herring, and squid (Perez and Big, 1986). The serum samples for this study were
10 collected from adult female fur seals with young pups. Provisioning pups with milk every
11 few days limits the distance that females can forage to relatively local waters near the
12 Pribilof Islands and may explain the relatively low occurrence of DA and STX (both at
13 5% of the animals tested; Tables 2 and 3).

14

15 4.2.2 *Steller sea lions*: Steller sea lions range throughout the Pacific Rim from
16 southern California, across the Gulf of Alaska, to Northern Honshu Island in Japan, and
17 north into the Bering Strait (Lander et al., 2009). In Alaska, they are harvested for food
18 and managed as two stocks, the eastern distinct population segment (DPS) and western
19 DPS. From 1980 to 2000 there was a greater than 80% population decline in the western
20 DPS, which included Russian and Alaskan waters of the Gulf of Alaska, North Pacific
21 Ocean, and Bering Sea, leaving fewer than 55,000 individuals (Lander et al., 2009).
22 Steller sea lions were listed as threatened under the ESA in 1990, and the western portion
23 of the population was reclassified as endangered in 1997. The cause of the decline is

1 unknown. The population has stabilized in the Gulf of Alaska and the eastern DPS, but
2 continues to decline in the western and central Aleutian Islands. Adult Steller sea lions
3 eat a wide variety of fish, with either walleye pollock or Atka mackerel (*Pleurogrammus*
4 *monopterygius*) predominant in most areas. Other prey consists of schooling fish,
5 including Pacific herring and salmon (*Oncorhynchus spp.*), with smaller numbers of
6 Pacific sand lance (*Ammodytes hexapterus*), capelin, eulachon, Pacific cod, Pacific hake
7 (*Merluccius productus*), flatfish, demersal fish, and cephalopods (Merrick et al., 1997).
8 Several of these prey species are planktivorous including herring, juvenile chum salmon,
9 walleye pollock and sand lance.

10

11 4.3 Harbor seals

12 In Alaska, harbor seals are primarily found in coastal waters throughout the Gulf
13 of Alaska, Aleutian Islands, and Southeastern Bering Sea where they are harvested for
14 food (Small et al., 2003). They are found in diverse habitats including glacial and non-
15 glacial areas and are generally non-migratory (Bigg, 1981). Harbor seals mainly forage
16 on fish including Pacific herring, rainbow smelt (*Osmerus mordax*), salmon
17 (Salmonidae), walleye pollock, Pacific cod, greenling (*Hexagrammidae*), sculpins
18 (*Cottidae*), Pacific sand lance, and flatfish (*Pleuronectidae*) (Pitcher, 1980a, 1980b).
19 Invertebrates such as octopus, squid, and shrimp are also consumed (Pitcher, 1980a,
20 1980b). The importance of these prey items varies by location. In the Bering Sea and
21 Gulf of Alaska, pollock and octopus are the most common prey items, whereas shrimp
22 and capelin are most common in the southeastern Gulf of Alaska (Pitcher, 1980b). No
23 harbor seals tested positive for STX, however six of nine animals tested positive for DA,

1 although the maximum concentration was low; 8 ng/g feces (Table 2). Harbor seals had a
2 much higher percentage of individuals that tested positive (67%) than spotted seals (3%)
3 even though they consume similar fish species. This could be due to the more southern
4 range of harbor seals compared to the spotted seal's more northerly range in the Bering
5 Sea. Most harbor seals sampled were from the Gulf of Alaska, which has warmer waters
6 and as such are more likely to be exposed to HABs, although no data are available on
7 HAB or shellfish toxicity for verification. Additionally, the samples sizes tested were
8 vastly different (n = 9 for harbor seals and n = 158 for spotted seals) making direct
9 comparisons difficult.

10

11 4.4 *Ice seals (Ringed, Bearded, Spotted and Ribbon)*

12 Ringed, bearded, and spotted seals are sea ice-associated seals that range
13 throughout the Bering, Chukchi, and Beaufort seas in Alaska (Burns, 1970). Of these
14 species, only ringed seals are currently ESA listed. Bearded seals were listed under ESA,
15 but a court overturned the decision, which is being appealed by the National Marine
16 Fisheries Service (NMFS). Ribbon seals are also ice-associated and occur throughout the
17 Bering and Chukchi seas, but are not often found in the Beaufort Sea (Burns, 1981).
18 Although movements in winter months are restricted by sea ice, these seals move widely
19 in spring, summer, and fall (Burns, 1970; Burns, 1981; Crawford et al., 2012; Harwood et
20 al., 2012a; Harwood et al., 2012b; Lowry et al., 2000). Ringed and bearded seals tend to
21 inhabit areas that are seasonally ice covered and are found in heavy pack ice. Spotted
22 and ribbon seals are less ice dependent at certain times of the year and can be found near
23 the ice edge, in the broken pack ice, of the Bering Sea in winter and spring. The

1 distribution of spotted seals shifts northward and toward the coasts as sea ice recedes in
2 May and June and many spotted seals enter bays and rivers and haul out on sand bars and
3 barrier islands (Burns et al., 1981). The distribution of ribbon seals also shifts northward
4 as sea ice recedes in May and June. When sea ice melts, however, the majority of the
5 ribbon seal population likely becomes pelagic in the North Pacific and the central Bering
6 Sea, although some seals follow receding ice into the Chukchi Sea (Burns et al., 1981).
7 All four species are harvested for food, mostly in spring and fall.

8 The diets of ringed, bearded, spotted, and ribbon seals vary widely. Ringed seals
9 feed mostly in the water column on pelagic and semi-demersal fish (including arctic cod,
10 *Boreogadus saida*; saffron cod, walleye pollock, and sculpins) and invertebrates
11 (including mysids, amphipods, shrimps and echiurids) (Crawford et al., 2015; Dunbar,
12 1941; Fedoseev, 1965; Johnson et al., 1966; Lowry et al., 1980; McLaren, 1958).
13 Bearded seals feed on a wide variety of benthic invertebrates (including bivalves,
14 gastropods, cephalopods, isopods, amphipods, shrimp, crab, echiurids and polychaetes)
15 and fish (including arctic and saffron cod; sculpins; snailfish (Liparidae); pricklebacks
16 (Stichaeidae); Pacific sandlance, and flatfish) (Antonelis et al., 1994; Burns, 1981;
17 Chapskii, 1938; Crawford et al., 2015; Dunbar, 1941; Quakenbush et al., 2011; Smith,
18 1981). Spotted seals eat mostly pelagic fish including arctic and saffron cod; Pacific
19 herring; and smelt (Bukhtiyarov et al., 1984; Frost and Lowry, 1981; Quakenbush et al.,
20 2009). Ribbon seal diet is less well documented because most have empty stomachs
21 when they are harvested in the late spring. But their diet is most similar to spotted seals
22 and includes fish (arctic and saffron cod and pollock), shrimp (*Crangonid* and *Pandalid*

1 species) and octopus (Dehn et al., 2007; Frost and Lowry, 1980; Quakenbush and Citta,
2 2008).

3 Bearded (25%) and ribbon seals (24%) were similar in the percent sampled that
4 contained DA. Fewer ringed seals (17%) were positive, but a female ringed seal pup had
5 the highest concentration of DA (127 ng/g) of any of the ice seals. Spotted seals were the
6 lowest (3% positive). Bearded and ringed seals were both 14% positive for STX and
7 again ringed seals had the higher concentration (172 ng/g feces). Spotted seals were
8 lower at 1% and STX was not detected in any ribbon seals. We would expect bearded
9 seals, as benthic feeders, to be most vulnerable to STX. We would also expect spotted
10 and ribbon seals, as fish-eaters, to be least vulnerable to STX. The higher values for both
11 DA and STX for ringed seals may be due to some individuals consuming larger volumes
12 of mysids, euphausiids, or amphipods (all of which eat algae and detritus) and may
13 explain their higher exposure to HABs.

14

15 4.5 *Pacific walrus*

16 Pacific walruses are migratory, following the southern margins of the pack ice
17 from the Bering Sea to the Chukchi Sea in the spring, where foraging is optimal in the
18 relatively shallow shelf waters (Estes and Gilbert, 1978; Fay, 1982; Gilbert, 1989).
19 Walruses are harvested for subsistence and in addition to walrus tissues, clams found in
20 the stomach during butchering are also eaten by harvesters. The Pacific walrus population
21 is currently a candidate species under the ESA due to concern regarding the species'
22 response to changes in summer sea ice habitat (Robards and Garlich-Miller, 2013;
23 USFWS, 2011). Walruses feed primarily on benthic invertebrates including marine

1 worms (e.g., polychaetes, sipunculids, echiurids priapulids), mollusks (e.g., bivalves and
2 gastropods), and crustaceans (e.g., amphipods, shrimp, crabs) (Born et al., 2003; Bowen
3 and Siniffand, 1999; Dehn et al., 2007; Fay, 1982; Sheffield et al., 2001; Sheffield and
4 Grebmeier, 2009) although fish and other vertebrates (including seals) are also
5 occasionally reported (Fay, 1982; Seymour et al., 2014; Sheffield et al., 2001; Sheffield
6 and Grebmeier, 2009). Walruses are not physiologically adapted for deep diving and
7 concentrate foraging efforts in shallower waters, typically using the sea ice as a resting
8 platform between feeding trips (Fay, 1982). Since 2007, walruses summering in the
9 Chukchi Sea have been hauling out in large numbers at two terrestrial haulout sites on the
10 eastern Chukchi Sea (Icy Cape and Point Lay) beginning in late summer when sea ice
11 over the Continental Shelf disappears (Robards and Garlich-Miller, 2013).

12 Stomach contents from walruses sampled near St. Lawrence Island had the
13 highest measured concentrations of both DA and STX of any species examined in this
14 study (Tables 2 and 3). That 41% and 28% of walruses sampled contained elevated
15 concentrations of DA and STX, respectively, is surprising due to the sampling location.
16 These walruses were sampled in the northern Bering Sea during May, as they were
17 moving northward with the receding sea ice (Fay, 1982). Water temperatures with sea ice
18 present are not considered favorable to support HABs, although the DA and STX could
19 have come from invertebrates eaten farther south. The elevated toxin concentrations in
20 walruses suggest that DA and STX producing phytoplankton are well established in
21 seasonally ice covered waters to accumulate in clams within the foraging range of
22 walruses. That the highest concentrations of both DA and STX in this study came from
23 walruses and that the walrus with the highest concentration of STX also had relatively

1 high DA is cause for continued monitoring and investigation. The DA concentrations
2 detected in walrus are similar to those detected in California sea lions suffering from
3 DA toxicosis, although hunters did not report abnormal behavior in any of the sampled
4 walrus (Figure 2; (Lefebvre et al., 1999; Scholin et al., 2000).

5 Walrus and bearded seals are typically benthic feeders with overlapping ranges,
6 but the percent positive and maximum concentration for both DA and STX was higher
7 for walrus than bearded seals. This could be because bearded seals are more generalist
8 foragers than walrus.

9

10 4.6 *Sea otters*

11 Three stocks of northern sea otters are recognized in Alaska: southeast,
12 southcentral, and southwest (Gorbics and Bodkin, 2001). The southeast and southcentral
13 stocks are considered to be increasing. The southwest stock, however, was listed as
14 threatened under the ESA in 2005, but is currently believed to have stabilized (USFWS
15 2014).

16 The primary prey of sea otters in the Gulf of Alaska (Southeast Alaska, Prince
17 William Sound, Kachemak Bay and Kodiak Island) are clams, such as butter clams
18 (*Saxidomus giganteus*) (Calkins, 1978; Doroff and Bodkin, 1994; Doroff and Degange,
19 1994; Hoyt et al., 2014; Kvitek et al., 1993). In contrast, the diet in the Aleutian Islands is
20 dominated by sea urchins and a variety of finfish, including those in the families of
21 Hexagrammidae, Gadidae, Cottidae, Cyclopteridae, and Scorpaenidae (Estes et al., 1982;
22 Kenyon, 1969). The majority of the sea otter carcasses recovered and sampled for this

1 study were from the northern Gulf of Alaska (i.e. Kachemak Bay) where clams are an
2 important prey item (Doroff et al., 2012; Newsome et al., 2015).

3 Given that clams are the predominant prey items for sea otters in Alaska, the
4 percentage of sea otters containing detectable concentrations of DA and STX were lower
5 than expected (Tables 2 and 3) and in the case of STX, may be due to avoidance
6 behaviors. Although sea otters are not immune to PSP toxins, they can detect and avoid
7 lethal amounts of toxic prey (Kvitek and Bretz, 2004; Kvitek, 1991). However, acute
8 toxicosis from STX may have contributed to at least two sea otters being struck and
9 killed by boats in November 2009 in the Kodiak boat harbor (Gill et al. in prep). Urine
10 from these two otters were included in this study and had the highest concentrations of
11 STX (45 and >100 ng/g) for all otters tested. Their behavior prior to being hit by the
12 boats was suggestive of intoxication as they were lethargic and non-reactive at the
13 surface of the water.

14 Additionally, between May 12-28, 2011 sea otters (n=21) were live-captured
15 around Kuiu Island in Southeast Alaska (Figure 1). Urine was collected and they were
16 implanted with VHF transmitters and released (Hoyt et al., 2014). Twenty of these otters
17 contained detectable concentrations of STX (3.0-28.4 ng/ml urine). During capture and
18 handling, none of these animals exhibited any clinical signs (i.e. paralysis, difficulty
19 breathing) associated with STX toxicity. After being released they were relocated and
20 feeding was observed between August 2011 and May 2013. Tagged sea otters consumed
21 a total of 32 unique prey types (Hoyt et al., 2014). In terms of biomass, the three most
22 common prey items were clams (primarily *Saxidomus giganteus*) followed by green
23 urchins (*Strongylocentrotus droebachiensis*) and Dungeness crab (*Metacarcinus*

1 *magister*). Continuing to track DA and STX concentrations in sea otters will be important
2 in understanding threats to their populations especially in the ESA-listed stock. As otters
3 are a nearshore, highly visible species that do haul out on land in some human inhabited
4 Alaskan locations, it may be prudent to set up protocols for documenting and reporting
5 signs of HAB-related toxicosis.

6

7 *4.7 Toxic Exposure Levels and Data Limitations*

8 An understanding of how the concentrations reported here relate to those known
9 to cause clinical signs of toxicity (behavioral neuroexcitotoxicity for DA and paralysis for
10 STX) in mammals from other regions is needed in order to assess health risks to Alaskan
11 marine mammals. Data on STX concentrations quantified in marine mammals
12 experiencing toxicosis are lacking, however, data for DA concentrations are prevalent
13 due to the regular occurrence of DA toxicosis in California sea lions along the central
14 California coast, USA. Figure 2 compares concentrations of DA quantified in feces and
15 urine of ten acutely exposed California sea lions exhibiting seizures with concentrations
16 quantified in Alaskan species. These data suggest that Alaskan marine mammals may
17 already be near toxic exposures particularly in humpback and bowhead whales, ringed,
18 bearded and spotted seals, Pacific walruses and sea otters (Figure 2A & B).

19 A major limitation in the assessment of health risks is that toxin concentrations in
20 marine mammal samples are not directly related to the magnitude of an animal's
21 exposure. For example, not all California sea lions with acute behavioral signs of toxicity
22 (e.g., seizures) have high concentrations of DA in feces and urine because elimination
23 rates are rapid (Lefebvre et al., 1999)(Figure 2). Passage rates for captive sea lions fed

1 Pacific herring averaged less than 5 hours (Helm, 1984) and laboratory studies have
2 reported 99% of algal toxin is eliminated through urine within 24 hours of dosing (Suzuki
3 and Hierlihy, 1993). Therefore, the concentrations presented here provide proof of
4 exposure risk and evidence for potential neurotoxic impacts to several marine mammal
5 species in Alaska.

6

7 **5. Conclusions**

8 Our results demonstrate that the algal toxins DA and STX are present in Alaskan
9 Subarctic and Arctic ecosystems and have the potential to affect most marine mammal
10 species in USA waters farther north than expected. Given the current trend of decreasing
11 sea ice and warming ocean waters that will extend the open water season favorable to
12 HABs, the prevalence and concentrations of DA and STX documented in this study are
13 expected to increase creating a greater risk to marine mammals. Clinical signs of
14 neurotoxicity were not confirmed in the present study, however many of the animals were
15 dead when sampled. Additionally, toxin effects could contribute to an increase in ship
16 strikes for large cetaceans and increased vulnerability to subsistence harvested seals,
17 walrus and whales, both of which would be difficult to detect due to concurrent
18 increases in ship traffic and changes in ice and weather patterns that affect hunting. Sea
19 lions along the central California coast provide a cautionary example of increasing HAB
20 impacts on marine mammal health. This threat to marine mammals was first recognized
21 in 1998, and now has a major impact on sea lions annually. Recent studies have
22 suggested that HABs are also affecting large cetaceans in southern latitudes such as the
23 Minke whale (*Balaenoptera acutorostrata*) (Fire et al., 2010). This study documents the

1 presence of HAB toxins in top predators from southeast Alaska to the Arctic Ocean
2 revealing a potentially growing exposure risk to northern marine mammal populations.
3 Unless unknown factors inhibit HABs in northern waters, warming water temperatures
4 and increased light availability due to loss of sea ice are likely to support more blooms
5 increasing toxin concentrations and the health risks they present for northern marine
6 mammal species as they have for southern species.

7
8

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5

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Figure 1: Locations where algal toxins were detected in stranded (s) and harvested (h) marine mammals. Red images represent species positive for domoic acid (DA) and purple images represent species positive for saxitoxin (STX). Marine mammal species are listed as follows; A) Humpback whales, B) Bowhead whales, C) Beluga whales, D) Harbor porpoises, E) Northern fur seals, F) Steller sea lions, G) Harbor seals, H) Ringed seals, I) Bearded seals, J) Spotted seals, K) Ribbon seals, L) Pacific walruses, and M) Northern sea otters.

Figure 2: Domoic acid (DA) concentrations quantified in A) feces and gastrointestinal (GI) contents, and B) urine for all Alaskan species sampled. Domoic acid concentrations detected in 10 California sea lions (CSL) exhibiting signs of DA toxicosis (seizures) are included for reference and shown in the box.

Figure 2

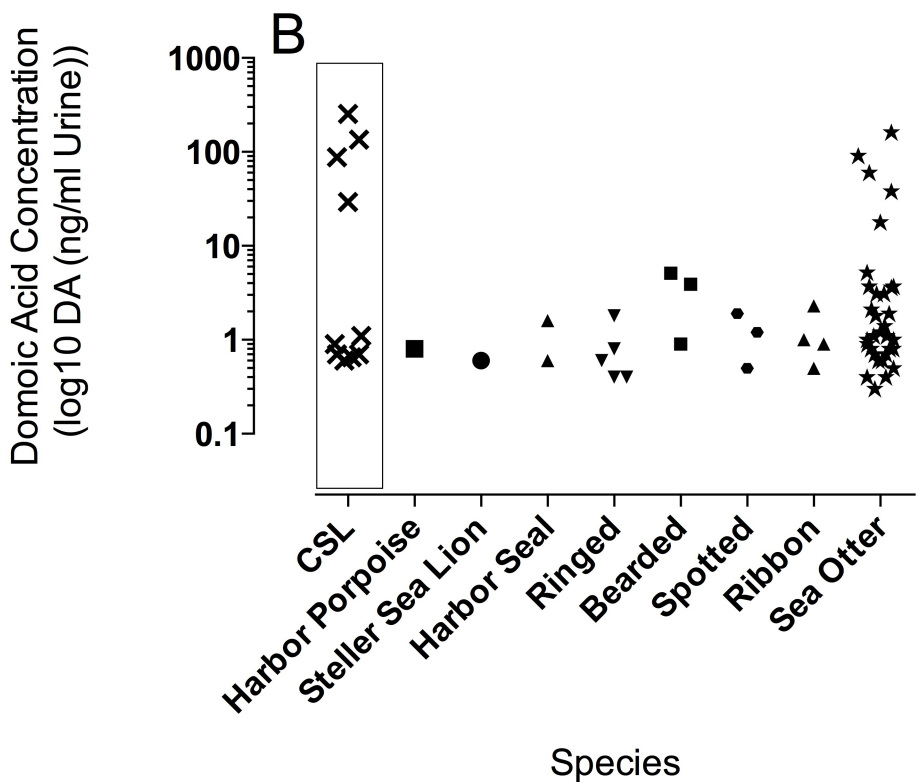
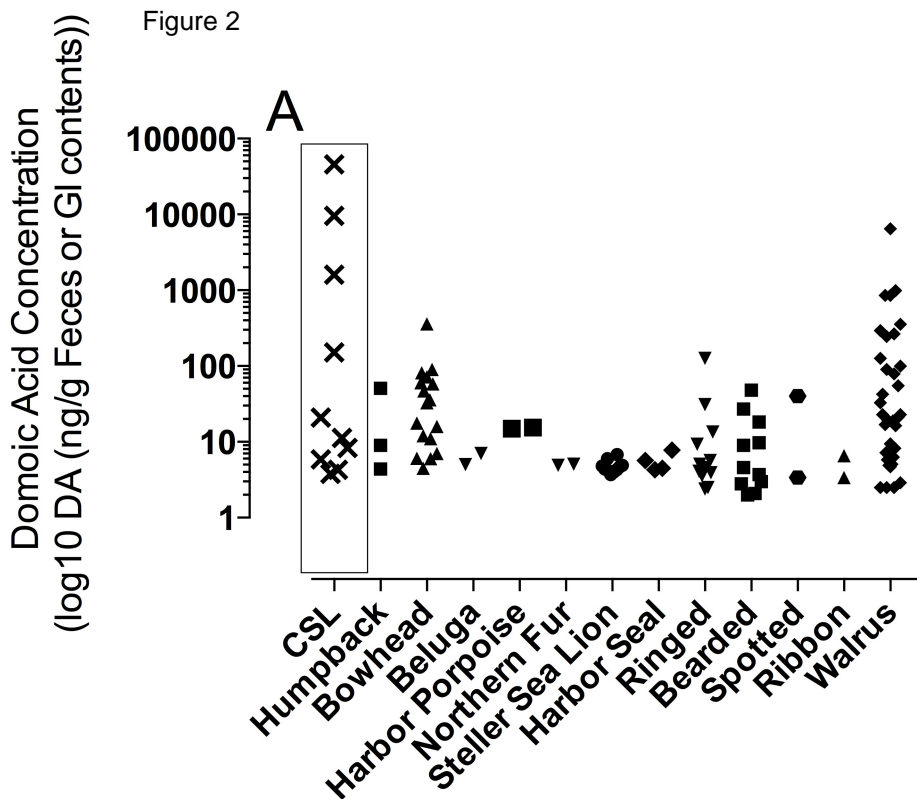


Table 1: List of species, collection status, period and locations, and total number of animals for each species of the 905 marine mammals sampled in Alaska (AK).

Species	Collection status	Collection period	Collection locations	Total # of animals
Humpback	Stranded	July 2007 to Sept. 2011	Kodiak, The AK Peninsula, Southeast	8
Bowhead	Harvested	Spring & Fall 2006 to 2011	Barrow	25
Beluga	Stranded & Harvested	Sept. 2005 to Oct. 2012	Cook Inlet, Hooper Bay	15
Harbor porpoise	Stranded	Aug. 2008 to July 2011	Cook Inlet	5
Northern fur seal	Harvested & Live Capture	2010	Saint George & Saint Paul Islands	179
Steller sea lion	Stranded	May 2004 to March 2013	Gulf of AK	42
Harbor seal	Stranded	May 2008 to Aug. 2012	Gulf of AK, Egegik	9
Ringed seal	Harvested	Nov. 2006 to Nov. 2012	Barrow, Chukchi Sea, Bering Sea	113
Bearded seal	Harvested	Oct. 2007 to June 2013	Barrow, Chukchi Sea, Bering Sea	55
Spotted seal	Harvested & Snow Urine	Nov. 2006 to Nov. 11	Barrow, Chukchi Sea, Bering Sea	158
Ribbon seal	Harvested & Snow Urine	May 2009 to Oct. 2012	Barrow, Chukchi Sea, Bering Sea, Yakutat	21
Pacific walrus	Harvested	May & June in 2012 & 2013	Saint Lawrence Island	82
Northern sea otter	Stranded & Live Capture	April 2004 to May 2011	Gulf of AK	193

Table 2: Summary of the number of domoic acid-positive individuals from 13 species of Alaskan marine mammals, including the sample matrix with the highest concentration. F = Feces, SC = Stomach Contents, S = Serum, IC = Intestinal Contents, U = Urine.

Species	Number of animals	Number positive	% Positive	Max conc. (ng/g or ml)	Sample matrix
Cetaceans					
Humpback whale	8	3	38%	51	F
Bowhead whale	25	17	68%	359	F
Beluga whale	15	2	13%	7	SC
Harbor porpoise	5	2	40%	15	F
Otariids					
Northern fur seal	179	8	5%	14	S
Steller sea lion	44	12	27%	7	SC
Phocids					
Harbor seal	9	6	67%	8	F
Ringed seal	113	19	17%	127	F
Bearded seal	55	14	25%	48	IC
Spotted seal	158	5	3%	40	SC
Ribbon seal	21	5	24%	7	F
Odobenids					
Pacific walrus	82	34	41%	6,457	SC
Mustelids					
Northern sea otter	172	43	25%	162	U
Total number	886	188	21%		

Table 3: Summary of the number of saxitoxin-positive individuals from 13 species of Alaskan marine mammals, including the sample matrix with the highest concentration. F = Feces, SC = Stomach Contents, IC = Intestinal Contents, U = Urine; na = not applicable.

Species	Number of animals	Number positive	% Positive	Max conc. (ng/g or ml)	Sample Matrix
Cetaceans					
Humpback whale	8	4	50%	62	F
Bowhead whale	25	8	32%	63	F
Beluga whale	12	1	8%	4	F
Harbor porpoise	5	0	0%	na	na
Otariids					
Northern fur seal	179	8	5%	42	F
Steller sea lion	42	4	10%	7	F
Phocids					
Harbor seal	8	0	0%	na	na
Ringed seal	110	15	14%	172	F
Bearded seal	44	6	14%	15	IC
Spotted seal	145	1	1%	3	SC
Ribbon seal	7	0	0%	na	na
Odobenids					
Pacific walrus	82	23	28%	240	IC
Mustelids					
Northern sea otter	163	37	23%	45	U
Total number					
	830	107	13%		

Table 4: Summary of animals (N = 46) that tested positive for both domoic acid (DA) and saxitoxin (STX) and mean (\pm sd) toxin values (ng/g or ml). Sample matrix includes: F= Feces, SC = Stomach Contents, IC = Intestinal Contents, and U = Urine.

Species	N	DA	STX	Matrix	Collection Locations
Humpback whale	3	21 \pm 26	30 \pm 28	F	Southeast Alaska
Bowhead whale	6	83 \pm 137	48 \pm 11	F	Barrow
Ringed seal	5	6 \pm 2	41 \pm 73	F/SC	Chukchi Sea, Bering Sea
Bearded seal	3	11 \pm 14	8 \pm 6	F/SC/U	Chukchi Sea, Bering Sea
Pacific walrus	20	524 \pm 1,432	64 \pm 80	IC	St. Lawrence Island
Northern sea otter	9	2 \pm 2	7 \pm 3	U	Kachemak Bay, Juneau, Glacier Bay
Total number	46				