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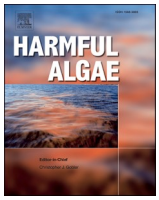

Paralytic shellfish toxins in Alaskan Arctic food webs during the anomalously warm ocean conditions of 2019 and estimated toxin doses to Pacific walruses and bowhead whales

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ABSTRACT

Climate change-related ocean warming and reduction in Arctic sea ice extent, duration and thickness increase the risk of toxic blooms of the dinoflagellate *Alexandrium catenella* in the Alaskan Arctic. This algal species produces neurotoxins that impact marine wildlife health and cause the human illness known as paralytic shellfish poisoning (PSP). This study reports Paralytic Shellfish Toxin (PST) concentrations quantified in Arctic food web samples that include phytoplankton, zooplankton, benthic clams, benthic worms, and pelagic fish collected throughout summer 2019 during anomalously warm ocean conditions. PSTs (saxitoxin equivalents, STX eq.) were detected in all trophic levels with concentrations above the seafood safety regulatory limit (80 μg STX eq. 100 g^{-1}) in benthic clams collected offshore on the continental shelf in the Beaufort, Chukchi, and Bering Seas. Most notably, toxic benthic clams (*Macoma calcarea)* were found north of Saint Lawrence Island where Pacific walruses (*Odobenus rosmarus*) are known to forage for a variety of benthic species, including *Macoma*. Additionally, fecal samples collected from 13 walruses harvested for subsistence purposes near Saint Lawrence Island during March to May 2019, all contained detectable levels of STX, with fecal samples from two animals (78 and 72 μg STX eq. 100 g^{-1}) near the seafood safety regulatory limit. In contrast, 64% of fecal samples from zooplankton-feeding bowhead whales (n = 9) harvested between March and September 2019 in coastal waters of the Beaufort Sea near Utqiaġvik (formerly Barrow) and Kaktovik were toxin-positive, and those levels were significantly lower than in walruses (max bowhead 8.5 μg STX eq. 100 g^{-1}). This was consistent with the lower concentrations of PSTs found in regional zooplankton prey. Maximum ecologically-relevant daily toxin doses to

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walruses feeding on clams and bowhead whales feeding on zooplankton were estimated to be 21.5 and 0.7 μg STX eq. kg body weight^{-1} day^{-1}, respectively, suggesting that walruses had higher PST exposures than bowhead whales. Average and maximum STX doses in walruses were in the range reported previously to cause illness and/or death in humans and humpback whales, while bowhead whale doses were well below those levels. These findings raise concerns regarding potential increases in PST/STX exposure risks and health impacts to Arctic marine mammals as ocean warming and sea ice reduction continue.

1. Introduction

Climate change-related ocean warming trends and continued loss of sea ice coverage, persistence, and quality in the Arctic are causing rapid and dramatic changes in Alaskan marine ecosystems (Danielson et al., 2020; Frey et al., 2014; Huntington et al., 2020). One of these changes is the potential for increased prevalence of the harmful algal bloom (HAB) species, *Alexandrium catenella.* This dinoflagellate species can produce potent neurotoxins known as saxitoxins (STXs) or paralytic shellfish toxins (PSTs) that can contaminate seafood and cause the human illness known as paralytic shellfish poisoning (PSP). In addition to forming toxic blooms in surface waters, *A. catenella* cells produce resistant resting cysts that sink and remain in benthic sediments until conditions are favorable for germination and subsequent seeding of blooms. *Alexandrium catenella* cells have been reported sporadically in Alaskan Arctic waters over the last several decades, with the likely source of bloom cells being from established populations in the northern Bering Sea that are transported northwards to the Chukchi and Beaufort Seas through Bering Strait (Natsuike et al., 2017). Recent studies have identified massive *A. catenella* cyst beds in the northeastern Chukchi Sea that are some of the largest and densest in the world (Anderson et al., 2021; Natsuike et al., 2013). Until recently, it was thought that bottom water temperatures were too cold to support significant cyst germination. As a result, cysts may have accumulated in bottom sediments over many decades as inputs from transported blooms exceeded losses through germination. The accumulation of cysts in areas that have been traditionally too cold for cyst germination has set the stage for significant concerns as Arctic waters continue to warm both at the surface and bottom. Hydrographic and bathymetric features that support high cell and cyst accumulations in the Chukchi Sea, coupled with warmer temperatures that promote bloom initiation from cysts in bottom sediments and cell division in surface waters, are likely to lead to larger and more frequent toxic blooms (Anderson et al., 2021).

Potential increases in *A. catenella* bloom prevalence in the Alaskan Arctic pose threats to wildlife and human health as well as food security. For example, STX has been detected in multiple marine mammal species from all regions of coastal Alaska (Lefebvre et al., 2016), so the potential for increased bloom incidence in Arctic waters raises wildlife health concerns. STXs are highly lethal, water-soluble neurotoxins that block voltage-gated sodium channels, thereby restricting signal transmission between neurons (Catterall et al., 1979). During blooms, the toxins quickly accumulate in filter-feeding organisms such as copepods and krill, both suspension and deposit feeding clams and worms, and several species of fish. The concentrated toxin loads are passed through the food web to marine mammals and other wildlife that consume these prey. Human consumers are also at risk of exposure by eating contaminated seafoods. Symptoms of STX poisoning in people are numbness around the lips, mouth, face and neck; muscular weakness; sensation of lightness and floating; ataxia; motor incoordination; drowsiness; incoherence; progressively decreasing ventilatory efficiency; and in high doses, respiratory paralysis and death (Kao et al., 1967). Though symptoms are difficult to observe in wild marine mammals, there have been a few confirmed and suspected mass-mortality events associated with STX poisoning, including humpback whales (*Megaptera novaeangliae*) in Cape Cod, New England (Geraci et al., 1989), sea otters (*Enhydra lutris L.*) in Kodiak, Alaska (Degange and Vacca, 1989), and Mediterranean monk seals (*Monachus monachus*) in Western Sahara, Africa (Costas and Lopez-Rodas, 1998).

Risks of STX exposure to marine mammals are directly related to the amount of toxin accumulated in prey during blooms, as exposure occurs through diet. In the present study, concentrations of PSTs were measured in multiple components of the food web throughout the US Bering, Chukchi, and Beaufort Seas, including phytoplankton, zooplankton, clams, worms, and fish during an anomalously warm summer in 2019. Fecal samples from walruses (*Odobenus rosmarus*) and bowhead whales (*Balaena mysticetus*) harvested for subsistence purposes in the Bering and Beaufort Seas, respectively, were also tested for PSTs to assess potential exposure. The food web data collected were used to characterize toxin presence in Alaskan Arctic ecosystems and to calculate average and maximum ecologically-relevant toxin doses to walruses and bowhead whales, which are important subsistence resources for the nutritional, cultural, and economic well-being of many communities and peoples throughout western and northern Alaska. These dose calculations are the first step towards predicting the potential health impacts of HAB toxins in Arctic marine mammals.

2. Methods

2.1. Collection of food web samples

Alaskan Arctic food web samples including phytoplankton, zooplankton, clams, worms, and fish were collected opportunistically during three research cruises in summer of 2019 and analyzed for the presence of PSTs (Fig. 1). Pacific walrus fecal samples were collected in collaboration with subsistence walrus hunters in the communities of Gambell and Savoonga on Saint Lawrence Island, Alaska (Fig. 1). Bowhead fecal samples were collected in collaboration with the Barrow (Utqiaġvik), Nuiqsut (Cross Island), and Kaktovik Whaling Captains' Associations, Alaska (Fig. 1). All fecal samples were analyzed for the presence of STX. Detailed collection procedures for each sample type and toxin quantification methods are described below.

2.1.1. Phytoplankton sample collection

During the HEALY19 cruise (Fig. 1), the ship's underway seawater system was used to collect algal cell pellets for toxin analysis at locations where high concentrations of *A. catenella* (~1000 cells L^{-1}) were detected through shipboard light microscopy observations. This occurred in three distinct regions: just north of the Bering Strait (DBO3-8), near Barrow Canyon (BCE-5), and offshore in Ledyard Bay (LB-12) (Fig. 1). At each location, a large volume of water (40-80 L) was concentrated from the underway seawater system using a 20 μm mesh plankton net and backwashed into a pre-weighed 15 or 50 mL conical tube. A subsample was collected and preserved to calculate cell density, after which the cell slurry was centrifuged for 10 minutes at 3,000 × g and supernatant was aspirated to obtain a cell pellet. Depending on the size of the pellet, it was resuspended and preserved with 1.5-5.0 mL 0.05 M acetic acid and then frozen at –20°C for future PST analyses via high performance liquid chromatography (HPLC). Samples were transported to the laboratory and thawed, and additional triplicate 25 μL cell count samples were collected and preserved with ~30 μL Utermohls solution in 1.0 mL filtered seawater. The remaining acetic acid cell mixture was sonicated in an ice water bath using a Branson Sonifier 250 D (Branson Ultrasonics Corp., Danbury, CT, USA) fitted with a micro-tip probe at a constant 40-watt output for 1 min. The samples were refrozen, thawed,

and sonicated two more times following the above protocol in order to thoroughly break open cells and release toxins.

2.1.2. Zooplankton sample collection

Zooplankton were collected on the HEALY19 and IES19 research cruises (Fig. 1) using oblique tows of paired bongo nets (20 cm frame, 153 μm mesh and 60 cm frame, 505 μm mesh) (Kimmel et al., 2018). The tows were within 5-10 m of the bottom depending on sea state, and the volume filtered was estimated using a General Oceanics flowmeter (General Oceanics, Miami, FL, USA) mounted inside the mouth of each net. Samples from one net were preserved in 5% buffered seawater formalin solution. Zooplankton were identified to the lowest taxonomic level and life stage possible at the Plankton Sorting and Identification Center (PSIC) in Szczecin, Poland (https://mir.gdynia.pl/o-instytucie/zaklad-sortowania-i-oznaczania-planktonu/?lang=en). Samples identified at the PSIC were verified at the Alaska Fisheries Science Center, Seattle, Washington, USA. For copepod and krill samples, the whole zooplankton sample from the 60 cm bongo net was concentrated on a 505 μm screen and re-suspended in seawater. Using a stereomicroscope, a minimum of 10 stage CIV or higher copepods of the species *Calanus glacialis* or *C. marshallae* (both species were potentially present and are difficult to distinguish morphologically (Campbell et al., 2016)) and a minimum of 5 individual juvenile or adult krill (*Thysanoessa raschii* or *T. inermis* (Hunt Jr. et al., 2016)) were selected using forceps and flash-frozen at -80°C. A scoop sample of zooplankton was also collected, where the whole sample from the 20 cm bongo net was concentrated onto a 153 μm screen. A spatula was then used to transfer a bulk zooplankton sample into a 5 mL microcentrifuge tube that was flash frozen at -80°C.

2.1.3. Clam and worm sample collection

Dominant clam and worm macrofaunal samples were collected on the HEALY19 and IES19 cruises (Fig. 1). During the HEALY19 cruise, surface sediments were collected via a weighted van Veen grab (0.1 m^2), and contents of the grab were sieved through a 1 mm metal mesh sieve with running seawater (as described in (Grebmeier et al., 2018). Dominant clams and worms were identified shipboard under a dissecting microscope, placed in plastic bags, and frozen at -20°C for post-cruise analyses. On the IES19 cruise, clams and worms were collected using a small mesh beam trawl (Abookkire and Rose, 2005) on the seafloor at a speed of 1.5 knots for a target time of 5 min.

2.1.4. Fish sample collection

Fish samples were collected on the IES19 and NBS19 cruises (Fig. 1). The following fish species were collected for algal toxin analyses (Murphy et al., 2021): sand lance (*Ammodytes hexapterus/personatus* not identified to species), Pacific herring (*Clupea pallasii*), gadids (*Gadus chalcogrammus, G. microcephalus, G. morhua, Eleginus gracilis*), and salmonids (*Oncorhynchus gorbuscha*). A Cantrawl 400/601 rope trawl (Cantrawl Pacific Ltd., Richmond, BC, Canada) was used to conduct surface trawl operations. All surface trawl tows were 30 min in duration and were towed at an average speed of 4 knots. Trawl dimensions were monitored during each tow with a Simrad FS70 net sounder (Kongsberg Mesotech Ltd., Port Coquitlam, BC, Canada), and the typical trawl opening was approximately 50 m horizontal by 20 m vertical. Surface trawl catches were sorted by species, and four specimens of each species, at each station as available, were frozen whole at -20°C in plastic bags. Samples remained frozen until toxin analysis. All biological data were recorded in an electronic catch logging system (Catch Logger for

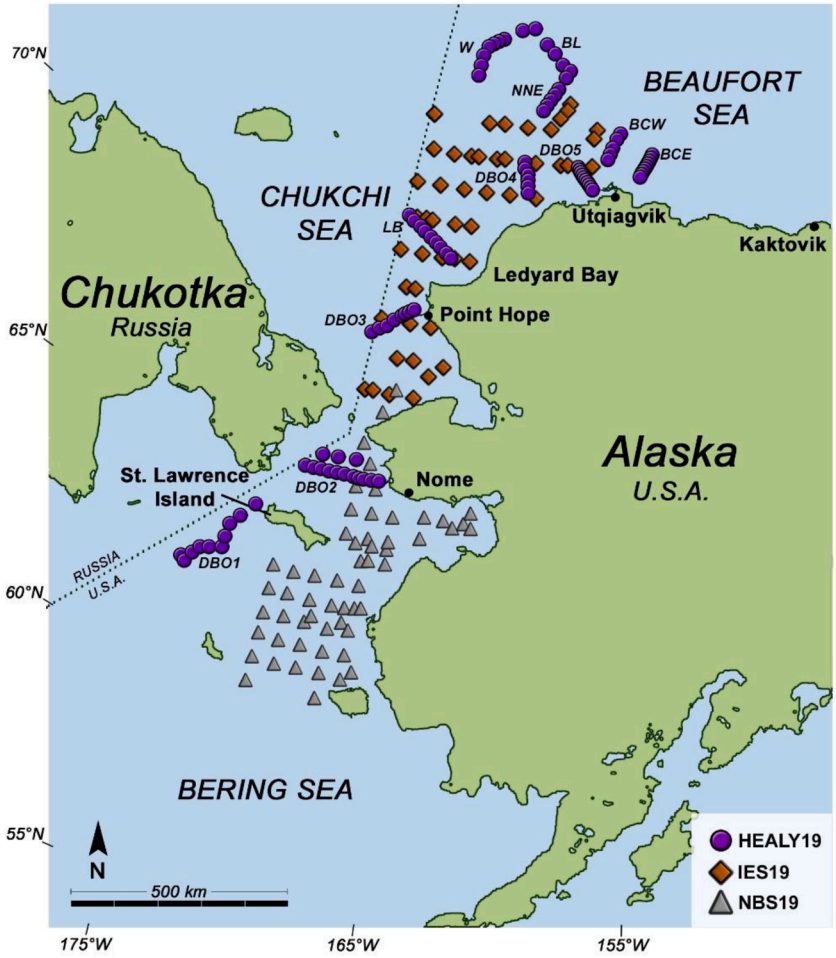

Fig. 1. Map of HEALY19, IES19, and NBS19 research cruise sampling stations during summer 2019. HEALY19 (U.S. Coast Guard ice breaker HEALY) cruise dates August 4th to 23rd, 2019; IES19 (Integrated Ecosystem Survey/North Pacific Research Board Arctic Program) cruise dates August 1st to October 3rd, 2019; NBS19 (Northern Bering Sea) cruise dates August 27th to September 29th, 2019. DBO = Distributed Biological Observatory stations. LB = Ledyard Bay. W = West. NNE = North-northeast. BL = Border Line. BCE = Barrow Canyon East. BCW = Barrow Canyon West.

Acoustic and Midwater Surveys (CLAMS). Specimens collected during the survey were assigned specimen numbers (barcode number) and/or a collection code to maintain a specimen record of the survey.

2.1.5. Walrus (Odobenus rosmarus) fecal sample collection – northern Bering Sea

Fecal samples were collected from 13 individual walruses (*Odobenus rosmarus divergens*) harvested for subsistence purposes during 28 March to 04 May 2019 near Saint Lawrence Island, Alaska in the northern Bering Sea. Hunters collected 30 cm lengths of intestine, retaining the contents and placing in labeled zip-close bags. Samples were given to a harvest monitor at the conclusion of the hunting trip, and placed in a consumer-grade chest freezer at approximately -17°C. Intestinal samples were originally collected to assess parasite load, and, when thawed, fecal material was removed from the intestine and subsampled for algal toxin analysis.

2.1.6. Bowhead whale (Balaena mysticetus) fecal sample collection – Beaufort Sea

In 2019, fecal samples were collected from nine bowhead whales (*Balaena mysticetus*) harvested for subsistence purposes during spring and fall whaling in the North Slope Borough region of Alaska. Fecal material was sampled from six whales harvested near Utqiaġvik between 19 April and 16 May 2019, from two whales harvested near Kaktovik on 30 August and 04 September 2019, and from one whale harvested near Cross Island on 30 August, 2019. Sections of colon were cut open and fecal matter was removed using plastic spoons. Samples were stored frozen in 50 mL Falcon™ centrifuge tubes (Corning Inc., Corning, NY, USA) and/or Whirl-Pak® bags (Nasco Sampling/Whirl-Pak, Madison, WI, USA) at -20°C until analyzed for algal toxins.

2.2. Quantification of PSTs in food web samples

Saxitoxin and PSTs were quantified in phytoplankton, zooplankton, clams, worms, and fish, as well as walrus and bowhead whale fecal samples via two methods. First, STX was quantified in zooplankton, clams, worms, fish and marine mammal fecal material using a commercially-available Enzyme-Linked Immunosorbent Assay (ELISA) kit. Additionally, multiple congeners in the suite of PSTs were individually quantified in phytoplankton pellets and clam samples using HPLC to validate ELISA findings in clams and to characterize the suite of toxins present in Arctic regions. Details of these measurement methods follow.

2.2.1. STX quantification via ELISA

Saxitoxin was quantified in marine mammal feces, zooplankton, clams, worms, and fish using the Eurofins Abraxis saxitoxin (PSP) ELISA kit (Eurofins Abraxis, Warminster, PA, USA). For extraction, walrus and bowhead fecal samples were partially thawed in a light proof cooler and stirred thoroughly. Aliquots of ~1 g were transferred to 14 mL polypropylene pop-cap tubes and weighed (Scout™ STX balance, Ohaus Corp., Parsippany, NJ, USA). Fifty percent methanol was added to each aliquot at 3 × the aliquot weight for a 1-in-4 dilution. Aliquots were vortexed until all material was fully thawed, then homogenized with a generator probe (GLH 850, 10 mm; Omni-International, Kennesaw, GA, USA) for 1 min at 2,100 rpm. Previous comparisons of marine mammal GI samples (n = 8) extracted in 50 % methanol and the traditional 80 % ethanol found no statistically significant differences between STX concentrations quantified in each extraction solvent type (data not shown). Extractions for zooplankton, clam, worm, and fish samples were modified from the fecal extraction protocol. Due to small size, zooplankton samples were not thawed prior to aliquoting. Before generator probe homogenization, samples were mashed using wooden stir sticks to break apart whole organisms. Small samples (<0.5 g) were homogenized for only 15 – 30 s to avoid sample heating. Clams, worms, and fish were thawed in a light-proof cooler until just soft enough to dissect. Fish were dissected using scalpels or dissection scissors, and the whole visceral mass was removed using clean dissection scissors and forceps for each fish. If visible, gonads and fat were removed from the visceral mass, and the remaining material was transferred to either a 14 mL (samples ≤ 2.5 g) or a 50 mL (samples >2.5 g) centrifuge tube. Viscera samples were minced with scissors after 50% methanol addition and vortexing, then homogenized with a generator probe as described above. Whole clams (minus shells) and whole worms were fragmented with scissors or scalpels and transferred with forceps to either a 14 mL or 50 mL tube, depending on sample mass as described above. Worms were carefully removed from any mud or benthic debris casing before being transferred. Clams and worms were minced with scissors between vortexing and homogenization as previously described.

After homogenization, all sample matrices were centrifuged at 3,063 × g for 20 min at 4°C (Jouan CR3i centrifuge, Thermo Electron Corp., Waltham, MA, USA), and up to 4 mL of supernatant was poured off into 4 mL glass amber vials. Remaining solids from centrifugation were discarded. Extracts were capped tightly and refrigerated until further analysis (within 1 week). Directly prior to toxin quantification, 200 µL extract subsamples were filtered (Millipore Sigma Ultra-Free Centrifugal filters, 0.22 µm; Merck KGaA, Darmstadt, Germany). Filtered extracts were quantified by ELISA according to the manufacturer's protocol with dilution modifications based on Hendrix et al. (2021): extracts were diluted 1:50 (extract: ELISA kit sample diluent) to avoid matrix effects. Any samples with results outside the absorbance range of the kit standards were diluted further and re-analyzed until all results fell within the working range. ELISA plates were washed with a BioTek ELx50 plate washer (BioTek Instruments, Winooski, VT, USA) and incubated on an orbital shaker. Well absorbance was quantified using a BioTek Epoch plate reader (BioTek Instruments, Winooski, VT, USA). The ELISA minimum detection limit for STX was 3 ng g^{-1}. The Abraxis ELISA kit is designed to detect STX (with very limited cross-reactivity with other PSP toxins); therefore, all ELISA results are reported as STX equivalents (eq.), and likely underestimate the presence of other congeners. STX eq. concentrations were interpolated using known standard absorbances and concentrations with the 4-parameter logistic curve fit model recommended in the Eurofins Abraxis STX ELISA protocol.

2.2.2. Suite of PSTs quantified via HPLC in phytoplankton cells

Prior to HPLC analysis, phytoplankton cell pellet extracts were passed through a Waters Sep-Pak® C18 light cartridge (Waters™ Corporation, Milford, MA, USA) per the manufacturer's protocol. Three hundred microliters of the cartridge eluate were added to an Amicon® Ultra 10,000 NMWL molecular weight filter (MilliporeSigma, Burlington, MA, USA) and centrifuged (13,000 × g, 10 min). The filtrate was pipetted into an autosampler vial and analyzed using a Waters 2695 HPLC coupled to a 2475 fluorescence detector (Waters™ Corporation, Milford, MA, USA) following post-column derivatization (Anderson et al., 1994; Oshima, 1995) with these additional changes: a 250 × 4.6 mm, 5 µm Waters Symmetry C18 column was used to separate the neosaxitoxin/saxitoxin congeners with a mobile phase acetonitrile concentration of 100:3 and a flow rate increase to 1.0 mL min^{-1}. The column temperature was set to 12°C and the post column reaction (PCR) reagents were infused at a rate of 0.5 mL min^{-1} at 50°C. For the gonyautoxin suite, a 150 × 4.6 mm, 5 µm InertSustain AQ-C18 column (GL Sciences, Torrance, CA, USA) was used at a temperature of 25°C with a mobile phase flow rate of 0.7 mL min^{-1} and a PCR flow rate of 0.4 mL min^{-1} maintained at 35°C. An Inertsil C8, 5 µm (4.6 × 150 mm) housed at 25°C and a PCR temperature of 45°C were used for C toxin analysis. All samples were kept at 4°C in an autosampler during HPLC analysis. Certified reference standard solutions purchased from the National Research Council Canada (Halifax, NS, Canada) used for sample quantitation, contained toxins N-sulfocarbamoyl gonyautoxin-2 and -3 (C1&2), gonyautoxins-1 through 6 (GTX1, 2, 3, 4, 5 and 6), decarbamoyl gonyautoxin-2 and -3 (dcGTX2&3), decarbamoyl neosaxitoxin (dcNEO), neosaxitoxin (NEO), decarbamoyl saxitoxin (dcSTX) and saxitoxin (STX) were run prior to, and following every fourth

sample. A triplicate, five-point standard curve for each standard set was run previous to sample analysis to assure linearity of a wide concentration range. Epimer pair toxins, C1&2, GTX2&3 as well as GTX1&4 were each combined for the mole percent comparisons presented within.

To better resolve similarities and differences between phytoplankton cell pellets extracted without hot acid hydrolysis, and clam samples that were extracted with hydrolysis, the sulfamate toxins C1&2 and GTX5 found in the phytoplankton pellets were stoichiometrically converted to their carbamate forms, GTX2&3 and STX, respectively, on a 1:1 mole percent basis to produce the pie charts in Fig. 2.

2.2.3. Suite of PSTs quantified via HPLC in clams

Clam samples were analyzed via HPLC with pre-column oxidation using the standard methods (Lawrence et al., 2005) with refinements (Ben-Gigirey et al., 2012) (Harwood et al., 2013). Briefly, samples were processed using a Kinematica Polytron model PT-MR 2500E homogenizer fitted with a 12 mm dispersing head (Kinematica, Inc., Bohemia, NY, USA). A 5 g subsample of homogenized tissue was extracted with 3 mL 1% acetic acid in a 100°C water bath for 5 min. After cooling at 4°C, the sample was centrifuged at 4,500 rpm for 10 min, and the supernatant was collected. The remaining pellet was re-extracted and the supernatants combined. One milliliter of the combined extract was passed through a conditioned SPE C18 cartridge, pH-adjusted to 6.5, and diluted to 4 mL for oxidation with periodate and peroxide. PSP toxins were quantified using Agilent 1100 (Agilent Technologies, Santa Clara, CA, USA) or Waters Aquity Arc (Waters™ Corporation, Milford, MA, USA) HPLC systems equipped with fluorescence detection and 5 μm C18 columns (150 × 4.6 mm, Phenomenex, Inc., Torrance, CA, USA). Concentrations of STX, neoSTX, decarbamoyl saxitoxin (dcSTX), gonyautoxins 2 and 3 (GTX2&3), decarbamoyl gonyautoxins 2 and 3 (dcGTX2, 3), gonyautoxins 1 and 4 (GTX1&4), gonyautoxin 5 (GTX5), and the di-sulfated toxins C1 and C2 were quantified using standards purchased from the National Research Council Canada (Halifax, NS, Canada). Isomers GTX 1&4, GTX 2&3 and C1&C2 are not separated using pre-column oxidation and are reported together. In keeping with the Alaska Department of Environmental Conservation's protocols, toxicity equivalency factors (TEFs) from the European Food Safety Authority 2009 ((EFSA), 2009) were used to convert congener concentrations to STX eq., with the higher TEF used for unresolved congener pairs (STX = 1, NeoSTX = 1, GTX1 = 1, GTX2 = 0.4, GTX3 = 0.6, GTX4 = 0.7, GTX5 = 0.1, GTX6 = 0.1, C2 = 0.1, C4 = 0.1, dc-STX = 1, dc-NeoSTX = 0.4, dc GTX2 = 0.2, GTX3 = 0.4, and 11-hydroxy-STX = 0.3). Throughout this study toxin concentrations in clams and tissue components are reported in total STX eq. The contribution of individual congeners to the clam toxin pool was calculated by weight based on STX eq. The fraction of clam toxin concentrations associated with specific tissues (% toxin) was calculated as the STX eq. in each tissue relative total toxin pool in that tissue component.

Quality assurance of toxin data was completed by instrument validation using homogenates of butter clams and mussels analyzed

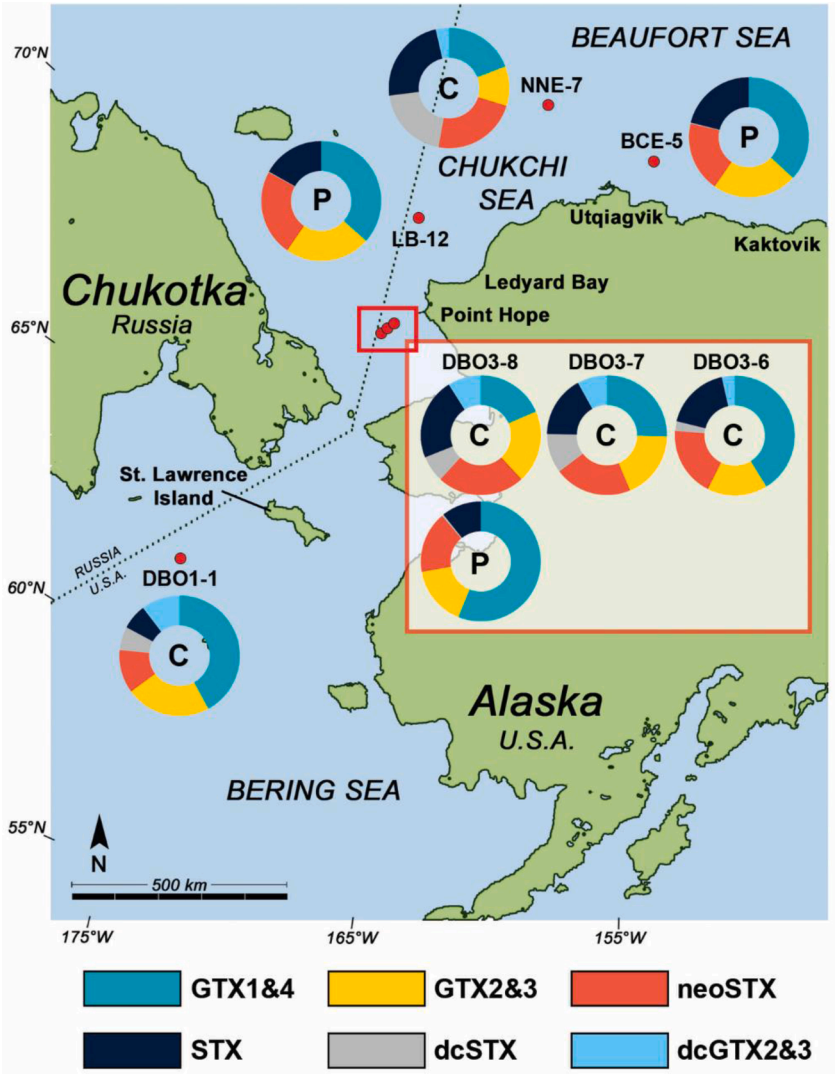

Fig. 2. Map showing the suite of paralytic shellfish toxins (PSTs), based on relative molarity, in the phytoplankton pellets and clams (*Macoma calcarea*) collected at sites in the northern Bering Sea, Chukchi Sea, and Beaufort Sea. "C" indicates a clam sampling site and "P" indicates a phytoplankton pellet; at station DBO3-8 both clams and phytoplankton were sampled. To facilitate comparison between clam samples that were hydrolyzed in hot acid to phytoplankton samples that were not, the sulfamate toxins C1&2 and GTX5 found in the phytoplankton pellets were stoichiometrically converted to their carbamate forms, GTX2&3 and STX, respectively, on a 1:1 mole percent to produce the plots. GTX = gonyautoxin; neoSTX = neo-saxitoxin, STX = saxitoxin, dcSTX = decarbamoyl saxitoxin, and dcGTX = decarbamoyl gonyautoxin. DBO = Distributed Biological Observatory stations. LB = Ledyard Bay. NNE = North-northeast. BCE = Barrow Canyon East.

previously by the Alaska Department of Environmental Conservation via post-column oxidation. Linear regression was used to compare results using pre- and post-column oxidation methods ($y = 0.94x - 6.04$, $r^2 = 0.985$). Daily quality assurance was performed by analyzing toxin standards pre- and post-analysis. In each case instrument response was within 97% of original standard curve results.

2.3. Estimation of ecologically-relevant STX doses to walruses and bowhead whales

Average and maximum ecologically-relevant STX doses to walruses and bowhead whales were calculated using published values for active daily metabolic rates (DMR) for each marine mammal species, known caloric values for prey, average and maximum toxin concentrations in prey quantified in this study, and published average total adult body weights for each marine mammal species. For walruses, these doses were calculated by taking the DMR for a 1,310 kg walrus (91,070 kcal d^{-1}; (Acquarone et al., 2006)) and dividing it by the caloric value of *Macoma calcarea* sampled from the Chukchi sea (5,330 kcal kg^{-1}; (Young, 2015)) to get the daily consumption requirement (DCR). The DCR was then multiplied by the average (40.2 ± 46 (SD) μg STX eq. 100 g^{-1}; n = 16) and maximum (165 μg STX eq. 100 g^{-1}) toxin levels detected in prey (*M. calcarea*) to get the total average and maximum daily doses per animal. Finally, these total doses were divided by total walrus body weight to get average and maximum realistic exposure doses expressed as μg STX eq. kg^{-1} d^{-1} (Table 1). The same procedure was used to calculate realistic daily STX doses to bowhead whales feeding on krill (*Thysanoessa inermis* and *T. raschii*) containing the maximum and average toxin concentrations in all krill with detectable levels of STX from this study. Additionally, the maximum toxin value in a zooplankton scoop sample was also used to calculate a realistic dose to bowhead whales. The DMR for a 30,000 kg bowhead (165,090 kcal d^{-1}; (Thomson, 2002) was divided by the average caloric value of krill sampled in summer in the Bering Sea (6,725 kcal kg^{-1}; (Harvey et al., 2012), then multiplied by the maximum (18.8 μg STX eq. 100 g^{-1}) and average (6.4 μg STX eq. 100 g^{-1}) krill toxin levels and maximum zooplankton scoop sample (85.1 μg STX eq. 100 g^{-1}) toxin levels quantified in this study, and finally divided by body weight to get average and maximum realistic exposure doses expressed as μg STX eq. kg^{-1} d^{-1} (Table 1).

3. Results

3.1. Suite of PSTs in phytoplankton (A. catenella)

Following mole-to-mole conversion of the sulfamated toxins C1&2 and GTX5 to their carbamate derivatives, GTX2&3 and STX, respectively, it was found that across all phytoplankton cell pellet samples, the GTX1&4 toxins had the greatest relative contribution, comprising over half (56%) of the total toxin molarity of the sample taken north of the Bering Strait near Pt. Hope (DBO3-8) and 37% in the samples from Ledyard Bay and east of Barrow Canyon (Fig. 2). The cell pellets also contained significant proportions of STX, GTX2&3, and neoSTX. While dcSTX was present in trace amounts, dcGTX2&3 were not detected in any of the phytoplankton samples.

Table 1
Maximum and average ecologically-relevant saxitoxin (STX) doses to Pacific walruses (*Odobenus rosmarus*) feeding on clams (*Macoma calcarea*) and bowhead whales (*Balaena mysticetus*) feeding on krill (*Thysanoessa raschii* and *T. inermis*). DMR = Daily Metabolic Rate (taken from Acquarone et al., 2006 for walrus [average body size = 1310 kg] and from Thomson et al., 2002 for bowheads [average body size = 30,000 kg]); [a]= average prey toxin concentration; [b]= maximum prey toxin concentration; [c]= maximum toxin concentration from a zooplankton scoop sample (Zp). bw = total body weight.

Species	DMR (kcal day^{-1})	Prey	Caloric Value of Prey (kcal kg^{-1})	STX Concentration in Prey (μg 100 g^{-1})	Daily STX Dose (μg STX eq. kg^{-1} bw)
Walrus	91,050	Clam	5,330	40.2[a]	5.2
Walrus	91,050	Clam	5,330	165[b]	21.5
Bowhead	165,090	Krill	6,725	6.4[a]	0.05
Bowhead	165,090	Krill	6,725	18.8[b]	0.15
Bowhead	165,090	Zp	6,725	85.1[c]	0.70

3.2. STX concentrations in zooplankton and species composition

STX was detected in copepods (*Calanus glacialis* or *C. marshallae*), krill (*Thysanoessa raschii* or *T. inermis*), and in bulk scoop zooplankton samples (Fig. 3). The highest zooplankton toxin concentration (85 STX eq. 100 g^{-1}) was quantified in a bulk zooplankton scoop sample taken near Ledyard Bay, a known hot spot for *Alexandrium* cysts (Anderson et al., 2021) (Fig. 3). Zooplankton abundances estimated by 20 cm bongo net tows were dominated by the copepods *Oithona* spp. and *Pseudocalanus* spp., the appendicularians *Fritillaria* spp., and meroplanktonic echinoderm and bivalve larvae (Fig. 4).

3.3. STX concentrations in benthic clams and worms

STX was detected in clams (n = 40 STX positive out of 43 samples) and polychaete worms (n = 33 positive out of 50 samples) collected from sediments of the Bering, Chukchi, and Beaufort Seas (Fig. 5). Toxin-positive clams contained significantly higher toxin concentrations than toxin-positive worms from the same collection sites (unpaired t test; $p = 0.0005$; Fig. 6A). In clams (*M. calcarea*), STX concentrations above the regulatory limit established for shellfish were detected at three locations: north of Utqiaġvik (80 μg STX eq. 100 g^{-1}), near Ledyard Bay (104 μg STX eq. 100 g^{-1}), and 113 km north of Saint Lawrence Island (165 μg STX eq. 100 g^{-1}) in the Bering Strait region (Fig. 5). The average and maximum toxin concentrations specifically for all *M. calcarea* samples with detectable levels of STX (n = 19) were 40.2 ± 46 (mean ± SD) and 165 μg STX eq. 100 g^{-1}, respectively.

3.4. Suite of PSTs in clams (Macoma calcarea)

Toxin composition of clam (*M. calcarea*) samples, calculated by relative molarity, varied by sample and location (Fig. 2). The southernmost clam samples (DBO1-1), collected in the northern Bering Sea, contained the highest relative proportion of GTX1&4 (42%) followed by GTX2&3 (23%). One of the clam locations north of Bering Strait region (DBO3-6) had a similarly dominant proportion of GTX1&4 (41%), while clams collected at adjacent stations to the west on the transect line (DBO3-7, DBO3-8) exhibited a more equal distribution between GTX1&4, GTX2&3, neoSTX and STX. The clams collected from the Northeast Chukchi shelf (NNE-7) had equal proportions of STX and neoSTX (23%) followed by dcSTX (20%). In all clam samples, dcSTX and dcGTX2&3 made up smaller but still significant proportions of the toxin composition ranging from a combined percentage of 6% (DBO3-6) to 24% (NNE-7).

3.5. Comparison of ELISA STX equivalents and HPLC toxicity equivalency factors

Results from clams collected from five locations (DBO1-1, DBO3-6, DBO3-7, DBO3-8, and NNE-7; Fig. 1) were used to compare ELISA-derived STX equivalents to toxicity equivalency factors (TEF) calculated from HPLC results (Table 2). In all cases, ELISA values were less than TEF values, confirming that ELISA methods underestimate potential toxicity due to limited cross reactivity of the antibody used in the kit for some of the PST congeners. Table 2 shows the percentage of ELISA values to HPLC-TEF values with the lowest value at 14% (DBO1-1) and the highest at 91% (DBO3-8). ELISA values at two stations (DBO3-8 and

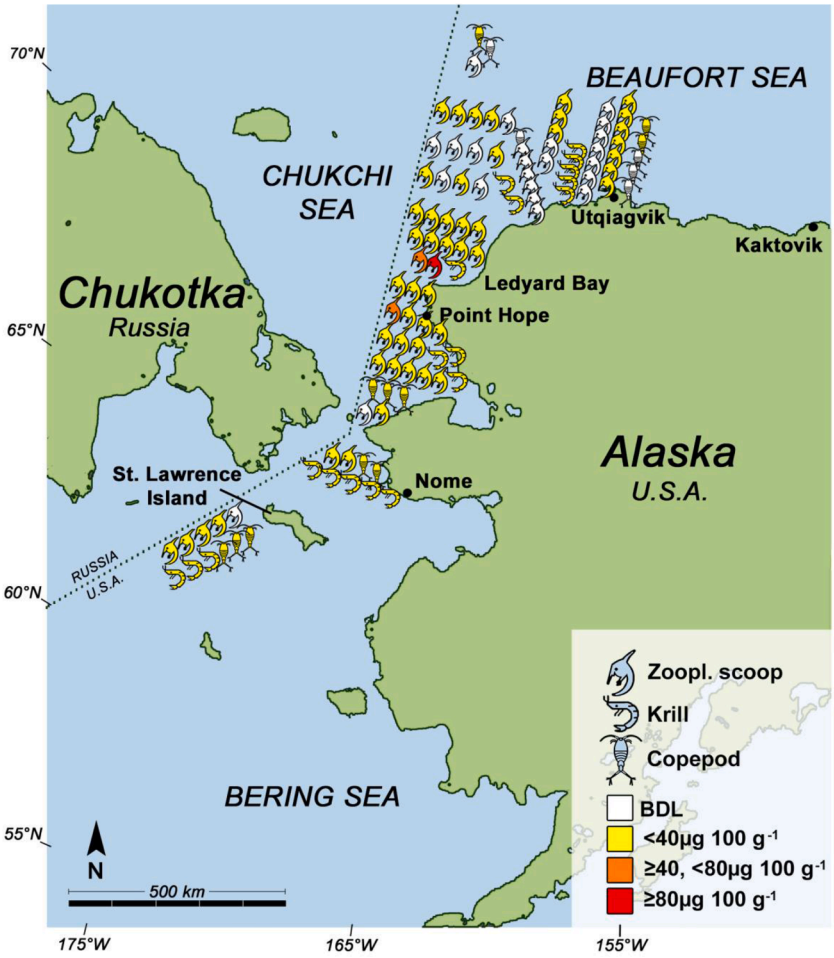

Fig. 3. Map shows saxitoxin (STX) concentrations in krill (*Thysanoessa raschii, T. inermis*), copepods (*Calanus glacialis* or *C. marshallae*), and bulk scoop samples of the entire zooplankton assemblage collected in the northern Bering Sea, Chukchi Sea, and western Alaskan Beaufort Sea. Toxin levels are categorized in relation to the seafood safety regulatory limit of 80 µg STX eq. 100 g^{-1}. Colors are defined as; white = BDL (below detection limit/not detected), yellow = low toxin levels (< ½ the regulatory limit), orange = moderate toxin levels (> ½ the regulatory limit and < 80 µg STX eq. 100 g^{-1}), and red = high toxin (≥ the regulatory limit).

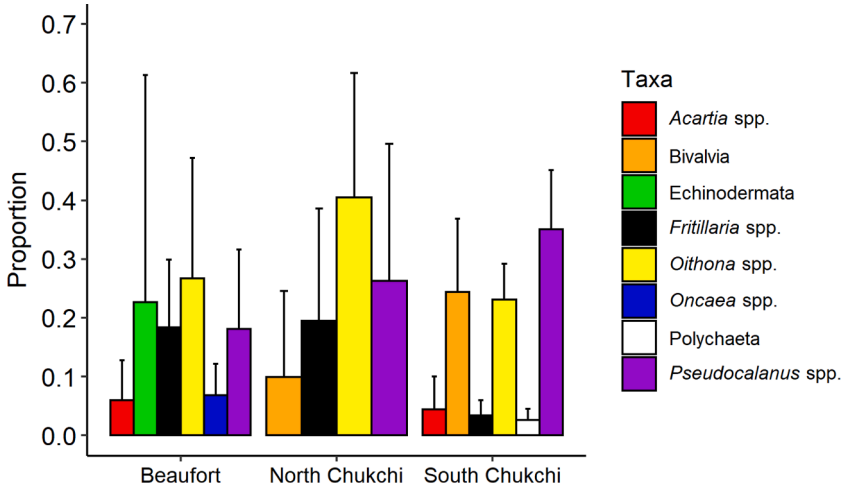

Fig. 4. Mean (± SD) proportion of the predominant taxa from the 20 cm, 153 µm mesh net.

NNE-7) fell within the standard deviation of the mean HPLC TEF values for each station (Table 2).

3.6. STX concentrations in fish

Low to moderate STX concentrations were detected in viscera samples from fish collected in the southern Chukchi and Bering Seas (Fig. 7): sand lance (*Ammodytes hexapterus*); herring (*Clupea pallasii*); gadids (*Gadus chalcogrammus, G. microcephalus, G. morhua, Eleginus gracilis*); and salmonids (*Oncorhynchus gorbuscha*). In general, pelagic fish contained much lower toxin concentrations than other parts of the food web including zooplankton (Fig. 3), and clams and worms (Fig. 5) from the same collection sites. This is notable considering that the fish toxin values calculated were for viscera only, while values in zooplankton, clams, and worms were from whole animals. Whole fish toxin loads per gram total body weight would be significantly lower than viscera values,

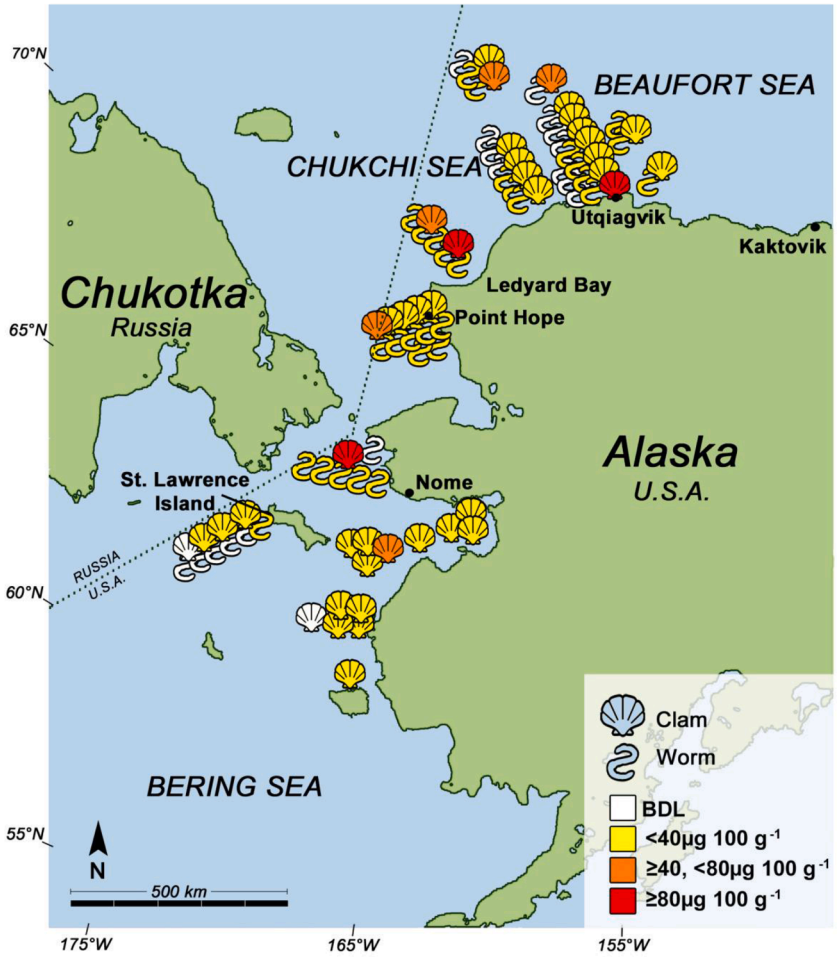

Fig. 5. Map shows saxitoxin (STX) concentrations in clams (*Macoma calcarea, Ennucula tenuis, Yoldia hyperborean, Clinocardium ciliatum, Astarte borealis, Serripes* sp., *Musculus* sp.) and worms (Maldanidae, Orbiniidae, *Nephtys* sp., *Pectinaria* sp., *Phyllodoce* sp., *Golfingia* sp., *Scoletoma* sp.) collected in the Bering Sea, Chukchi Sea, and western Alaskan Beaufort Sea. Toxin levels are categorized in relation to the seafood safety regulatory limit of 80 µg STX eq. 100 g^{-1}. Colors are defined as; white = BDL (below detection limit/not detected), yellow = low toxin levels (< ½ the regulatory limit), orange = moderate toxin levels (> ½ the regulatory limit and < 80 µg STX eq. 100 g^{-1}), and red = high toxin (≥ the regulatory limit).

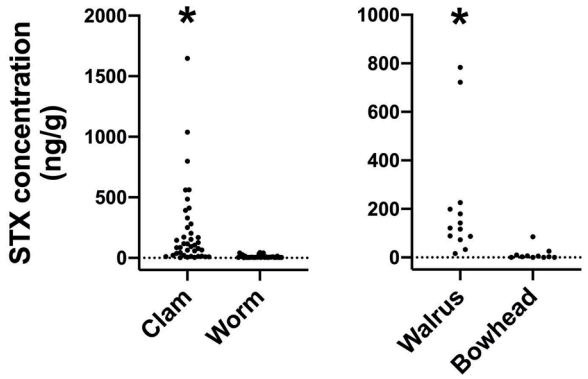

Fig. 6. Comparison of saxitoxin (STX) concentrations in (A) whole clams and worms and (B) fecal samples collected from Pacific walruses (*Odobenus rosmarus*) and bowhead whales (*Balaena mysticetus*) harvested for subsistence purposes.
* = significantly higher toxin levels (unpaired t-test; $p < 0.05$).

as STX is water soluble and expected to be at highest levels in the gastrointestinal tract. Viscera from sand lance collected north of Ledyard Bay in the northeast Chukchi Sea did not contain detectable levels of STX (Fig. 7).

3.7. STX concentrations in walrus and bowhead whale fecal samples

STX was detected in 13 of 13 walrus fecal samples and 7 of 9 bowhead fecal samples collected during subsistence harvests in summer/fall of 2019 (Fig. 8). Toxin concentrations were significantly higher in walrus fecal samples compared to bowhead fecal samples (unpaired t test; $p = 0.01$; Fig. 6B). Toxin concentrations quantified in bowhead whales ranged from undetectable (BDL = below detection limit) to 8.5 µg STX eq. 100 g^{-1} feces. Toxin concentrations quantified in walruses

Table 2
Comparison of saxitoxin (STX) equivalents (eq.) concentrations quantified via high performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA) for representative clams (*Macoma calcarea*) sampled at five stations. HPLC values are in toxicity equivalency factors (TEF) to express the analogues as STX equivalents based on acute intraperitoneal toxicity via mouse bioassay described in the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel; EFSA Journal, 2009). ELISA values as a percentage of HPLC values are shown in column five (ELISA % of HPLC). Individual clams (n = 2 to 6 per location) were analyzed via HPLC and pooled clams (n = 2 to 5 pooled per location) were analyzed via ELISA.

Species	Station	HPLC TEF values (µg STX eq. 100 g^{-1}; Mean ± SD)	ELISA values (µg STX eq. 100 g^{-1}; n = 1)	ELISA % of HPLC
Clam	DBO1-1	46 ± 11 (n = 5)	6.4	14 %
Clam	DBO3-6	36 ± 4.4 (n = 4)	28	78 %
Clam	DBO3-7	44 ± 19 (n = 6)	10.6	24 %
Clam	DBO3-8	54 ± 23 (n = 6)	49	91 %
Clam	NNE-7	67 ± 22 (n = 2)	56	84 %

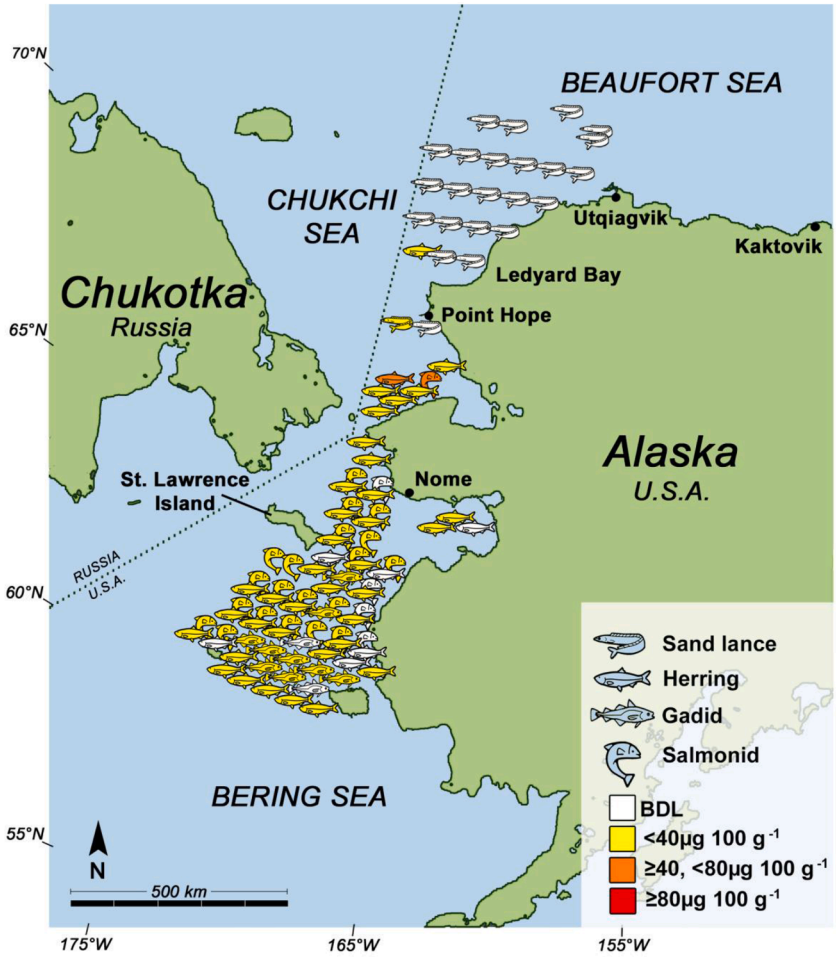

Fig. 7. Map shows saxitoxin (STX) concentrations in fish viscera (sand lance (*Ammodytes hexapterus*); Pacific herring (*Clupea pallasii*); gadids (*Gadus chalcogrammus, G. microcephalus, G. morhua, Eleginus gracilis*); salmonids (*Oncorhynchus gorbuscha*)) collected in the Bering Sea, Chukchi Sea, and western Alaskan Beaufort Sea. Toxin levels are categorized in relation to the seafood safety regulatory limit of 80 µg STX eq. 100 g^{-1}. Colors are defined as; white = BDL (below detection limit/not detected), yellow = low toxin levels (< ½ the regulatory limit), orange = moderate toxin levels (> ½ the regulatory limit and < 80 µg STX eq. 100 g^{-1}). All fish samples were below the seafood safety regulatory limit.

ranged from 1.6 to 78 µg STX eq. 100 g^{-1} feces and approached seafood safety regulatory limits in two animals at 78 and 72 µg STX eq. 100 g^{-1} (Fig. 8).

3.8. Ecologically-relevant STX doses to walruses and bowhead whales in 2019

Table 1 shows average and maximum ecologically-relevant daily STX doses calculated in this study at 5.2 and 21.5 µg STX kg^{-1} total body weight (bw) for walruses, respectively, and 0.05 and 0.70 µg STX kg^{-1} for bowhead whales, respectively. Average daily doses in walruses were 104 times higher than in bowhead whales and maximum daily doses in walruses were 30 times higher compared to bowhead whales, indicating that walruses in the northern Bering Sea may have higher exposures than bowhead whales in the Chukchi and Beaufort Seas.

4. Discussion

Recent findings of massive *A. catenella* cyst beds in the Alaskan Arctic, along with increasing cyst germination rates linked to warming ocean temperatures, highlight the emerging threat of large and recurrent toxic blooms in northern regions (Anderson et al., 2021). This can result in higher toxin concentrations throughout food webs as filter-feeding marine organisms consuming cells, and deposit feeders consuming cysts, accumulate the toxins and pass them on to higher trophic levels. In the present study, PSTs were found in all trophic levels tested, including phytoplankton, zooplankton, clams, worms, and fish, as well as walruses and bowhead whales harvested for subsistence purposes in the Bering, Chukchi, and Beaufort Seas in 2019 during an anomalously warm year (Figures 2, 3, 5, 6, 7 and 8) (Anderson et al., 2018). Collectively, these data presented here reveal elevated toxin exposure risks to marine wildlife that may negatively impact the health of important marine resources within Alaskan Arctic ecosystems.

4.1. Suite of PSTs in phytoplankton and clams

Representative samples of *A. catenella* cells collected in summer 2019 appeared to have a consistent suite and proportion of PST congeners in the northern Bering, Chukchi, and western Beaufort Sea regions, while clam toxin profiles were more variable, suggesting that bioconversion of toxins between trophic levels likely occurs (Fig. 2). After molar conversion of the sulfamated toxins to their carbamate forms, the suite of toxins detected in phytoplankton pellets consisted of four main congeners (GTX1/4; GTX2/3; STX and neoSTX) in similar proportions, while clams from comparable locations contained six congeners (GTX1/4; GTX2/3; STX; neoSTX; dcSTX; and dcGTX2/3; Fig. 2). After accounting for hydrolysis effects during extraction, toxin composition in clams will most closely resemble the source plankton just after consumption, with changes in form and concentration accumulating over time (Hall and Reichardt, 1984). The occurrence of dcSTX and dcGTX2&3 in clams, but not in plankton, indicates that some bioconversion had already occurred. Although there are no published bioconversion studies with *M. calcarea* specifically, both dcSTX and dcGTX2&3 have been reported to be produced by surf clams (*Spisula solidissima)*(Bricelj and Cembella, 1995). These data suggest that similar bioconversion occurs in *M. calcarea*, which likely explains why the toxin profiles of clams and cells collected at the same time north of Bering Strait (DBO3-8) were different from each other (Fig. 2). The toxin profile in the DBO3-6 clams

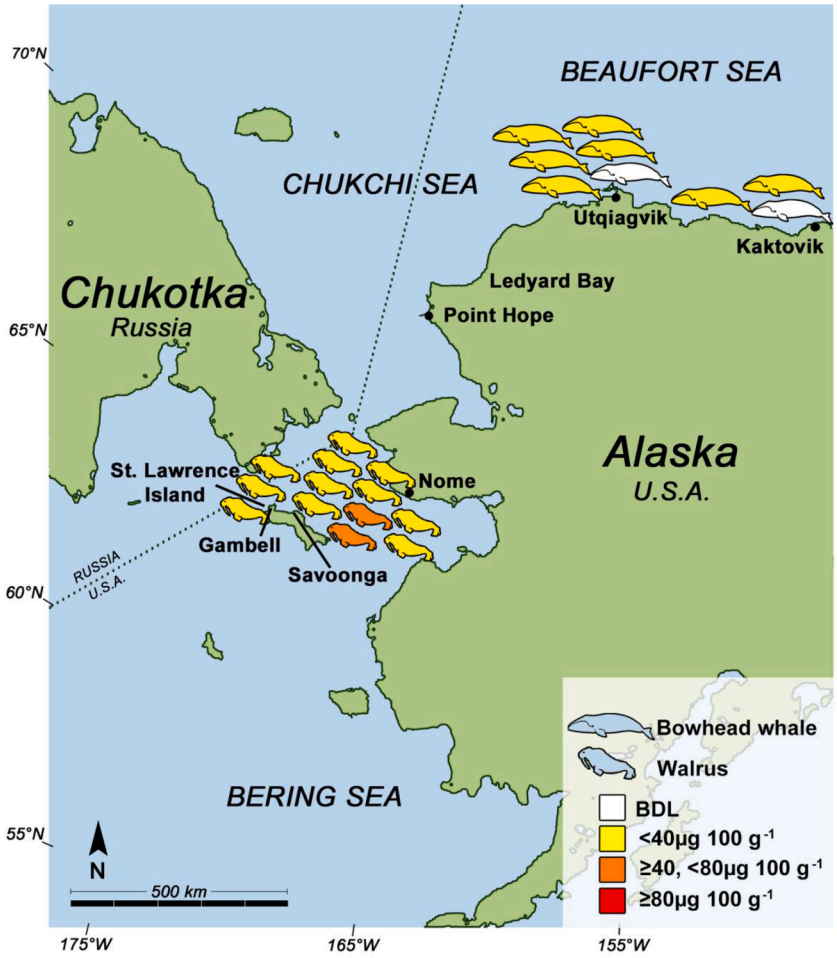

Fig. 8. Map shows saxitoxin (STX) concentrations in fecal samples from Pacific walruses (*Odobenus rosmarus*) and bowhead whales (*Balaena mysticetus*) harvested for subsistence purposes during 2019. Toxin levels are categorized in relation to the seafood safety regulatory limit of 80 µg STX eq. 100 g^{-1}. Colors are defined as; white = BDL (below detection limit/not detected), yellow = low toxin levels (< ½ the regulatory limit), orange = moderate toxin levels (> ½ the regulatory limit and < 80 µg STX eq. 100 g^{-1}). The two orange samples were near regulatory limits at 72 and 78 µg STX eq. 100 g^{-1}.

collected north of the Bering Strait most closely resembled the profile in phytoplankton cell pellets collected at the nearby DBO3-8 station (Fig. 2). These clams also had the lowest combined proportion of dcSTX and dcGTX2&3, potentially indicating less bioconversion and more recent consumption of *A. catenella* cells. In contrast, northeast Chukchi shelf clams (NNE-7) had the greatest combined proportion of dcSTX and dcGTX2&3, which may indicate a greater degree of bioconversion (Fig. 2). It is difficult to confirm the role of bioconversion at this time because some strains of *A. catenella* isolated from this region produce low molar concentrations of dcSTX (<10%) and dcGTX2&3 (<1%, D.M. Anderson, unpublished data), so there is a possibility that these toxins in the clams may have come from a cell population that was not sampled in this study. In any case, metabolism of toxins by clams is likely to result in some conversion, which would impact the overall toxicity of the clam and determine which toxin forms are passed on to bivalve consumers. Toxin profiles were generally consistent across phytoplankton samples collected in this study, while clam toxin profiles differed from phytoplankton and varied by region, with increased STX in the Chukchi Sea clams relative to the Bering Sea samples (Fig. 2). Toxin profiles in clams will have a direct impact on exposure risks and toxicity to upper trophic level organisms that feed on clams.

4.2. STX concentrations in zooplankton, clams, worms and fish

Clams appear to be the most toxic vectors of STX to walruses and other benthic consumers, with concentrations above the seafood safety regulatory limit detected in samples from all three regions: the northern Bering Sea, Chukchi Sea, and western Beaufort Sea (Fig. 5). Zooplankton contained the next highest level of STX with one sample collected near Ledyard Bay, a known accumulation zone for *A. catenella* cysts (Fig. 3; (Anderson et al., 2021)), above the regulatory limit. Benthic worms contained low to moderate concentrations of STX from all three regions, but direct comparisons revealed that benthic worms had significantly lower STX concentrations than clams collected at the same time and location (Figures 5 and 6A). In contrast, planktivorous fish sampled in this study contained very low levels of STX with none considered a risk for marine mammal consumption (Fig. 7). As noted above, the STX levels reported here for fish were from viscera samples and not whole-body burdens. In a previous study with another water-soluble algal toxin, domoic acid, toxin concentrations were compared between viscera and body tissue in anchovies (*Engraulis mordax*) and sardines (*Sardinops sagax*) revealing that 99.8 % of the total body toxin burden was located in the viscera and only 0.2 % in corresponding body tissue (Lefebvre et al., 2002). In addition, mean visceral masses for anchovies and sardines were approximately 9% of the total body weight, and therefore total body burdens were estimated to be 10 times lower than viscera concentrations (Lefebvre et al., 2002). A similar pattern is likely for STX in planktivorous fish, and emphasizes the very low toxin values detected in fish in this study compared to other trophic layers. Note, however, that the mobile free-ranging behavior of fish likely contributes to the drastic differences in toxin accumulation in fish when compared to sessile and planktonic filter-feeders collected in the same region. Had forage fish been actively feeding on an *A. catenella* bloom, toxin concentrations would likely have been higher as cells and toxin would be present in fish stomachs prior to digestion and elimination. It has been well documented that fish, when consumed whole, can be vectors of STXs at levels that can cause PSP (Deeds et al., 2008).

Toxin concentrations for trophic layer comparisons were made using

quantification of STX eq. via ELISA and are likely underestimates of total potential toxicity due to the lack of reactivity of the ELISA antibody to congeners other than STX. For example, dcSTX, GTX2&3, dcGTX2&3, NeoSTX, and GTX1&4 reactivities are reported as 29, 23, 1.4, 1.3, and < 0.2 %, respectively (Abraxis, 2021). A comparison of STX eq. via ELISA and TEFs via HPLC in comparable clams from five stations resulted in a range of 14 to 91% ELISA value to HPLC TEF values, confirming that ELISA consistently underestimates potential toxicity (Table 2). These differences can be partially explained by the suite of congeners present. For example, the DBO1-1 clam ELISA value was 14% of the HPLC TEF and the main congeners detected via HPLC were GTX1&4 (ELISA cross reactivity < 0.2%). By contrast, the DBO3-8 clam ELISA value was 91% of the TEF, and this sample had the highest proportion of STX (ELISA cross reactivity 100%; Fig. 2; Table 2). In addition to the potential for overall underestimation of toxicity in all trophic layers, organisms within a species containing different suites of congeners due to bioconversions could also have variable results due to cross reactivity differences. However, it is unlikely that these differences would dramatically change the relative toxicity comparisons made between the trophic layers reported here. Based on data from this study, the two most toxic trophic members, clams and zooplankton, are important prey for walruses and bowhead whales, respectively, and represent major STX exposure risk pathways to these important marine resources.

4.3. Ecologically-relevant STX doses to Pacific walruses and bowhead whales

It has been well documented that walruses and bowhead whales are exposed to PSTs via consumption of contaminated prey in Arctic food webs. However, neither the results of this study, nor previous studies (Lefebvre et al., 2016), are able to demonstrate whether the toxin doses have reached high enough levels to impact their health. An increased frequency and intensity of *A. catenella* blooms as a result of warming ocean conditions as well as increased cyst germination will increase toxin exposure doses as food webs become more contaminated. Data from this study were used to calculate daily ecologically-relevant STX doses for walruses and bowhead whales feeding on typical prey such as clams (*M. calcarea)* for walruses and zooplankton/krill (*T. inermis* and *T. raschii*) for bowhead whales (Table 1). Pacific walruses are known to forage on a variety of benthic taxa, including clams such as *Macoma* (Sheffield et al., 2001), while bowhead whales typically forage on dense patches of small, free-swimming zooplankton such as copepods and euphausiids (Sheffield and George, 2021). The STX doses calculated here suggest that walruses in the northern Bering Sea are at risk for higher STX exposure than bowhead whales in the Chukchi and Beaufort Seas due to higher toxin concentrations in clams than in zooplankton, and due to higher metabolic demands per kg bodyweight in walruses (Table 1; (Acquarone et al., 2006; Thomson, 2002). A previous study comparing STX levels detected in four ice-associated seal species revealed that bearded seals (*Erignathus barbatus*), primarily benthic feeders that consume clams, also had the highest prevalence of STX compared to the other ice-associated seal species that feed on fishes, krill, and/or shrimps throughout the water column such as ringed seals (*Pusa hispida*), spotted seals (*Phoca largha*), and ribbon seals (*Histriophoca fasciata*) (Hendrix et al., 2021). This provides additional evidence for the role of clams as one of the most toxic vectors of STX to marine mammals in Arctic ecosystems. Benthic clams such as *M. calcarea* are also surface deposit feeders that consume fine sediment material that likely contains *A. catenella* cysts. This suggests that clams could be year-round toxin vectors, even in the absence of a bloom event in surface waters.

It is not clear if the daily ecologically-relevant doses calculated in this study are sufficient to cause health impacts in marine mammals. Based on this study, it is likely that walruses are more at risk for higher exposure than bowhead whales, as average and maximum daily STX doses were estimated to be 104 and 30 times higher, respectively (Table 1; Fig. 6B). Although it is known that STX can impact marine mammal health (Costas and Lopez-Rodas, 1998; Degange and Vacca, 1989; Geraci et al., 1989), nothing is known regarding the precise doses of STX required to cause health impacts in a walrus or bowhead whale. There have been several laboratory studies with mammalian rodent models that define toxicological metrics such as the no observable adverse effect level (NOAEL), the lowest observable adverse effect level (LOAEL), and the median lethal dose (LD50) with oral STX exposure (Table 3; (McFarren et al., 1960; Munday et al., 2013; Wiberg and Stephenson, 1960)). Additionally, there are a few publications with dose estimates and some toxicological metrics extrapolated from human PSP cases (Table 3; (Fitzgerald et al., 1999; Gessner et al., 1997; Llewellyn et al., 2002)). None of these metrics have been fully defined in marine mammals. However, in a case study of the deaths of 14 humpback whales (*Megaptera novaeangliae*) in Cape Cod Bay, MA from November, 1987 to January, 1988, STX poisoning through consumption of contaminated mackerel (*Scomber scombrus*) was found to be the cause at an estimated daily dose of 3.2 μg STX eq. kg^{-1} bw (Table 3; (Geraci et al., 1989)). This estimated fatal daily dose to humpback whales is lower than both the average and maximum daily STX doses calculated in this study for walruses (5.2 and 21.5 μg STX eq. kg^{-1} bw, respectively), but several times higher than those calculated for bowhead whales. This suggests that walruses in the Bering, Chukchi and Beaufort Seas may already be at risk for toxic STX doses during a bloom event (Table 1).

The variability of reported toxic STX doses in laboratory models, humans, and humpback whales adds to the difficulty of definitively determining whether the oral STX doses calculated in this study are sufficient to cause health impacts in walruses and bowhead whales. It is not surprising that the published doses causing effect or no effect are highly variable between laboratory rodent models (NOAEL = 163 – 958 and LD50 = 212 – 420 μg STX eq. kg^{-1} bw) and actual human cases (LOAEL = 2.1 – 21 and Lethal dose = 1 – 411 μg STX eq. kg^{-1} bw), as well as within these categories (Table 3). It is well known that rodent mammalian models are typically less sensitive to oral exposure to algal

Table 3
Examples of published oral saxitoxin (STX) doses and toxicological metrics calculated from laboratory rodent studies, human cases of Paralytic Shellfish Poisoning (PSP), and one mortality event involving humpback whales (*Megaptera novaeangliae*). bw = body weight; NOAEL = no observable adverse effect level; LOAEL = lowest observable adverse effect level; LD50 = median lethal dose.

Study	Species	Toxicological metric/ exposure source	Daily Dose (μg STX eq. kg^{-1} bw)
Fitzgerald et al. 1999	Human	LOAEL/drinking water	2.1
Gessner et al. 1997	Human	Lowest dose to cause illness/shellfish	21
Gessner et al. 1997	Human	Lethal dose-respiratory arrest/shellfish	230 - 411
Llewellyn et al. 2002	Human	Lethal dose/crab	1 -2
Munday et al. 2013	Mouse	NOAEL/gavage	163
Munday et al. 2013	Mouse	NOAEL/feeding	958
Munday et al. 2013	Mouse	LD50/gavage	356
Wiberg et al. 1960	Mouse	LD50/gavage	260-263
McFarren et al. 1960	Mouse	LD50/gavage	420
McFarren et al. 1960	Rat	LD50/gavage	212
Geraci et al. 1998	Humpback	Lethal dose/mackerel	3.2
Max dose this study	Walrus	Unknown/clams	21.5
Max dose this study	Bowhead	Unknown/zooplankton	0.7

toxins than humans, and that conventional pharmacological dogma suggests that larger animals require proportionally lower doses for the same effect (Casarett, 1975). This study is the first to present ecologically-relevant STX doses to walruses and bowhead whales feeding in Alaskan Arctic marine ecosystems. Simple comparisons of the daily STX doses in Table 3 suggest that walruses may experience toxic exposures, as these doses are near STX levels known to cause illness in humans, and larger animals typically require less toxin per unit body-weight for the same toxic effect. The duration of exposure also impacts potential toxicity, as animals in the wild are not simply exposed to a single daily dose such as a meal or laboratory exposure, but to repetitive exposure over several days to weeks during an *A. catenella* bloom, or potentially much longer if clams consume toxic cysts year-round or retain PSTs for extended intervals, as is known for the butter clam (Shumway, 1990). Chronic versus acute toxicity is less well studied but negative effects on body mass and food intake have been observed in laboratory rodent models chronically-exposed to subacute doses of neoSTX (Vilariño et al., 2018). Additional research is essential to determine potential health impacts of long-term subacute exposure to PSTs in marine mammals.

Physiological factors may also influence toxin susceptibility in marine mammals. Although speculative, given their unique physiological diving adaptations (e.g. peripheral vasoconstriction while maintaining blood oxygen supply to central hypoxia-intolerant organs such as the heart and brain), marine mammals may be particularly vulnerable to central and peripheral cardiorespiratory effects of STX as exposure is amplified in those organs (García et al., 2004; Gessner et al., 1997). Additionally, the metabolism of STX is unknown in marine mammals, but toxicokinetic studies of PSTs in animal model species and humans demonstrate that metabolism of these toxins to potentially more toxic congeners can occur (Vilariño et al., 2018). Enhanced post-mortem analyses of tissues and body fluids from marine mammals for PSTs and congeners will be critical for determining what constitutes a toxic dose in marine mammals. It is clear that there are many factors involved in defining a toxic PST dose and these factors can vary greatly between exposure events and between species.

5. Conclusions

Climate-change driven reduction of sea ice and continuing ocean warming increase the risk of large and more frequent toxic blooms of *A. catenella* in Arctic regions, thereby escalating the risks to ecosystem and wildlife health due to potentially increasing toxin levels in food webs. During anomalously warm ocean conditions in 2019, PSTs were found in all levels of the Alaskan Arctic food web tested, including zooplankton, clams, worms, fish, Pacific walruses, and bowhead whales. In several cases, toxin concentrations were above the seafood safety regulatory limit in important prey for walruses and bowhead whales. The average and maximum ecologically-relevant daily toxin doses determined here for walruses were in the range of those known to impact humans. This suggests that walruses may already be experiencing doses that could impact their health. Furthermore, walruses are expected to require a lower toxin dose than humans for the same effect due to physiological differences and to the relationship between body size and toxicity, as larger animals typically require lower toxin doses per kg than smaller organisms for the same toxic effect.

In contrast, maximum daily doses calculated for bowhead whales were 30 times lower than those for walruses and toxin values measured in fecal samples were significantly lower in bowhead whales (Table 1; Fig. 6), suggesting that bowhead whales are not likely experiencing toxic PST doses under current environmental conditions. However, it is difficult to extrapolate definitively in the absence of observed morbidity or mortality data for these animals; that will require additional research incorporating observations of behavior and mortality events of walruses and bowhead whales that can be directly linked to toxin exposure to confirm toxic impacts. It is important to note that blooms can vary dramatically in geographic extent and cell concentration, so toxin doses calculated from other years and seasons might be higher or lower than those presented here from 2019.

As part of an ongoing ECOHAB study (*Trophic transfer and health impacts of algal toxins in Arctic food webs*), coastal communities in western and northern Alaska have been engaged to participate in sampling efforts, and to provide behavioral observations of marine wildlife, including during marine mammal harvests by Alaska Natives for subsistence purposes in order to help address this issue. The data presented here are the first step in assessing toxin exposure risks and potential health impacts from expected increases in toxic *A. catenella* blooms to Alaskan Arctic ecosystems and to critically important marine resources utilized for subsistence by communities throughout northern and western Alaska.

Disclaimers

The findings and conclusions of the authors are their own and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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