Pacific Walrus (*Odobenus rosmarus divergens*)
Saint Lawrence Island Harvest Sample Analyses, 2012–2014 and 2016

Technical Report to:

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and
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Executive Summary

The Alaska Department of Fish and Game (ADF&G) partnered with the U.S. Fish and Wildlife Service (USFWS), the Eskimo Walrus Commission (EWC), and the Native Villages of Savoonga (NVS) and Gambell (NVG) to collect information and samples from the subsistence walrus (*Odobenus rosmarus divergens*) harvest near Saint Lawrence Island, Alaska, in 2012–2014 and 2016.

Information and samples from the Alaska Native subsistence harvest are especially important because agencies have yet to overcome the logistical constraints necessary to estimate walrus abundance in remote, ice covered waters. As such, reliable estimates of walrus abundance and population trend are lacking. Retrospective analyses of data provided by this monitoring program allow us to examine how parameters that affect population size may vary in time and how current conditions compare with past conditions. Parameters such as body condition, diet, age distribution, sex ratio, and pregnancy rate can be useful in evaluating population health or status. This project also recorded hunter knowledge regarding walruses and analyzed tissue samples for contaminants and disease. Sample collection relied on the partnership with EWC, NVS, NVG and the walrus hunters of Saint Lawrence Island.

Hunter knowledge. Hunters evaluated the health of 208 of the walruses they harvested as very healthy (51%), average (47%), or unhealthy (2%).

Diet. Contents of intestines and stomachs from 116 walruses were analyzed; 57 (49%) were empty and 16 had prey items that could not be identified. Prey items were identified for 43 walruses including a minimum of 20 invertebrate prey species from seven major taxonomic groups: Polychaeta, Priapulida, and Echiurida (worms), Mollusca (snails and clams), Crustacea (amphipods, shrimp, and crab), and Cucumariidae (sea cucumbers), of which mollusks and echiurids were most common. Fish were uncommon, but three walruses had fish, two of the fish were identified as Pacific sand lance (*Ammodytes hexapterus*).

Whiskers from 31 walruses (28 collected from Gambell and Savoonga and three collected near Hooper Bay) were analyzed for diet using molecular techniques of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Although no specific prey items were identified using this method, the whiskers did show a seasonal isotopic pattern that likely corresponds to winter feeding in the Bering Sea and summer feeding in the Chukchi Sea. These walruses probably fed on similar prey, but the isotopic chemistry of those prey change with latitude driven by differences in the dominant water masses.

Contaminants. Liver, kidney, muscle, and blubber tissues from 42 walruses were analyzed for concentrations of 20 trace elements including potentially toxic elements (i.e., arsenic, cadmium, mercury, and lead) and except for arsenic, were found to be similar to or lower than previous studies and lower than concentrations of ringed, bearded, spotted, and ribbon seals harvested in the same region. For elements of concern, liver had the highest concentrations of mercury and lead, kidney had the highest concentrations of cadmium, and blubber had the highest concentration of arsenic. A separate analysis was conducted for methylmercury, total mercury, and selenium to better understand the most toxic form of mercury in walrus tissues.

Methylmercury concentrations were higher in liver than both muscle and kidney, which were similar; however the proportion of all mercury that was methylmercury was highest in muscle.

Persistent organochlorine compounds (e.g., CHL (chlordanes), HCH (hexachlorocyclohexane), DDT (dichlorodiphenyltrichloroethane), and PCB (polychlorinated biphenyls) were found to be similar or lower than previous walrus studies, lower than walruses in Canada, and lower than concentrations of the four sympatric seal species. In general, organochlorine concentrations in blubber tissue were an order of magnitude higher than in liver, which were an order of magnitude higher than kidney and muscle, which were similar. The relationship among the organochlorines was the same for blubber, kidney, and muscle; $\Sigma HCH > \Sigma PCB > \Sigma CHL > \Sigma DDT$.

Vitamins. Liver tissue from 37 walruses was analyzed for vitamin A and E concentrations. Concentrations of vitamin A were significantly higher in males than females, but concentrations of vitamin E were not.

Disease. Prevalence of all diseases (except herpesvirus) was low. No antibodies of phocine or canine distemper were detected. Antibodies for *Brucella* were detected at 5%, *Leptospira* at 4% and *Toxoplasma* at < 1%. Antibodies for Phocine herpesvirus-1 were detected in almost all (98%) of the 151 walruses tested, which is normal for mammals; herpesvirus is rarely symptomatic. Toxins (domoic acid and saxitoxin) from harmful algal blooms were found in 49% and 52% of the walruses tested, respectively. In addition to the high proportion that were positive, concentrations were also the highest measured of 13 Alaskan marine mammal species sampled (domoic acid 6,457 ng/g, saxitoxin 1,161.8 ng/g). No walruses were reported to be symptomatic. Parts of clams (feet and siphons) found in the stomachs of walruses that tested positive were also found to have detectable concentrations of domoic acid (range 2.8–29.0 parts per billion) and saxitoxin (range 14.3–60.0 parts per billion). These concentrations are below the regulatory limit for clams.

Age distributions. We analyzed age at harvest for 167 walruses sampled in 2012–2014. Ages for 2016 animals are still pending. Ages ranged from 3 to 34. Most females were 11–15 years old and most males were 16–25 years old.

Sex ratios. Sex ratios of walruses in the harvest are biased by hunter preferences and by movement patterns during migration. Although 68% more females than males were harvested in Gambell, the opposite was true for Savoonga resulting in an even overall sex ratio of 115 males and 106 females.

Pregnancy rate. Hunters provided information about the reproductive condition of the adult females sampled and harvested. Information included whether the female was pregnant or had a calf or yearling. The birth rate of adult female walruses is limited by the long (15 month) gestation period (actually diapause plus gestation), which results in a minimum inter-birth interval of one calf every two years but it is more likely to be one calf every three years. During the three consecutive years of our study 79–87% of adult females sampled had calves of the year (or near term fetuses) with them; well above the expected 33–50%. This pregnancy rate is higher than expected for the overall walrus population, likely due to hunter selection for females

with calves or the status of females that are available to hunters. It does show, however, that many females are becoming pregnant and that calves are being born.

Conclusions. Walrus body condition was described by hunters as good. Diet is similar to previous studies. Concentrations of trace elements and organochlorine contaminants were similar to or lower than concentrations of ice seal species harvested in Alaska and the prevalence of diseases were also lower than that of seals that share the same habitats. Walruses are exposed to harmful algae blooms through diet and have the highest concentrations of marine mammals tested in Alaska. The overall sex ratio of the harvest was similar when Gambell and Savoonga harvests were combined across years. Pregnancy rates of harvested females were higher than theoretically possible for the entire population due to hunter and availability bias.

These results are especially valuable because they provide information that allows us to detect changes in parameters that are useful for monitoring population status when estimating population size and trend is not possible. Overall walruses appear to be in good body condition, are reproducing, have lower concentrations of contaminants than seals of the same region, and do not show prevalence for diseases of concern. Walruses are ingesting toxins from harmful algal blooms but no adverse effects have been documented.

Introduction

Pacific walruses (*Odobenus rosmarus divergens*) are an important subsistence species to coastal Alaska Natives and they are important to the Bering and Chukchi marine ecosystems. Female walruses, their newborn calves, and juveniles use sea ice in the Chukchi Sea during summer as a resting platform between feeding bouts. Walruses feed over the continental shelf in waters less than 100 m deep. Reduced summer sea ice in the Chukchi Sea creates added stress for females and young when the sea ice retreats north beyond the 100 m isobath and is no longer useful to walruses for resting between feeding bouts. Without ice in the Chukchi Sea over the continental shelf, walruses must stay in the water or use land haulouts for resting. Unlike the sea ice, which is a moving platform that supports relatively small groups of walruses, land haulouts are much larger and prey resources close by can be quickly depleted. In addition to manmade disturbances (e.g., airplane and boat traffic), repeated disturbances from grizzly and polar bears create stampedes that cause high calf mortality (Fay 1982). In part because these conditions are likely to become more common in the future and are likely to have a negative impact on the population, the Pacific walrus was petitioned for listing as a threatened species under the Endangered Species Act in 2008, however listing was determined to be warranted but precluded in 2011 by higher priority listing actions (Federal Register 76(28):7634–7679). In addition to the effects of climate change, including increases in shipping, oil and gas activity was increasing within summer walrus habitat in the Chukchi Sea, until 2015 when exploration activities were halted. Many leases in the Chukchi Sea Oil and Gas Lease Area 193, within walrus summer habitat, were relinquished in May 2015.

The U.S. Fish and Wildlife Service (Service) has been working with the Eskimo Walrus Commission, (EWC), Native Villages of Gambell and Savoonga (NVG, NVS), and local walrus hunters to conduct a tissue sample collection program (Walrus Biosampling Program) annually at Gambell and Savoonga in conjunction with the spring harvest data collection program (Walrus Harvest Monitoring Project). Although the Service continues to support annual harvest data and sample collection activities they have not had funding to analyze samples other than teeth and have been dependent upon researchers with funded projects for those services. Teeth are collected annually for assessing age as most analyses are age dependent. Other recent sample collections have included female reproductive tracts, blubber, tongues, stomach contents, and nasal swabs depending upon what funded researchers have requested. Because of the dependence on funded research and the vagaries of such funding, the samples collected and analyzed are not consistent through time and are not working toward a long-term dataset that will allow retrospective comparisons to monitor whether walruses are faring better or worse than in the past (Garlich-Miller et al. 2006). This information is especially important because we have no measure of population abundance or trend (Speckman et al. 2011). Population estimates for walruses are difficult to obtain due to problems related to conducting surveys over large areas of ice-covered waters and their highly clumped distribution. Although it can be cost effective to have samples analyzed this way there is an urgent need to systematically collect and analyze samples that will allow the status and health of the walrus population to be monitored through time.

The Alaska Department of Fish and Game (ADF&G) recognizes the importance of walruses to Alaskans and the need to monitor their status and trend during the current changes in sea ice and

has chosen to use funds provided to the State of Alaska by the Service under Section 6 of the Endangered Species Act for this purpose. In this report we analyze walrus data and samples collected during the spring harvest at Gambell and Savoonga, Alaska, in 2012–2014 and from limited sampling in 2016 to evaluate contaminants, disease, diet, body condition, age distribution, and productivity. The purpose of this report is to provide current information that will allow the status and health of the walrus population to be evaluated and monitored through time and to provide current information to the Service to use during a proposed listing determination for Pacific walruses in 2017.

Methods

Collection and handling of specimens

Walruses from the spring (May) subsistence harvest were sampled at Gambell and Savoonga, Alaska, during 2012–2014 and in 2016 (Fig. 1). Sampling occurred during subsistence hunts by trained hunters. Samples were transported from the harvest location back to the community and transferred to a local crew trained to process, freeze, and ship them to the ADF&G laboratory in Fairbanks. Biological information collected included location, date harvested, date sampled, sex, and hunter assessed health condition. Data collected for adult females included those related to pregnancy, such as lactation (milk present), and if accompanied by a newborn calf or yearling. In 2012, samples collected included teeth, whiskers, skin, blubber, liver, kidney, muscle, heart, spleen, intestine, blood, and nasal swabs. In 2013, samples collected included teeth, whiskers, skin, blubber, liver, kidney, muscle, heart, spleen, intestine, blood, and nasal and anal swabs. In 2014, samples collected included teeth, whiskers, skin, blubber, liver kidney, muscle, spleen, intestine, blood, stomach content, bone, and amniotic fluid. In 2016, samples collected included only stomach contents and urine for toxic algae screening. Most samples were frozen in the field and shipped to ADF&G in Fairbanks for processing. Blood was spun in a centrifuge and sera was collected in cryovials and frozen. Teeth were loosened from the lower mandible by a blow at the base with a hatchet or hammer. Both lower canines were removed and put in a plastic bag pre-labeled with the specimen number and then placed within the larger sample bag. Biological samples were collected voluntarily by hunters when time and conditions allowed.

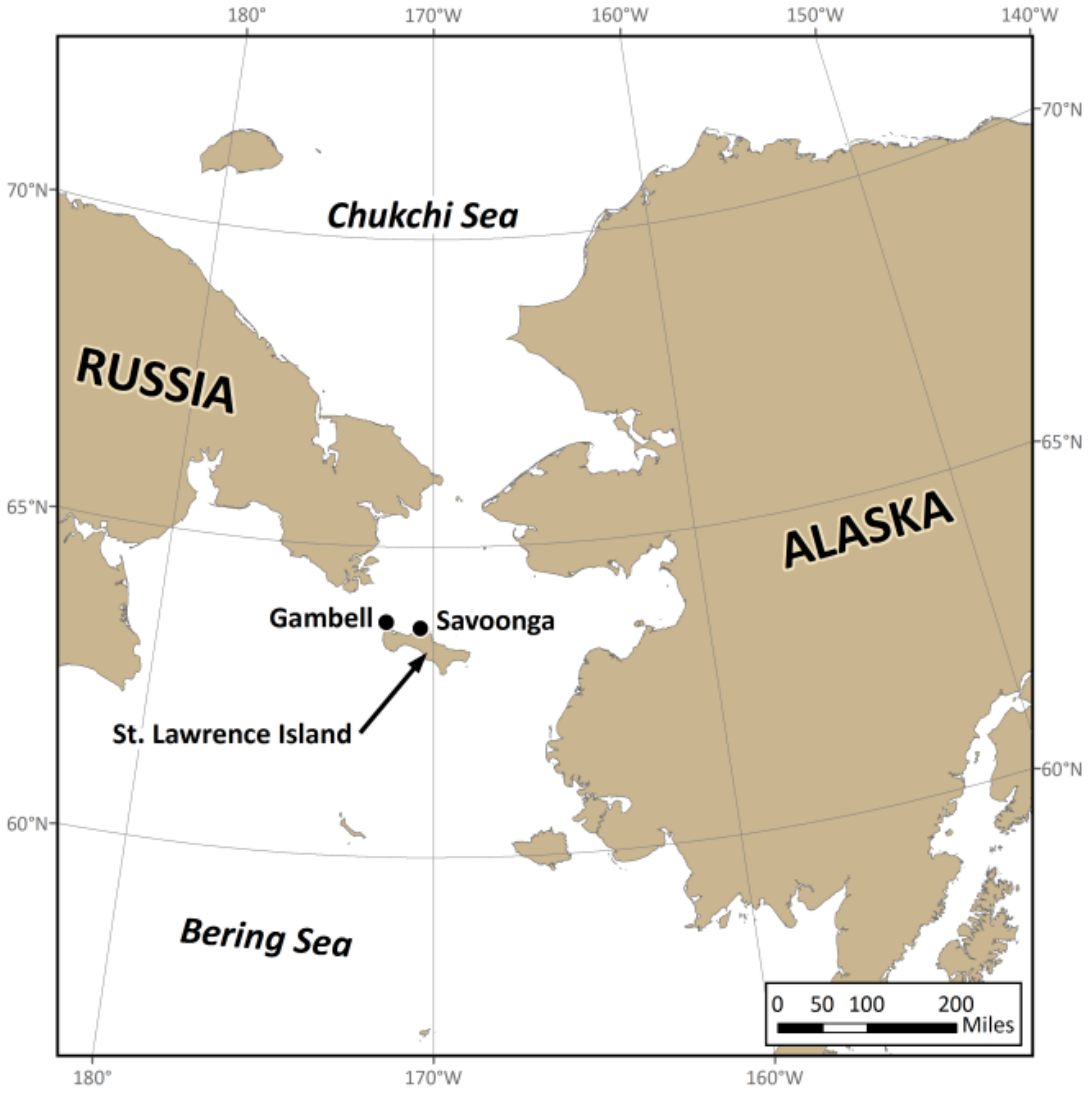

Figure 1. Walrus sample collection locations.

Hunter knowledge

Walrus hunters have extensive experience regarding the biology, general health, and behavior of the animals they harvest. Hunters recorded age class of walrus sampled based on tusk morphology. Males were considered subadults if tusks were < 12 inches long and adults if tusks were ≥ 12 inches long. Females were considered subadults if tusks were < 8 inches long and adults if tusks were ≥ 8 inches.

Age determination

Once samples arrived at the beach, the teeth were washed with water, wiped clean, dried, and placed in labeled manila envelopes. Envelopes were sent to Matson's Laboratory, Manhattan, Montana, where they were sectioned and aged by counting cementum layers (Fay 1982, Garlich-Miller et al. 1993).

Diet analyses

Intestinal and stomach contents. Walrus stomachs are large and many are empty during spring migration and clams found in stomachs are often kept by the hunters for food, therefore, in 2012–2014 a ~30 cm (1 ft) length of lower intestine from the lower gastrointestinal tract was collected for analysis of prey items. String was provided to tie off the ends or the section and its contents were placed into a pre-labeled plastic bag. In 2014 and 2016, a subsample of stomach contents (up to 250 ml) was also collected in a Nalgene bottle. Both intestines and stomach contents were frozen until analysis. In the lab, the intestine was cut longitudinally with scissors to remove contents. Both intestine and stomach contents were rinsed with freshwater through two stacked sieves with mesh sizes of 1.0 mm and 0.5 mm and prey items were sorted, and identified to the lowest taxonomic level.

To provide a general description of walrus prey items, we calculated the frequency of occurrence (FO) for each item of prey. FO is calculated as the number of intestines/stomachs that contain a prey taxa, divided by the number of intestines/stomachs with contents (i.e., we did not include empty stomachs/intestines in the calculation). Due to biases in digestion time, volume measurements were not considered representative of the true volume of prey consumed and were not analyzed.

Stable isotopes. Hunters were asked to cut off a small piece of a cheek pad containing five or six of the longest whiskers and place it in a pre-labeled plastic bag to be frozen with the rest of the samples. Once in the lab, the longest whisker was removed and sent to Dr. Seth Newsome at the University of New Mexico. The whisker was then subsampled by removing 0.5 mg every 0.25 cm along the length of the whisker, resulting in an average of 16 subsamples per whisker (range 11–31).

Muscle incorporates molecules from diet continually and reflects an average of recent (weeks) diet. Walrus muscle was freeze-dried (VirTis Sentry) for a minimum of 48 hrs and homogenized into a fine powder at the UAF Marine Mammal Laboratory. A subsample of 0.2–0.4 mg (dry weight) of ground muscle was placed into a tin capsule using a mico-balance (Sartorius Model MP2) and weighed (Dehn et al. 2007). Whisker subsamples were analyzed without pulverizing.

The stable isotope values were determined using a Thermo Scientific Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) coupled to a Costech Elemental Analyzer (ESC 4010). Whisker samples were analyzed at the University of New Mexico, muscle samples were analyzed at the Alaska Stable Isotope Facility at UAF. The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are expressed as delta (δ) notation in parts per thousand (‰).

$$\delta R‰ = (Rsample/Rstandard)-1) \times 1{,}000$$

where δR represents the difference between stable isotope ratios of the sample and the standard. Standards were Vienna Pee Dee Belemnite (VPDB) and atmospheric N2 for carbon and nitrogen, respectively.

Contaminants

Tissue preparation. Walrus samples collected in 2012–2014 were analyzed for contaminants. Blubber, liver, kidney, and muscle tissues were clean-sampled at the ADF&G lab following protocols established by the National Institute of Standards and Technology (Becker et al. 1991) and organochlorine contaminants were quantified by TDI–Brooks International, Inc., B&B Laboratories, Inc., College Station, TX. Subsamples of liver, kidney, and muscle tissue analyzed for organochlorines were analyzed for trace metals by Trace Element Research Lab (TERL) also in College Station, TX. Individual walruses were selected for contaminants testing only 1) if blubber, liver, kidney, and muscle tissues were available in sufficient quantity after clean-sampling each tissue, 2) if a tooth was available for aging, and 3) if the sex and age fit with the objective to analyze across all classes represented.

Essential and non-essential elements. Liver, kidney, and muscle tissue were analyzed for 20 elements, and blubber samples were analyzed for arsenic. Tissue samples were freeze-dried and then homogenized with a ball-mill. Percent moisture was calculated by comparing the weight of the wet sample with that of the dry sample. Samples of 0.5 g were digested as described in Quakenbush and Citta (2009).

Briefly, samples were homogenized with a meat grinder. An aliquot of approximately 100 g was weighed and freeze-dried and then further homogenized using a blender prior to extraction. Percent moisture was calculated by comparing the weight of the wet sample with the weight of the dry samples before a 0.5 g sample was extracted and digested in a microwave wet ash procedure using, H_2O_2, and HCl. Microwave digestion was used for all metals except As and Se.

Samples analyzed for As and Se were digested using magnesium dry ash digestion methods. This method uses methanol, HNO_3, HCl, and heat for digestion. After digestion As and Se were analyzed using Hydride Generation AA. Calibration was done at 0, 1.0, 5.0, 15.0 parts per billion (ppb) and the QC check was 10.0 and a known reference sample. The 5.00 ppb standard was checked every tenth sample and if the value differed by >5% from 5.00 the instrument was recalibrated. If the value was >10% different from 5.00 the last 10 samples were re-analyzed. Pb was analyzed using Graphite Furnace AA. Calibration was done at 0 and 1.0 ppb and then 3–5 standards were run to check the calibration. All other metals were analyzed using ICP on a Perkin-Elmer 4300 DV.

For total mercury, a 10 ml aliquot was removed immediately after dilution, HCl was added and concentrations were determined using Cold Vapor AA. Calibration was done at 0, 1.0, 5.0, 30.0 ppb and the QC checks were 10.0, 20.0, and a known reference sample. The 5.00 ppb standard was checked every tenth sample and if the value differed by >5% from 5.00 the instrument was recalibrated. If the value was >10% different from 5.00 the last 10 samples were rerun.

Quality control included analyses of certified reference materials (DOLT 2, 3, 4 and TORT 2, provided by the National Research Council of Canada (NRC) and NIST 1566b provided by the National Institute of Standards and Technology (NIST)), spiked samples, and duplicate digestions. Spikes, duplicates, blanks, and reference materials were analyzed for quality control with each batch of 20 samples or less. No analytes exceeded 2 times the method detection limits. The recovery for all analytes is between 80-120% for valid spikes. The criterion for valid

duplicates and spiked duplicates is ± 30%; where concentrations are greater than 3 times the method detection limit (MDL). The certified limit for reference materials NIST SRM is ± 20%; again where concentrations are greater than 3 times the MDL.

In addition to the elemental analysis described above, total Hg (THg), methylmercury (MeHg), and %MeHg were analyzed in liver, kidney, and muscle. Frozen tissues were subsampled, weighed, freeze-dried, and analyzed at the University of Alaska Fairbanks (UAF) Wildlife Toxicology Laboratory (WTL). Triplicate subsamples of each tissue were analyzed for greater precision of measured concentrations. THg was analyzed on a DMA80 direct mercury analyzer (Milestone Inc., Shelton, CT; EPA method 7473) (Knott et al., 2011). MeHg was analyzed by cold vapor atomic fluorescence spectroscopy with a Brooks Rand MERX 4400 (EPA Method 1630) after digestion in 25% KOH in methanol (Moses et al., 2009). Detection limits for THg in muscle was 0.016 µg/g dry weight (dw) (~0.003 µg/g wet weight (ww)) for a 0.030 g dw sample; in liver 0.05 µg/g dw (~0.01 µg/g ww) for a 0.010 g dw sample; and in kidney 0.030 µg/g dw (~0.006 µg/g wet mass) for a 0.015g dw sample. Detection limits for MeHg in muscle was 0.003 µg/g dw (~0.0006 µg/g ww) for a 0.15 g dw sample; in liver 0.008 µg/g dw (~0.0016 µg/g ww) for a 0.06 g dw sample; and in kidney 0.005 µg/g dw (~0.001 µg/g ww) for a 0.1 g dw sample. Quality control included liquid standards, certified reference materials (DOLT4 and TORT2, provided by the NRC and NIST 1946 provided by NIST), spikes and duplicate digestions. Quality control for THg was good with recoveries (% mean ±SD) 97.4 ± 2.1 (100 ng/g liquid standard), 99.3 ± 5.7 (NIST 1946) and 95.4 ± 2.8 (DORM4). For MeHg, %mean recoveries were 85.2 ± 9.0 (1µg/g liquid standard), 106.7 ± 10.7 (NIST 1946), 95.0 ± 4.7 (DORM4) and 105.7 ± 7.6 (TORT2). Recoveries for spiked samples were 101.2 ± 3.1. Mean relative standard deviation for duplicates was 3.8 ± 3.0.

Concentrations in dw from the TERL report were converted to concentrations in ww using sample specific water content measured for each sample. Detection limits were also converted to ww using 74% moisture content for all elements except arsenic where 56% moisture content was used. For statistical analysis, values that were below the detection limit (BDL) were entered into the dataset as half the detection limit. Elements that were BDL in more than half the samples by tissue were eliminated from the statistical analyses. The dw concentrations were then log transformed and ANOVA's were used to identify differences in concentrations by sex. All statistical tests were run in R (version 3.1.0, R Development Core Team 2014).

Organochlorines. Blubber, liver, kidney, and muscle were analyzed for organochlorines (e.g., PCBs and pesticides). Tissue samples were homogenized using a stainless steel blender with titanium blades. Aliquots of approximately 15 g of wet tissue were chemically dried using Hydromatix® and extracted with 100% dichloromethane using a Dionex Accelerated Solvent Extractor (ASE200) operated at 100°C and 2,000 psi. The extracts are reduced to 3 mL by evaporative solvent reduction. A 100 µL aliquot is removed and weighed to determine lipid weight. The remaining sample portion is purified using alumina/silica gel column chromatography and gel permeation column (GPC)/high performance liquid chromatography (HPLC). After HPLC purification, the eluents were reduced to 0.5 mL and analyzed for PCBs and pesticides by either gas chromatography/mass spectrometry (GC/MS) or gas chromatography/electron capture detector (GC/ECD).

A GC/ECD, coupled to two capillary columns, was used to resolve and detect chlorinated hydrocarbons (polychlorinated biphenyls and pesticides) in tissues. Samples were injected into a temperature-programmed GC/ECD, operated in splitless mode. The capillary columns are DB-5 (30 m x 0.25 mm ID and 25 μm film thickness) and DB-17HT (30 m x 0.25 mm ID and 0.15 μm film thickness). The DB-17HT column was used for analyte confirmation. A data acquisition system continuously acquired and stored all data for quantitation. This method is capable of producing data at parts-per billion (ppb) and parts-per trillion (ppt) concentrations. The surrogate spiking solution includes 4,4'-dibromooctaflurobiphenyl (DBOFB), 2,2',4,5',6 pentachlorobiphenyl (PCB 103), and 2,2',3,3',4,5,5'6 octachlorobiphenyl (PCB 198). Surrogate solution (100 μL) is added to all samples and quality control samples prior to extraction. Surrogate compounds are resolved from, but elute in close proximity to, the analytes of interest. The recovery of PCB 103 is used to correct analyte concentrations. Spikes, duplicates, and blanks were analyzed for quality control with each batch of 20 samples or less.

The laboratory report identified analytes that were: 1) not detected, 2) detected below the MDL, 3) detected in the procedural blanks greater than 3X MDL, and 4) detected in duplicate samples where the relative percent difference (RPD) was < 2X MDL.

For statistical analysis, ANOVA's were used to detect differences in total wet concentrations of chlordane, HCH, DDT, and PCB in blubber by sex. All statistical tests were run in R (version 3.1.0, R Development Core Team 2014).

Vitamins

Liver tissue from 37 walruses analyzed for contaminants were also analyzed for vitamin A and E concentrations. A subsample between 5–10 g was taken from each liver and placed in I-Chem glass jars and frozen at -40°C. Samples were analyzed for vitamins at Michigan State University, Diagnostic Center for Population and Animal Health. Retinol, a form of vitamin A, was extracted from the liver by first weighing the sample and then the sample was homogenized in de-gassed methanol containing butylated hydroxytoluene (BHT) as an antioxidant. Potassium hydroxide (40%) was added and the sample was then heated to 100°C in a nitrogen atmosphere for 10 minutes. This saponifies retinyl esters resulted in free retinol which was extracted in hexane. A known aliquot of hexane was removed and dried under vacuum and then the remaining retinol was re-dissolved in chromatographic mobile phase and placed in autosampler vials. Alpha-tocopherol, a form of vitamin E, was extracted from the liver by first weighing the sample and then homogenizing it in distilled, deionized water (1:4 weight to volume). Lipids were then extracted from the homogenate with equal volumes of ethanol and hexane. BHT was then added to the ethanol as an antioxidant. After thorough mixing the samples were centrifuged and a known aliquot of hexane was removed. The hexane was dried under vacuum and then the remaining alpha-tocopherol was re-dissolved in chromatographic mobile phase and placed in autosampler vials.

Samples were then analyzed chromatographically using a Waters Acquity separation module, Waters 996 photodiode array detector, and Waters Empower Pro Chromatography Manager software. Elution was isocratic using a mobile phase of acetonitrile: methylene chloride: methanol (70:20:10, v/v/v) and a Symmetry C18, 3.5 μm, 4.6X75 mm analytical column. The system also contained a Sentry guard column, C18, 3.5 μm. The flow rate was 1.2 mL/min and

detection was by UV absorption at 325 nm (retinol) and 292 nm (alpha-tocopherol). Peak integration is by the ApexTrack method of Empower Pro. All peaks were reviewed manually after initial auto integration. Peaks with large shoulders or peaks that were otherwise questionable were reviewed for purity using photodiode array data.

For statistical analysis, ANOVA's were used to detect differences in the concentrations of vitamin A and E in liver by sex. All statistical tests were run in R (version 3.1.0, R Development Core Team 2014).

Disease

Blood was collected from inside the heart when possible by scooping pooled blood into pre-labeled blood tubes with spin beads. If blood from the heart was not available it was collected from another location in the body cavity or from the bullet exit wound. Blood vials were placed in the sample bag and transported back to the community where it was centrifuged and serum was transferred to sterile cryovials. The cryovials were frozen and stored at –20°C for one or two weeks and then at –40°C for several months before shipping to Athens Veterinary Diagnostic Laboratory in Athens, Georgia for testing.

We tested blood serum for antibodies to six diseases known to affect walruses and seals (phocids). We tested for *Brucella* spp., phocine herpesvirus-1 (PhHV-1), canine distemper (CDV), phocine distemper (PDV), Leptospira, and Toxoplasma.

Brucella is known to cause reproductive problems for terrestrial animals (Rhyan et al. 2001) and can cause reproductive problems for pinnipeds and cetaceans (Foster et al. 2002, Miller et al. 1999); abortions have been documented in cetaceans (e.g., Miller et al.1999). Although, it is known there are *Brucella* spp. specific to marine mammals (e.g., *B. pinnipedialis* and *B. ceti*; Larsen et al. 2013), the tests that are commercially available, have been developed for domestic animals and their efficacy for testing in marine mammals is unknown. We tested for *Brucella abortus* (a terrestrial mammal *Brucella* spp.) using the standard card agglutination test (SCA) developed for swine.

PhHV-1 usually affects pups and immunocompromised or diseased adults (Zarnke et al.1997). PDV is a morbillivirus known to cause large seal die-offs. PDV infected seals exhibit symptoms of respiratory distress and the most common post-mortem finding is pneumonia (Kennedy 1998). In Alaska, PDV has previously been identified in harbor seals (Zarnke et al. 2006). For PDV and CDV, serum was tested for the presence of antibodies by using a serum neutralization test and for PhHV-1 serum was tested using an ELISA test. Threshold titers of ≥16 were considered positive for all three of these tests.

Leptospirosis is a zoonotic disease caused by Leptospira bacteria that affects a variety of animals including marine mammals (Gulland et al. 1996). This disease is known cause reproductive failure and renal disease (Gulland et al. 1996) in pinnipeds (Gerber et al. 1993, Delaney et al. 2014) and has been isolated from a Southern right whale (*Eubalaena australis*) kidney (Loffler et al. 2015). Leptospira was tested for using microscopic agglutination test. Titers of ≥100 were considered positive for Leptospira antibodies.

Toxoplasma is a protozoan that can be fatal for many terrestrial mammals and can cause encephalitis (swelling of the brain) in marine mammals (Dubey et al. 2003). Serum was tested for the presence of toxoplasma antibodies using antibody latex AG test. Titers of <1:32 are interpreted as negative, titers of 1:32 are considered weak positive, and titers of 1:64 are considered positive. These titers, however, were developed for pigs and cats and the titers for marine mammals may be different.

Coccidian parasitic protozoans, *Toxoplasma gondii* and *Sarcocystis* sp., were also tested for, using molecular PCR. To determine protozoal parasite infection status, tissue (liver, muscle) samples from Pacific walrus (n=35) were processed. Approximately 25 mg of each tissue type was digested overnight at 57°C with Proteinase K. DNA extractions were then conducted using the spin-column protocol for purification of total DNA from animal tissues (Qiagen DNeasy Blood and Tissue Kit). DNA was eluted in 30 µL of 1:10 dilution of Qiagen EB buffer and molecular grade water. Extracted DNA samples were stored at -20°C between PCR reactions. Previously published pan-coccidian ApiITS1 primers (Gibson et al. 2011) anchored in the 18S and 5.8S small subunit (SSU) rDNA gene array were used to screen all samples. The ApiITS1 primers amplify across the internal transcribed spaces 1 (ITS-1) region to distinguish between closely related and novel species of tissue-encysting coccidian parasites.

We conducted PCR using 3 µL of each DNA extraction with 5 µL of PCR buffer (10x containing $MgCl_2$; Sigma, St Louis, Missouri, USA), 5 µL of 2 mM dNTP (Sigma-Aldrich, DNTP100-1KT), 20 pmol of each primer, and 1.5U of Taq DNA Polymerase (Sigma-Aldrich, D1806), in a total reaction volume of 50 µL. We then carried out PCR amplification for 35 cycles (94C for 40 sec, 58C for 40 sec, 72C for 40 sec, followed by one 10-min extension at 72C). All PCRs were nested, and amplicons were visualized in gel-red (Biotium Inc, Hayward, CA, USA), stained 1% agarose gels and purified using Exo SAP-IT (USB, Cleveland, Ohio, USA) according to manufacturer's instructions.

DNA sequencing was performed by Rocky Mountain Laboratory Genomics Unit DNA Sequencing Center, Division of Intramural Research, Hamilton, Montana. We visualized, aligned, and analyzed the sequences using the Seqman component of the Lasergene software package (DNASTAR Inc., Madison, Wisconsin, USA) and identified the sequences by alignment with known reference sequences and verified via nucleotide BLAST search in GenBank.

We also tested intestinal and stomach contents from walruses collected during 2012–2014 for domoic acid and saxitoxin, toxic by-products of algae responsible for harmful algal blooms (HABS). In the lab, prior to sorting intestines and stomachs for prey, 5 ml of content was removed from each, placed in centrifuge tubes with screw caps, and refrozen at -20 °C until analyzed for algal toxins. The subsamples of content were then shipped to Northwest Fisheries Science Center, Seattle, WA in 2012 and 2013 and to U.C. Santa Cruz, California in 2014 for analysis. Methods were the same for both labs; algal toxins were quantified using commercially available kits, Biosense® DA ELISA and Abraxis saxitoxin ELISA for domoic acid and saxitoxin, respectively (Lefebvre et al. 2016). In 2016, stomach contents paired with urine samples were tested for both domoic acid and saxitoxin at Northwest Fisheries Science Center. In 2016, parts of bivalves found in the stomachs (i.e., feet and siphons) were removed from the

stomach sample without rinsing in fresh water, identified to the lowest taxonomic level possible, refrozen, and shipped for testing.

Population parameters

Age and sex ratio of harvest. We summarized the age distribution of the walruses sampled by plotting the number of walruses in each age class. To compare age distributions over time, we categorized our sample into six groups. The groups were 1–10, 11–15, 16–20, 21–25, 26–30, and 31–35 years of age. The sex ratio of sampled walruses was measured as the proportion of females in the adult harvest.

Productivity. We used information recorded by the hunters and by the local beach monitors regarding the reproductive status of the females harvested. Because the calves and yearlings are almost always harvested with the females and fetuses are obvious during butchering, the reproductive status of females can be determined by inspection.

Results

Walruses sampled

During 2012–2014 and 2016, a total of 225 walruses were sampled (116 in Gambell and 109 in Savoonga) (Table 1). Of the 116 sampled in Gambell, 28 were male and 88 were female, and of the 109 sampled in Savoonga, 87 were male and 18 were female (sex was unknown for four). Although the sex ratio of sampled walruses was skewed by village with ~68% more females sampled than males in Gambell, the reverse was true for Savoonga, so that overall the sex ratio of the samples for both villages was similar (115 males:106 females).

Table 1. Number of harvested walruses by sex (male = M, female = F, unknown = U) sampled near Gambell and Savoonga in 2012–2014 and 2016.

	Gambell			Savoonga				**Total**			
Year	M	F	Total	M	F	U	Total	**M**	**F**	**U**	**Total**
2012	4	49	53	20	9	1	30	**24**	**58**	**1**	**83**
2013	18	21	39	25	2	0	27	**43**	**23**	**0**	**66**
2014	6	9	15	31	6	3	40	**37**	**15**	**3**	**55**
2016	0	9	9	11	1	0	12	**11**	**10**	**0**	**21**
Total	**28**	**88**	**116**	**87**	**18**	**4**	**109**	**115**	**106**	**4**	**225**

Hunter knowledge

In 2012, 92% (76 of 83) of walruses sampled from the harvests of Gambell and Savoonga received health scores by hunters; in 2013, it was 89% (59 of 66), in 2014, 95% (52 of 55), and in 2016, it was 100% (of 21). Overall, 98% (203 of 208) were scored as average or very healthy (Table 2).

Table 2. Results of hunter health scores for walruses sampled near Gambell and Savoonga in 2012–2014 and 2016.

Year	Unhealthy (%)	Average health (%)	Very healthy (%)	Total
2012	3 (4)	23 (30)	50 (66)	76
2013	1 (2)	38 (64)	20 (34)	59
2014	1 (2)	30 (58)	21 (40)	52
2016	0 (0)	6 (29)	15 (71)	21
Total	**5 (2)**	**97 (47)**	**106 (51)**	**208**

Diet

Intestinal and stomach contents. Contents of the intestines and stomachs from 116 walruses (51 in 2012, 29 in 2013, 15 in 2014, and 21 in 2016) were analyzed; 57 (49%) were empty and 16 had prey items that could not be identified. Identifiable prey items were found for 43 walruses (14 in 2012, 12 in 2013, 9 in 2014, and 8 in 2016). Identifiable prey items included hard parts resistant to digestion (e.g., otoliths and opercula) and some soft parts (e.g., clam feet and syphons). The most common prey taxa identified and their frequency of occurrence (%FO), from both intestines and stomach contents, is given in Table 3.

Fishes. Fish were uncommon in our samples. Three samples, however had fish, two were identified as Pacific sand lance, *Ammodytes hexapterus*, but the third was not identifiable.

Invertebrates. Epibenthic invertebrates were commonly consumed by walruses harvested near Saint Lawrence Island. We identified a minimum of 20 species including representatives from seven taxonomic groups (Polychaeta, Gastropoda, Bivalvia, Crustacea, Echiurida, Cucumariidae, and Priapulida), of which mollusks and echiurids were most common.

Table 3. Percent frequency of occurrence (%FO) of prey identified from walrus intestine and stomach contents collected in Alaska, 2012–2014 and 2016.

Prey	n	%FO 43
All Fish		7
Pacific sand lance (*Ammodytes hexapterus*)		5
All Invertebrates		100
All Polychaeta		14
Polynoidae		2
Nephtyidae, *Nephtys* spp.		12
Nereidia, *Nereis* spp.		2
All Mollusca		86
Gastropoda		51
Buccinidae		26
Buccinum spp.		14
Naticidae		2
Cryptonatica spp.		35
Bivalvia		63
Cardiidae, *Serripes* spp.		9
Mactridae, *Mactromeris polynyma*		9
Myidae, *Mya* spp.		19
Nuculanidae		2
Nuculana pernula		2
Nuculidae, *Ennucula tenuis*		5
All Crustacean		33
All Amphipoda		9
Uristidae, *Anonyx* spp.		2
Pontoporeiidae, *Pontoporeia* spp.		2
Gammaridae		2
All Decapods		28
All Shrimp		7
Caridea		2
Crangonidae, *Argis* spp.		2
All Crab		23
Oregoniidae		7
Hyas spp.		5
Chionoecetes spp.		7
Paguridae		2
All Cucumariidae		5
All Echiuridae		60
All Priapulida		2
Minimum number of species eaten = 21		

Stable isotopes from whiskers. Whiskers grow continuously and are made up of molecules, including stable isotopes of carbon and nitrogen, from diet. Once the whisker is formed its chemical makeup remains biochemically unchanged and can be measured. Analyzing isotopes over the length of a whisker can provide a general record of what was eaten or where foraging occurred during the period of whisker growth. Although whisker growth rate is unknown, a distinctive pattern of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values was found and was consistent among whiskers analyzed (Figs. 2 and 3). This pattern is likely due to seasonal movements between winter and summer feeding areas.

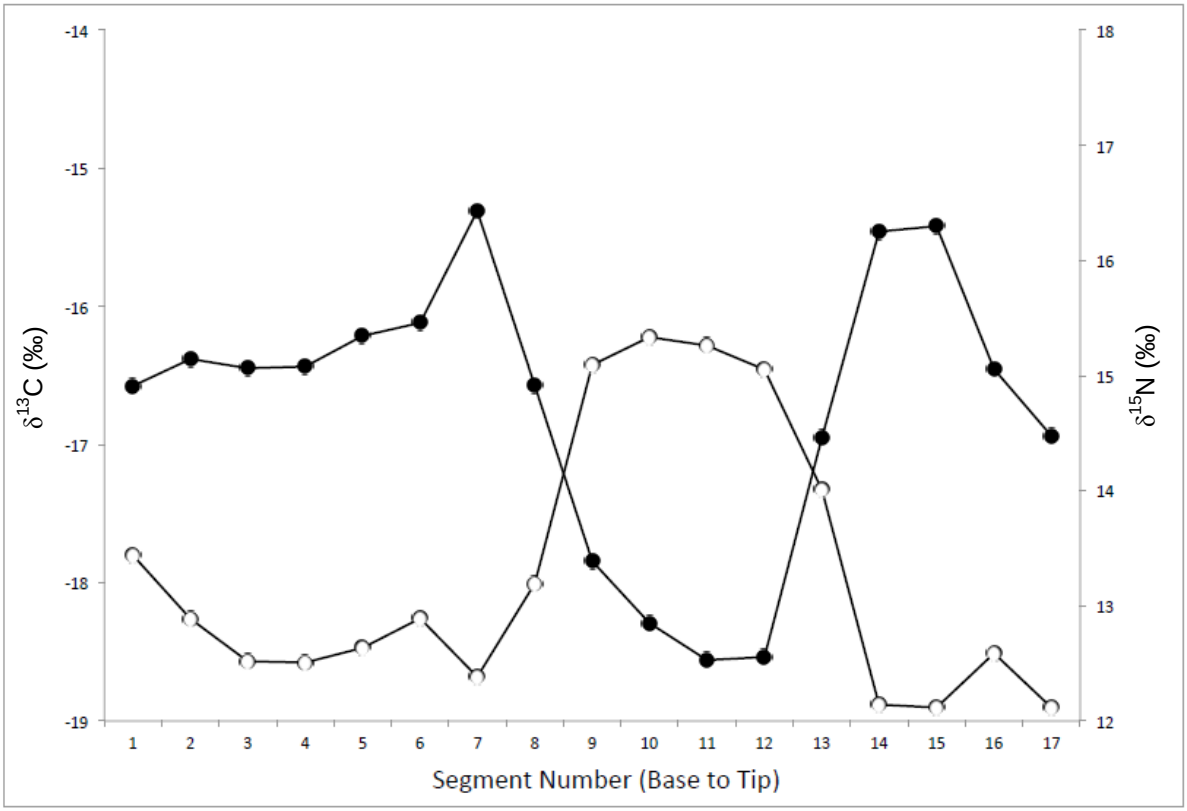

Figure 2. Stable carbon ($\delta^{13}C$, closed circles) and nitrogen ($\delta^{15}N$, open circles) values of a whisker from an 11-yr-old female walrus (G12-0086) harvested near Gambell in May 2012. Figure prepared by Seth Newsome.

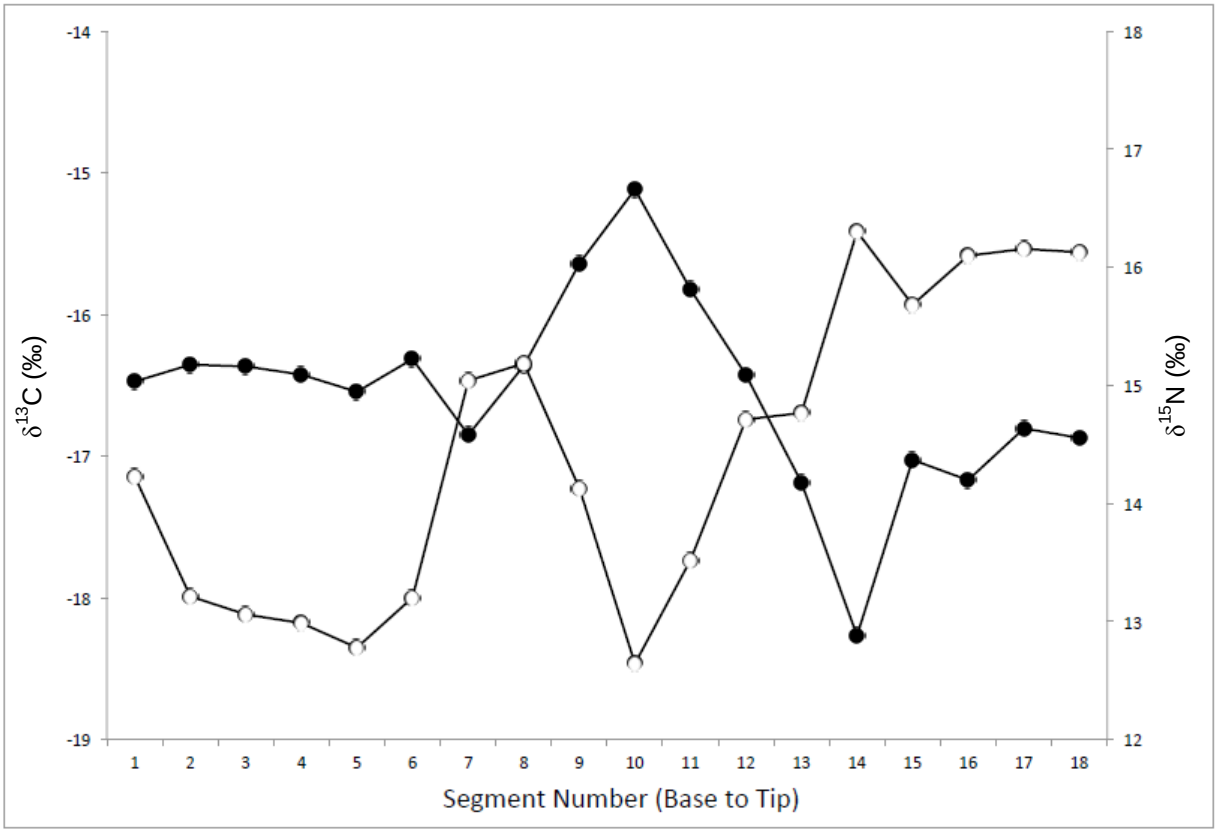

Figure 3. Stable carbon ($\delta^{13}C$, closed circles) and nitrogen ($\delta^{15}N$, open circles) values of a whisker from a 10-yr-old female walrus (G12-0088) harvested near Gambell in May 2012. Figure prepared by Seth Newsome.

A plot of the mean values for whiskers from 31 walruses (three of which were collected at Hooper Bay and not included in the rest of this study) shows high variability in individuals for both stable carbon and nitrogen (Fig. 4), which is expected given the fluctuations across the whiskers in Figures 2 and 3. The $\delta^{15}N$ values for walruses shown in Figure 4 indicate that some walruses consume higher trophic level prey than others. Low trophic filter feeders like clams (e.g., *Mya* spp. and *Serripes* spp.) have lower nitrogen values than high trophic scavengers/carnivores like crab (e.g., Paguridae and *Chionoecetes* spp.) and gastropods (e.g., *Buccinum* spp.) (Dehn et al. 2007, Iken et al. 2010). Additionally carbon changes with latitude and is likely partly responsible for the wide range of $\delta^{13}C$ values in Figure 4. Northern waters such as the Beaufort Sea are more depleted in $\delta^{13}C$ (Schell et al. 1998), and environmental $\delta^{13}C$ can change seasonally depending on the contribution of primary production from sea ice algae (Wang et al. 2014). Known seasonal movement patterns (i.e., in the Bering Sea October–April and in the Chukchi Sea May–September) could be used to estimate whisker growth rates so that stable isotopes can be analyzed and compared during specific time periods.

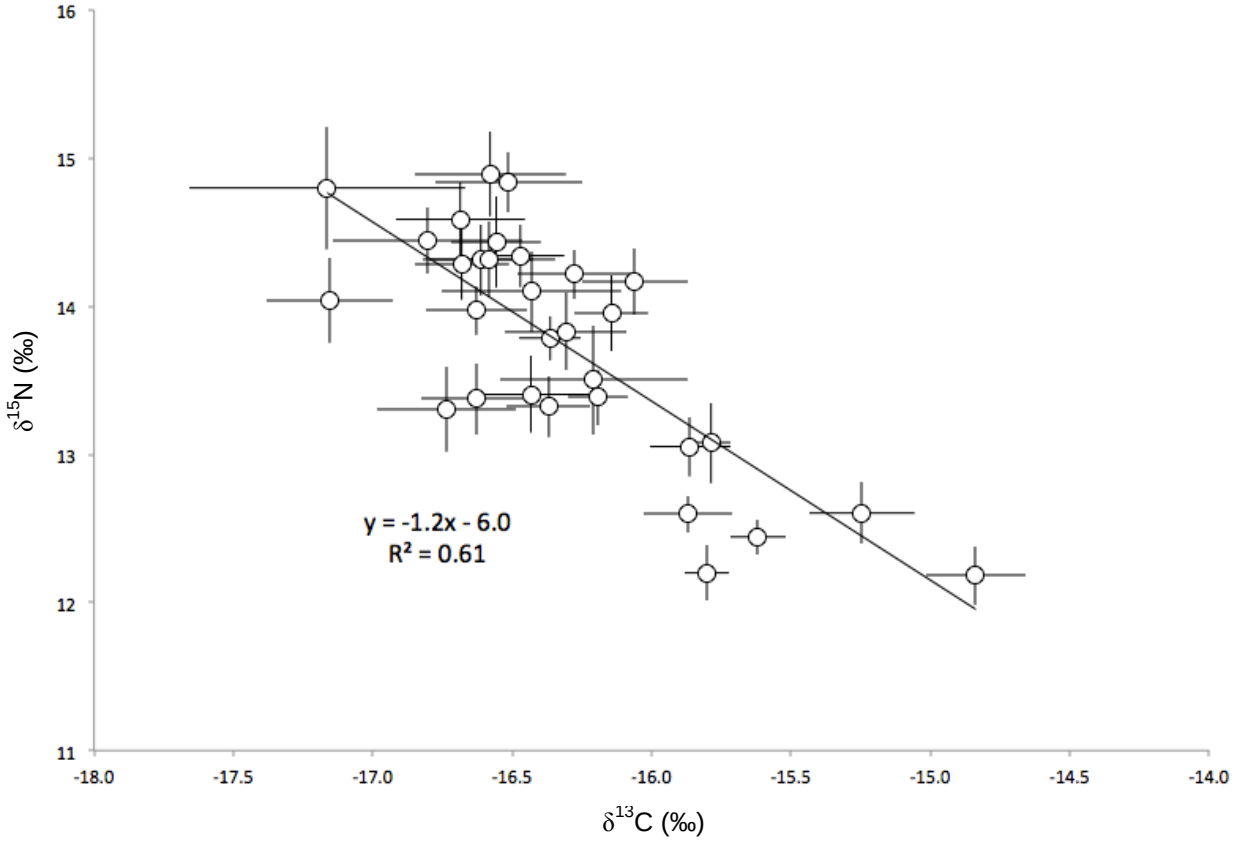

Figure 4. Mean stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values of whiskers of 28 walruses harvested in May 2012 near Saint Lawrence Island and three near Hooper Bay. Error bars are standard error. Figure prepared by Seth Newsome.

Contaminants

Essential and non-essential elements. Concentrations of 20 trace elements were quantified in tissues of 42 walruses during 2012–2014; liver and kidney were analyzed in all years and muscle was only analyzed in 2012 for 14 walruses (10 females and 4 males). One methylated element (MeHg) was quantified in liver and kidney of all 42 of these walruses, and from muscle tissue of 41 of these walruses. In addition, As was quantified in blubber, where it is known to concentrate in marine mammals (Woshner et al. 2001a, Ebisuda et al. 2002, Moses et al. 2009, Woshner et al. 2001b, Wagemann et al. 1984). Females ranged in age from 9 to 23 years (mean 15.9 ± 4.2); males from 7 to 34 years (mean 19.9 ± 7.9). Some of the elements we tested are potentially toxic at high levels (i.e., elements of concern). These included As, Cd, Hg, MeHg, and Pb (Tables 4a, 4b, 5a, and 5b) and others are essential nutrients which are only toxic at extreme concentrations (e.g., Cu, Fe, and Mg; Tables 6a and 6b). We presented results as wet weight (ww) (Tables 4a, 5a, and 6a) and dry weight (dw) (Tables 4b, 5b, and 6b) for comparison with other studies. Concentrations of elements analyzed in this study provide a temporal comparison with previous analyses (e.g., Warburton and Seagars 1993, Seagars et al. 1994, Taylor et al. 1989) as well as a current baseline for apparently healthy walruses harvested for subsistence. For elements of concern, Hg and Pb were highest in liver, Cd was highest in kidney, and As was highest in blubber (Tables 4a and 4b).

A separate analysis was conducted for MeHg, total Hg (THg), and selenium (Se) to better understand this most toxic form of mercury in walrus tissues. We found MeHg concentrations to be highest in liver (0.053 µg/g ww), however, in proportion to concentrations of THg, liver had the lowest %MeHg (8.8%) (Tables 5a and 6a). In contrast, MeHg concentrations in muscle were much lower (0.019 µg/g dw), but the proportion of MeHg within THg was highest (93.2%; Table 5b). We also found that the mean molar ratio of Se:Hg was highest in muscle at 259 and lowest in liver at 6.6. It is thought that Hg toxicity can be reduced in some tissues when it is associated with Se, such as when the molar ratio is > 1 (Koeman et al. 1975, Dietz et al. 2013, Correa et al. 2015).

Table 4a. Arithmetic mean, standard deviation, and range of concentrations (µg/g = parts per million ***dry weight***) for elements of concern in liver, kidney, muscle, and blubber of walruses harvested near Saint Lawrence Island, Alaska, 2012–2014. The tissue with the highest value for each element is in bold. Concentrations that were below detection limits are denoted by BDL.

Element (detection limit)	Liver (n=42) Mean ± SD (range)	Kidney (n=42) Mean ± SD (range)	Muscle (n=14) Mean ± SD (range)	Blubber (n=42) Mean ± SD (range)
Arsenic, As (0.004-0.02)	0.90 ± 0.29 (0.50-1.61)	1.38 ± 0.69 (0.52-3.46)	0.56 ± 0.30 (0.21-1.15)	**3.24 ± 1.45 (1.48-7.09)**
Cadmium, Cd (0.002)	14.63 ± 5.79 (3.94-34.70)	**109.17 ± 43.63 (16.80-241.0)**	0.21 ± 0.12 (0.07-0.58)	–
Mercury, Hg (0.00005)	**3.69 ± 5.30 (0.58-29.3)**	0.83 ± 0.55 (0.28-3.65)	0.10 ± 0.08 (0.04-0.38)	–
Lead, Pb (0.01)	**0.17 ± 0.10 (0.05-0.50)**	0.07 ± 0.10 BDL-0.63	0.00 ± 0.01 BDL-0.06	–

Table 4b. Arithmetic mean, standard deviation, and range of concentrations (µg/g = parts per million ***wet weight***) for elements of concern in liver, kidney, muscle, and blubber of walruses harvested near Saint Lawrence Island, Alaska, 2012–2014. The tissue with the highest value for each element is in bold. Concentrations that were below detection limits are denoted by BDL.

Element (detection limit)	Liver (n=42) Mean ± SD (range)	Kidney (n=42) Mean ± SD (range)	Muscle (n=14) Mean ± SD (range)	Blubber (n = 42) Mean ± SD (range)
Arsenic, As (0.002-0.02)	0.27 ± 0.09 (0.13-0.49)	0.29 ± 0.15 (0.11-0.75)	0.16 ± 0.08 (0.06-0.28)	**2.74 ± 1.13 (1.28-5.72)**
Cadmium, Cd (0.0005-0.0016)	4.37 ± 1.75 (1.21-10.38)	**23.06 ± 8.83 (3.78-48.68)**	0.06 ± 0.04 (0.02-0.16)	–
Mercury, Hg (0.000013)	**1.12 ± 1.70 (0.18-9.84)**	0.18 ± 0.12 (0.05-0.80)	0.03 ± 0.02 (0.01-0.09)	–
Lead, Pb (0.003-0.008)	**0.05 ± 0.03 (0.02-0.17)**	0.02 ± 0.02 BDL-0.14	0.00 ± 0.01 BDL-0.02	–

Table 5a. Arithmetic mean, standard deviation, and range of concentrations (µg/g = parts per million ***wet weight***) for total mercury (THg) and methylmercury (MeHg) in liver, kidney, and muscle of walruses harvested near Saint Lawrence Island, Alaska, 2012–2014.

Element	Liver (n=42) Mean ± SD (range) Detection limit	Kidney (n=42) Mean ± SD (range) Detection limit	Muscle (n=41) Mean ± SD (range) Detection limit
THg	1.172 ± 1.857 (0.214-10.513) 0.001	0.195 ± 0.142 (0.060-0.939) 0.006	0.021 ± 0.012 (0.009-0.084) 0.0003
MeHg	0.053 ± 0.028 (0.022-0.177) 0.0016	0.019 ± 0.011 (0.007-0.076) 0.001	0.019 ± 0.011 (0.010-0.081) 0.006

Table 5b. Arithmetic mean, standard deviation, and range of concentrations (µg/g = parts per million ***dry weight***) for total mercury (THg), methylmercury (MeHg), percent methylmecury (%MeHg), and the molar ratio of total selenium (TSe) to THg in liver, kidney, and muscle of walruses harvested near Saint Lawrence Island, Alaska, 2012–2014.

Element	Liver (n=42) Mean ± SD (range) Detection limit	Kidney (n=42) Mean ± SD (range) Detection limit	Muscle (n=41) Mean ± SD (range) Detection limit
THg	4.091 ± 6.155 (0.700-33.502) 0.05	0.956 ± 0.670 (0.327-4.428) 0.003	0.079 ± 0.047 (0.041-0.333) 0.016
MeHg	0.188 ± 0.097 (0.086-0.634) 0.008	0.094 ± 0.052 (0.041-0.357) 0.005	0.073 ± 0.046 (0.038-0.322) 0.003
% MeHg	8.8 ± 5.1 (0.5-19.4)	11.2 ± 5.1 (3.4-30.9)	93.0 ± 9.2 (63.9-110.1)
Molar TSe:THg	6.6 ± 3.0 (1.2-13.0)	79 ± 36 (9-194)	259 ± 119* (36-482)

* Molar TSe:THg ratio for muscle was analyzed for 14 walruses collected in 2012.

Table 6a. Arithmetic mean, standard deviation, and range of concentrations (µg/g = parts per million ***dry weight***) for other essential and non-essential elements in liver, kidney, and muscle of walruses harvested near Saint Lawrence Island, Alaska, 2012–2014. Concentrations that were below detection limits are denoted by BDL.

Element (detection limit)	Liver (n=42) Mean ± SD (range)	Kidney (n=42) Mean ± SD (range)	Muscle (n=14) Mean ± SD (range)
Silver, Ag (0.002)	**2.71 ± 1.50 (0.34-7.80)**	0.05 ± 0.02 (BDL-0.10)	BDL
Aluminum, Al (0.02)	**1.44 ± 1.50 (BDL-7.46)**	0.44 ± 0.57 (BDL-2.37)	0.59 ± 0.51 (BDL-1.62)
Boron, B (0.1)	BDL	0.08 ± 0.23 (BDL-1.10)	**0.12 ± 0.32 (BDL-1.08)**
Barium, Ba (0.01)	0.06 ± 0.14 (BDL-0.84)	**0.07 ± 0.06 (BDL-0.29)**	0.02 ± 0.04 (BDL-0.11)
Berylium, Be (0.01)	BDL	BDL	BDL
Chromium, Cr (0.01)	0.23 ± 0.26 (BDL-1.72)	**0.34 ± 0.79 (0.07-5.22)**	0.29 ± 0.23 (0.10-0.94)
Copper, Cu (0.02-0.99)	**71.27 ± 37.80 (7.63-182.0)**	20.32 ± 3.85 (14.70-31.80)	2.92 ± 0.42 (2.11-3.47)
Iron, Fe (0.10-0.20)	**863.48 ± 350.38 (303-1610)**	325.17 ± 83.69 (117-493)	549.71 ± 71.47 (384-664)
Magnesium, Mg (0.20)	543.24 ± 45.7 (454-647)	571.14 ± 75.17 (495-937)	**720.93 ± 64.15 (641-886)**
Manganese, Mn (0.01-0.04)	**7.72 ± 1.75 (5.29-13.10)**	2.57 ± 0.44 (1.80-3.47)	0.35 ± 0.15 (BDL-0.49)
Molybdenum, Mo (0.01-0.20)	**1.15 ± 0.16 (0.83-1.49)**	0.55 ± 0.13 (0.35-0.97)	BDL
Nickel, Ni (0.01-0.02)	0.07 ± 0.10 (BDL-0.43)	**0.26 ± 0.16 (BDL-0.83)**	0.04 ± 0.04 (BDL-0.15)

Element (detection limit)	Liver (n=42) Mean ± SD (range)	Kidney (n=42) Mean ± SD (range)	Muscle (n=14) Mean ± SD (range)
Selenium, Se (0.01-0.02)	6.40 ± 3.47 (2.44-18.60)	**24.67 ± 9.78 (13.40-68)**	7.93 ± 3.42 (3.43-14.5)
Strontium, Sr (0.01-0.02)	0.15 ± 0.12 (BDL-0.45)	**0.64 ± 0.36 (0.26-2.17)**	0.31 ± 0.33 (0.12-1.3)
Tin, Sn (0.004)	**0.04 ± 0.03 (BDL-0.19)**	0.01 ± 0.02 (BDL-0.10)	0.0 ± 0.02 (0.0-0.06)
Vanadium, V (0.01-0.10)	**4.37 ± 3.10 (1.02-15.80)**	1.18 ± 1.51 (BDL-6.82)	BDL
Zinc, Zn (0.04)	153.69 ± 25.09 (105-210)	146.33 ± 30.11 (103-233)	**192 ± 33.79 (119-239)**

Table 6b. Arithmetic mean, standard deviation, and range of concentrations (µg/g = parts per million ***wet weight***) for other essential and non-essential elements in liver, kidney, and muscle of walruses harvested near Saint Lawrence Island, Alaska, 2012–2014. Concentrations that were below detection limits are denoted by BDL.

Element (detection limit)	Liver (n=42) Mean ± SD (range)	Kidney (n=42) Mean ± SD (range)	Muscle (n=14) Mean ± SD (range)
Silver, Ag (0.0005-0.002)	**0.81 ± 0.47 (0.10-2.39)**	0.01 ± 0.01 (BDL-0.02)	BDL
Aluminum, Al (0.005-0.08)	**0.43 ± 0.44 (BDL-2.27)**	0.09 ± 0.12 (BDL-0.52)	0.17 ± 0.15 (BDL-0.49)
Boron, B (0.03-0.08)	BDL	0.02 ± 0.05 (BDL-0.22)	**0.03 ± 0.09 (BDL-0.31)**
Barium, Ba (0.003-0.008)	**0.02 ± 0.04 (BDL-0.26)**	0.01 ± 0.01 (BDL-0.06)	0.01 ± 0.01 (BDL-0.03)
Berylium, Be (0.003-0.008)	BDL	BDL	BDL
Chromium, Cr (0.003-0.008)	0.07 ± 0.08 (BDL-0.54)	0.07 ± 0.16 (0.01-1.04)	**0.08 ± 0.06 (0.02-0.25)**

Element (detection limit)	Liver (n=42) Mean ± SD (range)	Kidney (n=42) Mean ± SD (range)	Muscle (n=14) Mean ± SD (range)
Copper, Cu (0.005-0.04)	**21.32 ± 11.43 (2.34-54.24)**	4.32 ± 0.83 (2.98-6.81)	0.85 ± 0.22 (0.53-1.30)
Iron, Fe (0.03-0.11)	**257.31 ± 107.49 (96.05-529.69)**	69.63 ± 19.60 (22.23-111.42)	160 ± 40.3 (111.78-234.43)
Magnesium, Mg (0.05-0.16)	161.94 ± 16.06 (126.96-193.42)	121.32 ± 15.47 (103.95-200.52)	**207.95 ± 35.93 (155.43-270.13)**
Manganese, Mn (0.003-0.02)	2.29 ± 0.50 (1.50-3.54)	**0.55 ± 0.09 (0.37-0.75)**	0.10 ± 0.05 (BDL-0.16)
Molybdenum, Mo (0.003-0.01)	**0.34 ± 0.05 (0.26-0.44)**	0.12 ± 0.03 (0.08-0.20)	BDL
Nickel, Ni (0.003-0.01)	0.02 ± 0.03 (BDL-0.14)	**0.06 ± 0.03 (BDL-0.18)**	0.01 ± 0.01 (BDL-0.04)
Selenium, Se (0.003-0.01)	1.92±1.11 (0.72-6.25)	**5.25±2.09 (2.84-13.94)**	2.32±1.09 (1.00-3.92)
Strontium, Sr (0.003-0.013)	0.05 ± 0.04 (BDL-0.14)	**0.14 ± 0.07 (0.05-0.43)**	0.09 ± 0.09 (0.03-0.37)
Tin, Sn (0.001-0.003)	**0.01 ± 0.01 (BDL-0.06)**	0.00±0.00 BDL-0.02	BDL*
Vanadium, V (0.003-0.05)	**1.31 ± 0.95 (0.29-4.63)**	0.25 ± 032 (BDL-1.49)	BDL
Zinc, Zn (0.01-0.03)	45.79 ± 7.78 (31.29-65.10)	31.07 ± 6.23 (21.12-50.10)	**55.88 ± 15.83 (33.08-84.74)**

*One sample at 0.02 parts per million, all others BDL.

Females had significantly higher mean concentrations of 11 of the 18 elements (that were above detection limits in 50% of the samples) in liver, including three elements of concern (Cd, Hg, and Pb) (Table 7). This was also true for 10 elements in kidney; however for the elements of concern, Pb was not significantly different from males. For 14 elements in muscle, females were significantly higher than males for four of these (Cd, Fe, Mn, and Se); Zn was the only element for which males were higher than females. For As, the only element measured in blubber, females were significantly lower than males (Table 7). MeHg was not significantly different by sex in liver, kidney, blubber, or muscle.

Table 7. Trace element concentration differences by sex in liver, kidney, blubber, and muscle. Elements below detection in greater than 50% of samples for each tissue were not included. Significant differences ($P \leq 0.05$) are noted with a *. NS = not significant.

Tissue type	Element	Difference by sex
Liver (M = 24, F = 18)	As	NS
	Cd	F>M*
	Hg	F>M*
	MeHg	NS
	Pb	F>M*
	Al	F>M*
	Ag	NS
	Cr	NS
	Cu	F>M*
	Fe	NS
	Mg	NS
	Mn	NS
	Mo	NS
	Ni	F>M*
	Se	F>M*
	Sn	F>M*
	Sr	F>M*
	V	M>F*
	Zn	F>M*
Kidney (M = 24, F = 18)	As	NS
	Cd	F>M*
	Hg	F>M*
	MeHg	NS
	Pb	NS
	Ag	M>F*
	Al	NS
	Ba	NS
	Cr	NS
	Cu	NS
	Fe	NS
	Mg	NS
	Mn	M>F*
	Mo	F>M
	Ni	M>F*
	Se	F>M*
	Sr	F>M*

Tissue type	Element	Difference by sex
	V	M>F*
	Zn	F>M*
Blubber (M = 24, F = 18)	As	M>F*
Muscle (M =4, F= 10)	As	NS
	Cd	F>M*
	Hg	NS
	MeHg**	NS
	Al	NS
	Cr	NS
	Cu	NS
	Fe	F>M*
	Mg	NS
	Mn	F>M*
	Ni	NS
	Se	F>M*
	Sr	NS
	V	NS
	Zn	M>F*

** Methylmercury (MeHg) concentrations in muscle were analyzed for 41 walruses (M =23 and F=18).

Organochlorines. Concentrations of organochlorines (OC) were measured in the blubber, liver, kidney, and muscle tissue of 42 walruses (18 females and 24 males). Females ranged in age from 9 to 23 years; males from 7 to 34. We examined four compounds of hexachlorocyclohexane (HCH; Alpha-HCH, Beta-HCH, Delta-HCH, and Gamma-HCH), seven compounds of chlordane (CHL; Heptachlor, Heptachlor-Epoxide, Oxychlordane, Alpha-Chlordane, Gamma-Chlordane, Trans-Nonachlor, and Cis-Nonachlor), seven compounds of dichlorodiphenyltrichloroethane (DDT: DDMU; 2,4'-DDD; 4,4'-DDD; 2.4'-DDE; 4,4'-DDE; 2,4'-DDT; and 4,4'-DDT), and >80 congener and congener groups of polychlorinated biphenyls (PCB) in all four tissues. The ΣPCB for all tissues ranged from 81-83 congeners, 11 of which were below detection for all tissues. Sum PCB_{10} (ΣPCB_{10}) for all tissues included congeners 28, 31, 52, 101/90, 105, 118, 138/160, 153/132, 156/171/202, and 180. Concentrations of congeners below detection are reported as zero and not replaced with 50% detection limits in the sums.

In general, OC concentrations in blubber tissue were an order of magnitude higher than in liver. Also, in general, OC concentrations in liver were an order of magnitude higher than kidney and muscle, which were similar (Table 8). The relationship among the compounds was the same for blubber, kidney, and muscle; ΣHCH > ΣPCB > ΣCHL > ΣDDT. In liver, ΣHCH remained the highest and ΣDDT the lowest, however ΣCHL was higher than ΣPCB (Table 8).

Total DDT concentrations were highest in blubber, where six of the seven compounds composing ΣDDT were identified, followed in decreasing order by muscle, liver and then

kidney. In blubber, the most dominant DDT compound detected was 4,4' DDT (51.4%) followed by 4,4" DDE (36%). In liver, the most dominant compound was 4,4' DDT (38.4%), followed by 4,4" DDE (34%) and DDMU (27.6%). In kidney, 4,4" DDD was the dominant compound at 43.3% followed by 4,4" DDE (33.3%) and 2,4' DDT (23.3%). In muscle, 4,4' DDT (73.1%) was the most dominant compound, followed by 4,4' DDE at 18.8%.

Of the more than 80 PCB congener and congener groups that were quantified, three made up the more than half (57.3%) of the ΣPCBs in blubber. They were, in decreasing dominance, 153/132 (41.2%), 138/160 (9.3%), and 118 (6.7%). Five compounds made up more than half (50.5%) of the ΣPCBs in liver; they were 86 (16.5%), 153/132 (11.1%), 105 (9.0%), 7/9 (7.7%), and 95 (6.1%). Two compounds made up more than half (60.0%) of the ΣPCBs in kidney; 7/9 (31.9%) and 153/132 (28.2%). In muscle, three compounds made up more than half (53.5%) of the ΣPCBs; in decreasing order, these were 31 (24.9%), 77 (16.1%), and 110/77 (12.4%).

Males had significantly higher mean concentrations of ΣHCH, ΣCHL, and ΣPCBs in blubber. There were no differences between sexes in ΣDDT.

Table 8. Arithmetic mean (SD), and range of concentrations (ng/g or parts per billion wet weight for total organochlorines by chemical category in three tissues (blubber, liver, kidney, and muscle) from walruses harvested in Alaska, 2012–2014. Contaminants that were not detected during analysis are denoted by BDL. The ΣPCB for all tissues ranged from 81–83 congeners, 11 of which were below detection for all tissues. The ΣPCB_{10} includes congeners 28, 31, 52, 101/90, 105, 118, 138/160, 153/132, 156/171/202, and 180.

Contaminant category	(detection limit)	Blubber (n=42)		Liver (n=42)		Kidney (n=42)		Muscle (n=14)	
		Mean ± SD	(range)	Mean ± SD	(range)	Mean ± SD	(range)	Mean ± SD	(range)
∑ HCH	0.36	70.3 ± 66.32	1.37-234.63	4 ± 2.55	0-15.85	0.83 ± 0.67	0-2.04	0.96 ± 0.58	0.22-2.42
Alpha-HCH	0.21	3.52 ± 1.94	0.99-8.54	0.16 ± 0.24	0-0.81	0.02 ± 0.05	0-0.17	0.16 ± 0.09	0-0.44
Beta-HCH	0.22	65.98 ± 66.35	0-231.07	3.66 ± 2.68	0-15.85	0.8 ± 0.67	0-2.04	0.7 ± 0.38	0-1.48
Delta-HCH	0.11	0.04 ± 0.1	0-0.33	BDL	BDL	0.02 ± 0.05	0-0.16	0.02 ± 0.04	0-0.14
Gamma-HCH	0.09	0.75 ± 0.57	0-2.45	0.18 ± 0.32	0-1.23	0 ± 0	0-0.01	0.08 ± 0.22	0-0.83
∑ CHL	0.76	38.7 ± 26.35	8.65-152.73	3.56 ± 3.83	0-23.67	0.3 ± 0.41	0-2.03	0.26 ± 0.28	0-0.74
Heptachlor	0.13	0.06 ± 0.28	0-1.77	0 ± 0.02	0-0.13	BDL	BDL	BDL	BLD
Heptachlor-Epoxide	0.16	4.1 ± 2.47	1.32-13.56	0.33 ± 0.38	0-1.54	0.02 ± 0.05	0-0.2	0.03 ± 0.06	0-0.17
Oxychlordane	0.12	32.76 ± 24.18	4.81-132.65	3.2 ± 3.63	0-22.13	0.28 ± 0.38	0-1.89	0.21 ± 0.22	0-0.67
Alpha-Chlordane	0.19	0.28 ± 0.36	0-1.93	0.01 ± 0.02	0-0.08	BDL	BDL	0 ± 0.01	0-0.02
Trans-Nonachlor	0.15	1.5 ± 1.07	0-6.52	0.02 ± 0.05	0-0.29	BDL	0-0.02	0.01 ± 0.03	0-0.1
Cis-Nonachlor	0.14	0 ± 0.02	0-0.13	0.01 ± 0.04	0-0.18	BDL	BDL	BDL	BDL
∑ DDT	0.82	4.72 ± 7.19	0-29.81	0.07 ± 0.13	0-0.49	0.01 ± 0.02	0-0.11	0.18 ± 0.21	0-0.61
DDMU	0.13	0.02 ± 0.09	0-0.44	0.02 ± 0.08	0-0.44	BDL	BDL	BDL	BDL
2,4'-DDD	0.21	BDL	BDL	BDL	BDL	BDL	BDL	0 ± 0.01	0-0.02
4,4'-DDD	0.13	0.44 ± 0.68	0-2.99	BDL	BDL	0 ± 0.02	0-0.11	0.01 ± 0.02	0-0.05
2,4'-DDE	0.12	0.02 ± 0.1	0-0.47	BDL	BDL	BDL	BDL	0 ± 0.01	0-0.04
4,4'-DDE	0.14	1.7 ± 2.45	0-15.4	0.02 ± 0.06	0-0.3	0 ± 0.01	0-0.06	0.03 ± 0.05	0-0.15
2,4'-DDT	0.16	0.12 ± 0.23	0-0.78	BDL	BDL	0 ± 0.01	0-0.03	0 ± 0.01	0-0.02
4,4'-DDT	0.17	2.43 ± 5.66	0-25.81	0.03 ± 0.06	0-0.19	BDL	BDL	0.13 ± 0.21	0-0.59

Contaminant category	(detection limit)	Blubber (n=42)		Liver (n=42)		Kidney (n=42)		Muscle (n=14)	
		Mean ± SD	(range)	Mean ± SD	(range)	Mean ± SD	(range)	Mean ± SD	(range)
$\sum$ PCB	3.96	52.88 ± 29.91	19.68-211.93	3.22 ± 2.94	0.18-10.57	0.5 ± 0.78	0-4.31	0.47 ± 0.78	0-2.15
$\sum$ PCB_{10}	3.96	38.61 ± 25.13	14.13-169.74	1.17 ± 0.99	0-4.23	0.24 ± 0.42	0-2.33	0.24 ± 0.47	0-1.62
Aldrin	0.12	0.04 ± 0.17	0-0.93	BDL	BDL	BDL	BDL	BDL	BDL
Dieldrin	0.19	22.89 ± 13.08	6.36-57.96	1.44 ± 1.68	0-8.33	0.33 ± 0.32	0-1.39	0.31 ± 0.23	0.03-0.83
1,2,3,4-Tetrachlorobenzene	0.15	BDL	BDL	BDL	BDL	0 ± 0.01	0-0.06	0.02 ± 0.08	0-0.29
1,2,4,5-Tetrachlorobenzene	0.27	1.21 ± 3.64	0-17.47	BDL	BDL	0.03 ± 0.09	0-0.35	0.14 ± 0.12	0-0.32
Hexachlorobenzene	0.23	0.27 ± 0.35	0-1.12	0.01 ± 0.03	0-0.15	BDL	BDL	0.03 ± 0.09	0-0.33
Pentachloroanisole	0.15	0.17 ± 0.29	0-0.93	0.08 ± 0.13	0-0.35	0 ± 0.01	0-0.03	0 ± 0.01	0-0.02
Pentachlorobenzene	0.11	0.47 ± 0.79	0-2.59	0.04 ± 0.08	0-0.29	BDL	BDL	0.02 ± 0.06	0-0.23
Endosulfan I	0.15	0.03 ± 0.14	0-0.86	0.02 ± 0.06	0-0.25	BDL	BDL	0 ± 0.01	0-0.04
Mirex	0.12	5.11 ± 2.86	1.06-13.6	0.21 ± 0.25	0-1.27	0.03 ± 0.06	0-0.26	0.07 ± 0.08	0.02-0.35
Chlorpyrifos	0.28	0.19 ± 1.26	0-8.15	0.03 ± 0.18	0-1.19	0.01 ± 0.09	0-0.61	BDL	BDL

Vitamins

Vitamin E and A concentrations were analyzed in liver samples from 37 walruses (Fig. 5). Although higher concentrations of vitamin E are expected in blubber, we could not find a lab willing to analyze blubber tissue due to accuracy issues with blubber as a matrix. Vitamin E concentrations were similar for males and females and averaged 26.8 ppm (range 3.5–104.7) for females and 27.4 ppm (range 4.6–75.0) for males. Average vitamin A concentrations, however, were significantly higher (P <0.05) for males (404.1 ppm, range 34.9–1,391.5) than females (148.7 ppm, range 76–339.9).

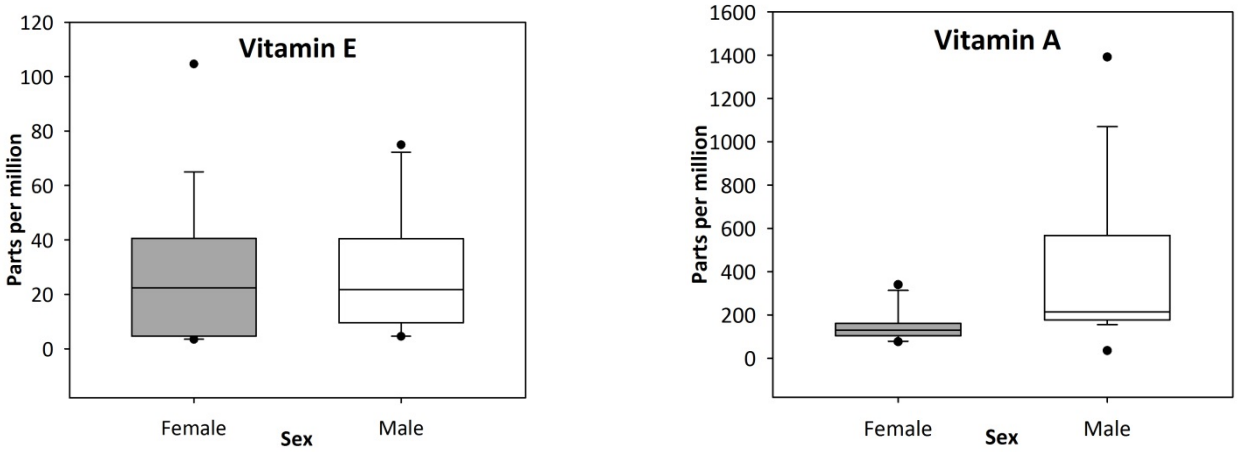

Figure 5. Vitamin E and A concentrations in liver from 37 walruses harvested at Gambell and Savoonga during 2012–2014.

Disease

Blood serum was analyzed from 151 walruses sampled during 2012–2014 for evidence of exposure (antibodies) to diseases known to cause health concerns for pinnipeds and some that can be transmitted to humans (zoonotic). All walruses tested for *Brucella* antibodies in 2012 and 2014 (n=90) were negative, however, 12.3% of walruses sampled in 2013 (n=57) were positive. All walruses tested for CDV (n=148) and PDV (n=149) were negative regardless of year harvested (Table 9). PhHV-1 antibodies were found in all of the walruses tested in 2012 and most tested in 2013 (96.5%) and 2014 (97.7%). We found no antibodies for four of the six *Leptospira* species tested for in any year, however 5 of 47 (10.6%) walruses were positive for *Leptospira bratislava* in 2012 and 1 of 44 (2.3%) was positive for *Leptospira canicola* in 2014 (Table 9). Toxoplasma antibodies were identified in 1 of 151 walruses tested by serology, however, a higher prevalence of *Toxoplasma gondii* was found in liver and muscle samples in 10 of 32 (31.3%) walruses tested for coccidian parasites using polymerase chain reaction (PCR) methods. A novel parasite, *Sarcocystis pinnipedi*, closely related to *S. canis*, was found in one walrus muscle sample (Haman et al. 2015).

Table 9. Serum antibody prevalence for 12 disease agents in up to151 walruses harvested near Saint Lawrence Island, Alaska, 2012–2014.

	Antibody prevalence No. positive/No. tested (%)			
Disease Agent	**2012**	**2013**	**2014**	**Total**
Brucella abortus	0/46 (0)	7/57 (12.3)	0/44 (0)	7/147 (4.8)
Canine distemper virus	0/47 (0)	0/57 (0)	0/44 (0)	0/147 (0)
Phocine distemper virus	0/47 (0)	0/56 (0)	0/44 (0)	0/147 (0)
Phocine herpesvirus-1	47/47 (100)	58/60 (96.7)	43/44 (97.7)	148/151 (98.0)
Leptospira bratislava	5/47 (10.6)	0/60 (0)	0/44 (0)	5/151 (3.3)
Leptospira canicola	0/47 (0)	0/60 (0)	1/44 (2.3)	1/151 (0.7)
Leptospira grippotyphosa	0/47 (0)	0/60 (0)	0/44 (0)	0/151 (0)
Leptospira hardjo	0/47 (0)	0/60 (0)	0/44 (0)	0/151 (0)
Leptospira icterrohemorrhagiae	0/47 (0)	0/60 (0)	0/44 (0)	0/151 (0)
Leptospira pomona	0/47 (0)	0/60 (0)	0/44 (0)	0/151 (0)
Toxoplasma spp.	0/47 (0)	1/60 (1.7)	0/44 (0)*	1/151 (0.7)

***** Two walruses had weak positives with a titer at lower limit of 1:32.

Testing for the toxin domoic acid using at least one sample matrix (i.e., stomach content, intestinal content, urine, or amniotic fluid) was conducted on 116 walruses and 57 (49%) had concentrations above the detection limit of 2.0 ng/g for stomach and intestinal content and 0.04 ng/ml for urine. Domoic acid concentrations, in stomach and intestinal content, ranged from 2.5 to 6,457 ng/g. Domoic acid concentrations in urine ranged from 0.6 to 49 ng/g. The only sample of amniotic fluid collected was below detection. Of the 116 walruses tested for domoic acid, 66 were also tested for saxitoxin and 34 (52%) had concentrations above the detection limit of 3.0 ng/g for all matrices. Saxitoxin concentrations for all matrices ranged from 3.8 to 1,161.8 ng/g. Toxic algae results from 2012 and 2013 are included in Lefebvre et al. (2016), which was an overview of toxic algae exposure for multiple species of marine mammals in northern Alaska.

In 2014, we collected paired stomach content and intestinal content samples, and intestinal content had higher values for both domoic acid and saxitoxin in 5 of 9 (55.6%) walruses tested (Table 10). In 2016, we tested paired stomach content and urine samples; domoic acid concentrations were higher in urine for 6 of 9 (66.7%) walruses, but for saxitoxin only 4 of 9 (44.4%) were higher (Table 10). In 2016, we also tested paired stomach content and bivalve parts (i.e., feet and siphons) found in the stomach contents of five walruses that tested positive for either domoic acid or saxitoxin. All bivalve parts had detectable concentrations of both domoic acid and saxitoxin and concentrations of domoic acid were higher than what was found in the general stomach content samples (Table 11).

Table 10. Domoic acid and saxitoxin concentrations in different matrices from the same individual walruses harvested near Saint Lawrence Island, Alaska, 2012, 2014, and 2016.

	Domoic acid			Saxitoxin		
Walrus ID	Stomach content (ng/g)	Intestinal content (ng/g)	Urine (ng/ml)	Stomach content (ng/g)	Intestinal content (ng/g)	Urine (ng/ml)
G12-0029	BDL	BDL	-	-	-	-
G14-0061	3.78	2,537.37	-	BDL	23.56	-
G14-0064	BDL	2.49	-	BDL	13.76	-
G14-0070	BDL	245.78	-	BDL	1,161.80	-
G14-0072	19.95	BDL	-	BDL	BDL	-
G14-0085	BDL	BDL	-	BDL	BDL	-
G14-0090	3.51	16.56	-	BDL	7.8	-
S14-0051	9.79	488.9	-	BDL	499.04	-
S14-0056	49.95	12.3	-	97.49	8.76	-
S14-0062	BDL	BDL	-	BDL	BDL	-
S16-010	3.5	-	3.4	28.9	-	BDL
S16-011	4.3	-	0.6	7.5	-	BDL
S16-014	BDL	-	3.3	27.2	-	6.2
S16-016	BDL	-	1	BDL	-	BDL
S16-028	10.1	-	2.3	28.8	-	BDL
S16-032	BDL	-	1.1	BDL	-	14.1
S16-037	BDL	-	49	BDL	-	4.2
S16-038	0.0	-	2.0	BDL	-	3.8
S16-039	BDL	-	19.6	BDL	-	4

Table 11. Domoic acid and saxitoxin concentrations in three genera of clams removed from the stomachs of walruses harvested near Saint Lawrence Island, Alaska in 2016.

			Domoic acid		Saxitoxin	
Walrus ID *Bivalve from stomach*	# of bivalves analyzed	Bivalve part analyzed	Stomach content (ng/g)	Urine (ng/ml)	Stomach content (ng/g)	Urine (ng/ml)
S16-007			**7.6**	**-**	**30**	**-**
Serripes spp.	2	Feet	21.0		17.6	
Mactromeris polynyma	1	Foot	29.0		12.4	
S16-010			**3.5**	**3.4**	**28.9**	**BDL**
Serripes spp.	3	Feet	3.6		33.2	
Mya spp.	~6	Feet and siphons	4		24.4	
S16-014			**BDL**	**3.3**	**27.2**	**6.2**
Mya spp.	1	Foot and siphon	2.8		20.4	
S16-028			**10.1**	**2.3**	**28.8**	**BDL**
Serripes spp.	4	Feet	26.7		14.3	
Mactromeris polynyma	2	Feet and siphons	20.9		20.0	
S16-031			**4.8**	**-**	**81.1**	**-**
Unidentified bivalve	~20	Feet and tissue	19.2		60	

Population parameters

Age at harvest. Age was determined by cementum analysis of teeth collected from 68 walruses sampled in 2012 (47 females, 21 males). Females ranged in age from 9 to 23 with an average age of 13.7 years. Males ranged in age from 12 to 29 with an average age of 20 years (Fig. 6).

In 2013, 56 walruses (18 females, 38 males) were aged from teeth. Females ranged from 11 to 25; average 15.4 years. Males ranged from 7 to 34; average 21.3 years (Fig. 7).

In 2014, 43 walruses were aged (10 females, 30 males, and 3 of unknown sex). Females ranged in age from 3 to 18 with an average age of 13.3 years. Males ranged in age from 4 to 28 with an average age of 17.8 years. The walruses of unknown sex were 10, 16, and 18 years old (Fig. 8).

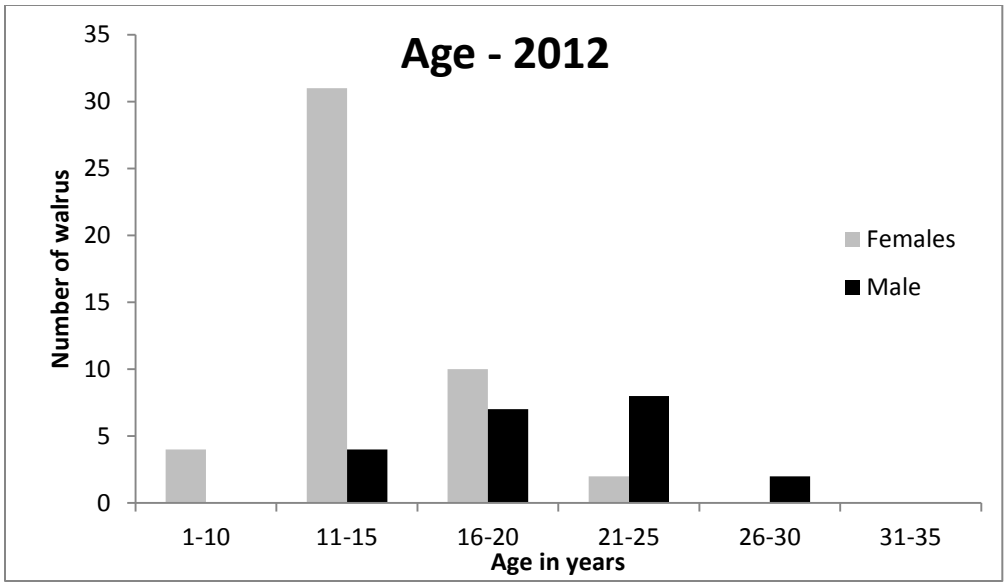

Figure 6. Harvested walruses (n=68) by sex and age category sampled at Gambell and Savoonga in 2012.

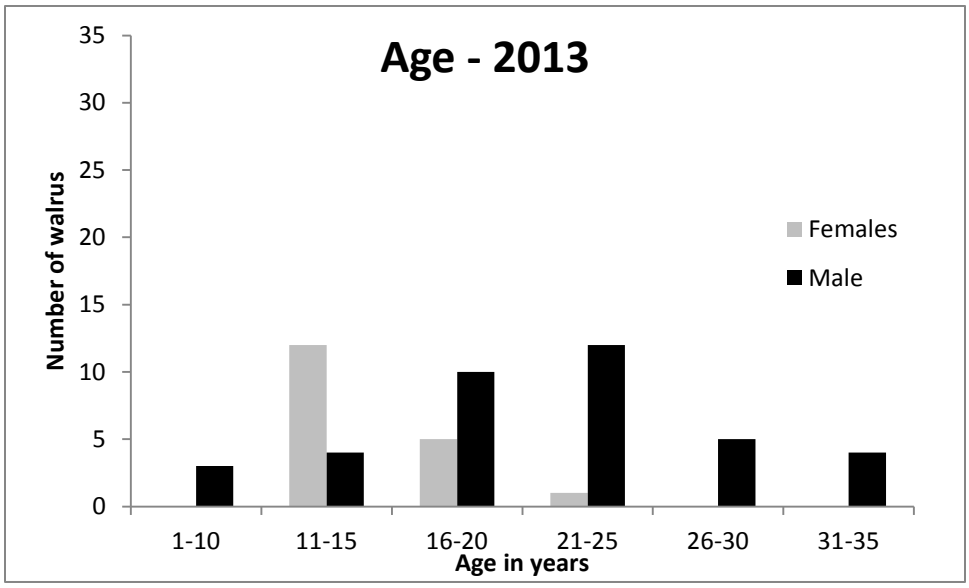

Figure 7. Harvested walruses (n=56) by sex and age category sampled at Gambell and Savoonga in 2013.

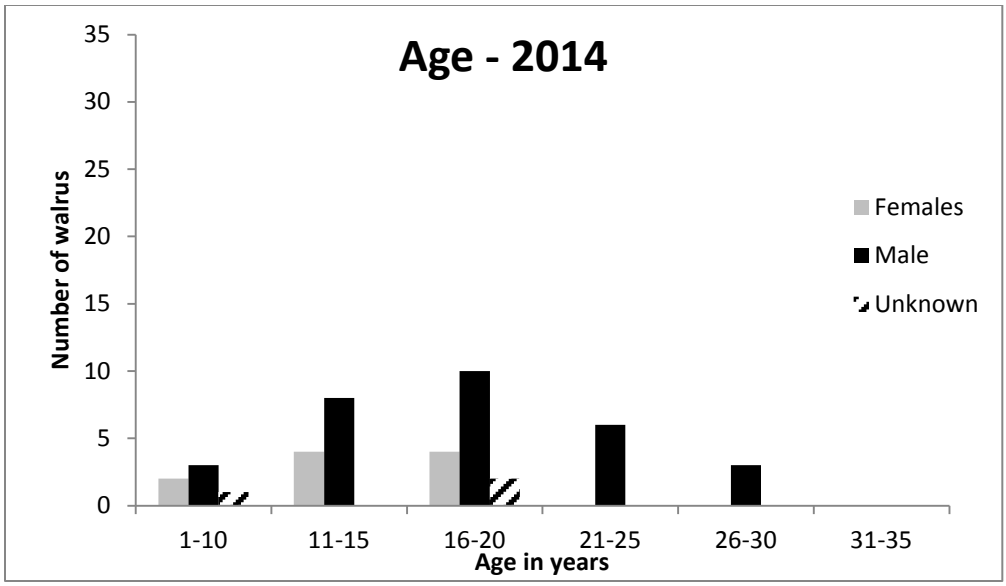

Figure 8. Harvested walruses (n=43) by sex and age category sampled at Gambell and Savoonga in 2014.

Sex ratio. In 2012, less than half as many males (24) were sampled than females (58) for a sex ratio of 24:57 = 0.42. Whereas in 2013, almost twice as many more males (43) were sampled than females (23) for a sex ratio of 43:23 = 1.9. In 2014, again more males (37) were sampled than females (15) for a sex ratio of 37:15 = 2.5. In 2016, slightly fewer males (10) were sampled than females (11) for a sex ratio of 10:11= 0.91. For all years combined, however, the sex ratio was nearly equal (1.08 in favor of males).

Pregnancy rate. From information provided by the hunters regarding reproductive status of harvested adult female walruses in 2012, at least 45 of 57 (79%) females had calves with them. In 2013, at least 19 of 23 (83%) had calves, and in 2014, 13 of 15 (87%) were accompanied by calves.

Discussion

Less sea ice for females and young walruses to haul out on in the Chukchi Sea during summer is expected to result in an increase in energy needed to travel from terrestrial haulouts to preferred feeding areas. Increased energetic costs are expected to decrease body condition and reproductive capacity, and increase disease prevalence to an unknown degree. During this study we collected information and samples from the subsistence walrus harvest in 2012, 2013, 2014, and 2016 to evaluate some walrus health parameters. Our sampling began five years after the first large (> 10,000 walruses) terrestrial haulout event occurred near Point Lay, Alaska in 2007. Less sea ice has resulted in females and young walruses hauling out on land near Point Lay in August and September in 7 of 10 years since 2007. No walruses hauled out there in 2008 or 2012 because remnant ice was available throughout the summer. In 2016, the haulout did not form until October when a relatively small number (~6,000) hauled out for a few days. Large

terrestrial haulouts of thousands of walruses, however, occurred in 2013, 2014, and 2015; therefore our sampling occurred during a time of predicted stress.

Although there are reasons to be concerned about walrus health related to changes in habitat, the results of our study were mostly positive. Body condition of 208 walruses sampled in April and May of 2012–2014 and 2016 were evaluated by the hunters to be of average health (47%) and very healthy (51%); only 2% were thought to be unhealthy. Hunters often wrote "fat" or "fat, no lesions" in the comments of walruses rated as very healthy.

In this study, sampling occurred during the spring migration in the Bering Sea, prior to summering in the Chukchi Sea. If walruses were sampled during the fall migration, after the predicted stressful summer period, results might have been less positive. However, if body condition declined during consecutive summer periods, we would not expect our results to be positive in all years of this study unless body condition recovered during winter in the Bering Sea.

The birth rate of adult female walruses is limited by the long (15 month) gestation period (actually diapause plus gestation), which results in a minimum inter-birth interval of one calf every two years, but is more likely one calf every three years (Fay 1982). Therefore the expected annual birth rate for a healthy female of prime breeding age would be between 33% and 50%. Thus, during the harvest (May), a third of the females would have just given birth, a third would be nursing a calf and not pregnant, and a third would be newly pregnant but in diapause and have a yearling with them. During diapause, newly pregnant females are not identifiable by our sampling methods and would be recorded as females with a yearling or barren females. During the three consecutive years of our study, 79–87% of the adult females sampled had calves of the year (or near term fetuses) with them; well above the expected 33–50%. If this higher than expected rate happened in one year, or in alternate years, some synchrony in estrous or birthing could be responsible, however, it appeared in all three consecutive years of this study and is consistent with past harvest data and has been interpreted to be some combination of hunter selectivity and access to the pregnant female spring migration route (Garlich-Miller et al. 2006). A lower proportion (40%) of females with calves (or near term fetuses) was documented in the subsistence harvest during the early 1980s when the population was thought to be less productive and beginning a decline (Fay 1982, Fay et al. 1989, 1997), possibly indicating that although an inflated metric, females with calves in the harvest may be a general indicator of productivity. Certainly the pregnancy rate of harvested females in three consecutive years observed during this study indicates that calves are being produced annually.

A trend in the mean age of harvest could be indicative of a change in the age distribution of the population. A population of older age animals may indicate a less productive, declining population, whereas a population of younger animals may indicate a growing population (Garlich-Miller et al 2006). The average age of sampled females (14.1 yrs) and males (19.7 yrs) was similar to ages reported by Garlich-Miller et al. (2006, Appendix 2) between 1996 and 2002 (i.e., 15.0 females and 19.4 males). No trend was evident across the three years of this study.

Less sea ice may also affect the timing and distribution of phytoplankton production and whether grazers are present in the water column to consume them or if much falls to the bottom to

support the benthic infauna walruses depend on (Arrigo and Dijken 2015, Bluhm and Gradinger 2008, Grebmeier et al. 2006, Moore et al. 2003, Mueter and Litzow 2008). As such, a shift in prey items or frequency of occurrence of prey may be expected. Dominant prey items found in this study included bivalves (63% FO) mostly *Mya*, gastropods (51%), both *Buccinium* spp. and *Cyptonatica* (formerly *Natica* spp.), echiurids (60%) and decapods (28%), mostly crabs (Table 3). Although our sample size was relatively limited, we did not find major differences in prey species or frequency of occurrence relative to other studies (Fay 1982, Sheffield and Grebmeier 2009).

Disease screening showed no elevated prevalence for diseases of concern. *Brucella* was only detected in 2013 (Table 9). It is unclear why more walruses were positive in 2013 but none were positive in 2014. Testing is available for the terrestrial form of *Brucella* (i.e., *B. abortus*) although it is more likely that *B. pinnipedialis* is the species carried by walruses. Thus it is not known how accurate this type of *Brucella* testing is for marine mammals. To improve *Brucella* testing for marine mammals we provided serum samples from this study and earlier studies to compare *Brucella* test results using the standard Rose Bengal test and an Indirect ELISA test developed for seals. Results indicated that the more specific Indirect ELISA test had a higher detection rate (18.8% positive vs. 8.7% positive) indicating standard testing is under detecting Brucella prevalence. The reliability of serological screening for other marine mammal diseases is unknown but thought to be valuable for general comparisons of prevalence.

Distemper (i.e., morbillivirus) can be a devastating disease to pinnipeds and several seal die offs have been attributed to both canine and phocine distemper (Kennedy et al. 2000, Earle et al. 2011). No distemper was detected in any year of this study. Herpesvirus was detected in most walruses, but this was expected because most mammal populations, including humans, are exposed to and carry species-specific herpesviruses. Zarnke et al. (1997) found a lower prevalence of herpesvirus in sera collected during 1981–1987 than our study (55% vs (89%), which could indicate an increase since the 1980s, however it is also likely that the lab methods we used were more sensitive. Of six species of *Leptospira*, two had positive results at low levels (*L. bratislava* in 2012 and *L. canicola* in 2014) (Table 9). We did not test for *Trichinella* during this study, although it has been documented from tissue at low levels of 0–2% prevelance (Fay 1960, Kozlov 1966, Bukina and Kolevatova 2007, Seymour et al. 2014a). A novel parasite, *Sarcocystis pinnipedi,* was found in 1 of 37 muscle samples from this study (Haman et al. 2015). It was also found to be enzootic in 15 of 68 (22%) ringed seals sampled. The high prevalence and lack of associated pathology suggests that ringed seals are a natural host. This parasite, although apparently harmless for ringed seals in Alaska, was identified as the cause of death for more than 400 grey seal pups in the Atlantic waters off Novia Scotia, Canada in 2012 (Haman et al. 2015).

With less sea ice and resulting warmer waters in summer it was also predicted that Arctic marine mammals could become more exposed to the toxic products of harmful algal blooms (domoic acid and saxitoxin). These compounds are produced by species of phytoplankton (diatoms and dinoflagellates) that reproduce rapidly under certain warm water conditions. These blooms are common in tropical and temperate oceans, including the Pacific Ocean. For example, sardines that feed on toxic phytoplankton are eaten by California sea lions (*Zalophus californianus*) causing seizures and mortality (Gulland et al. 2002). Bivalves, primary walrus prey, are also

known to concentrate algal toxins, however, little toxicosis caused by domoic acid and saxitoxin has been reported in Alaskan marine mammals. Concentrations of domoic acid (2.5 to 6,457 ng/g) and saxitoxin (3.8 to 1,162 ng/g) were present in walruses at higher than expected concentrations, especially considering that sampling occurred in the spring when sea ice was present and prior to when a bloom could occur. Concentrations from walruses sampled in 2012 and 2013 were the highest measured of 13 northern marine mammal species sampled and published in a comprehensive review (Lefebvre et al. 2016), however, an even higher concentration of saxitoxin (1,162 ng/g) was measured in a walrus sampled in 2014.

It is unknown at what concentrations these toxins are harmful to walruses; domoic acid is known to pass through amniotic fluid and milk of other marine mammals (Rust et al. 2014) and thus could be harmful to walrus calves. Domoic acid and saxitoxin do not reside in muscle, blubber or other walrus tissues commonly eaten by humans. The toxins are, however, found in the clams in walrus stomachs, which are highly favored as food by hunters and their families. These clams are the likely source of the algal toxins in the walruses. Although sample collection for this project was scheduled to end in 2014, we extended the study into 2016 to sample urine and prey items to better understand where the highest concentration of algal toxins might be, and to determine prey items that might be sources. The highest concentrations of both toxins were found in intestinal contents (Table 10). Domoic acid in urine was above detection limits for all walruses tested (n = 9), even when stomach contents were below detection (5 of 9). Saxitoxin in urine was more variable and was above detection limits for 5 of 9 tested but only matched concentrations found in stomach contents for two of them. In one animal both were below detection and in the other both had detectible concentrations with stomach contents at a higher concentration (Table 10). Therefore it appears that urine may be the best matrix to detect domoic acid, and intestinal content may be best for saxitoxin. Unfortunately, we were unable to test all three matrices in the same individuals, doing so is necessary to determine if urine is better than intestinal content for either toxin.

In an effort to determine the source of the toxins we analyzed feet and siphons of several species of clams found in five walrus stomachs that tested positive. All clam parts from all stomachs tested had measurable concentrations of domoic acid (range 2.8–29.0 ng/g) and saxitoxin (12.4–60 ng/g). All concentrations of domoic acid and saxitoxin measured in these clams were below the regulatory limits for human consumption; 20,000 ng/g and 800 ng/g respectively (Wekell et al. 2004). However, additional testing of walrus stomach contents, intestinal contents, urine, milk, amniotic fluid, and prey items in stomachs and intestines should be conducted to better understand and monitor HABs in walruses. Because these toxins depurate rapidly it is likely that any detection in any matrix indicates higher concentrations likely occurred prior to sampling, therefore low concentrations should not be assumed to indicate low exposure.

In addition to the high concentrations, we also found the mechanism for the transfer of algal toxins to walruses of interest. Walruses were sampled during spring migration in the vicinity of sea ice in late April and May before water temperatures that support HABs would occur, suggesting that the toxins come from blooms that occurred previously or were transported into the Bering Sea from the Pacific and are being stored in prey.

Contaminants

Although contaminant (e.g., trace element and organochlorine) concentrations are less directly related to climate warming than other health parameters, marine mammals are known to have higher concentrations of some contaminants than terrestrial mammals do (e.g., Kubota 2001). Marine mammals, however also have a variety of antioxidant and chemical binding mechanisms that make the toxic forms less biologically available (Kubota 2001, Dietz et al. 1998, Das et al. 2003). Regardless, these higher concentrations have raised concerns about what they mean for the health of Arctic marine mammals and for the people that consume them. Also of concern is how concentrations may be changing through time as sources and environments change. These are the most comprehensive contaminants data for Pacific walruses to date and include trace elements and persistent organic pollutants for 42 walruses. There are many purposes for analyzing contaminants data and too many ways to present them all here. Therefore, these data are available upon request for specific analyses and summary data are presented here.

Although we can measure contaminants at concentrations of parts per million (elements) and parts per billion (organochlorines), we know very little about what concentrations in what tissues cause problems for marine mammals or for people. We can however make general comparisons to concentrations measured in this study to those measured in Alaska in the past, to walruses in Canada, and to other marine mammal species sharing the same waters (e.g., seals from the Bering and Chukchi seas) for some perspective. Specific or statistical comparisons are not recommended due to potential differences in laboratory and analytical methods.

Essential and non-essential elements. Of the 20 trace elements we analyzed, many are essential elements that are regulated by physiological processes that prevent elevated concentrations in tissues. There are, however, four non-essential elements (sometimes called heavy metals and elements of concern) that can elevate and become toxic if they are biologically available at high concentrations. Those elements are As, Cd, Hg (in the form of MeHg), and Pb.

Arsenic, the first element of concern, can be harmful (i.e., teratogenic and carcinogenic) at higher concentrations (Kubota et al. 2001) and sources can be natural (volcanic) and anthropogenic (agricultural and industrial; Azcue and Nriagu 1994). Marine algae can remove inorganic As from seawater and convert it to organic As providing a mechanism for it to enter the marine food chain (Francesconi and Edmonds 1993, 1997). Although most trace elements are found in their highest concentrations in liver and kidney, As had higher concentrations in blubber in seals (Woshner et al. 2001a, Ebisuda et al. 2002, Moses et al. 2009), beluga whales (Woshner et al. 2001b), and narwhals (Wagemann et al. 1984). In our study, As was measured in liver, kidney, muscle, and blubber; and was ~10 times higher in blubber than liver or kidney, and 17 times higher than muscle (Tables 4a and 4b). A similar relationship and values were reported for blubber and liver for Saint Lawrence Island walruses collected in 2005–2009, although muscle values were more similar to liver (Welfinger-Smith et al. 2011). For 18 ringed seals from Canada, mean blubber concentrations (range 0.60–1.76 μg/g ww) were 2.4 times higher than liver concentrations (range 0.19–0.74 μg/g ww) (Ebisuda et al. 2002). Considering, for example, that blubber makes up 29% (in summer) to 39% (in fall and winter) of the body mass of a bearded seal (Burns 1981), blubber could account for a significant portion of the body burden of As in pinnipeds.

Marine mammal blubber (oil) is an important component of subsistence food and therefore monitoring As in blubber should be considered, however, several forms of As are known, and the form most commonly found in marine mammals is a relatively nontoxic organic form called arsenobetaine, which accounts for 68–98% of total As in marine mammal tissues (Kunito et al., 2008). Therefore, health risks of As to walruses and their consumers may be lower than expected relative to the concentrations of total As detected in tissue (Thatcher et al. 1985, Ponce et al. 1997, O'Shea 1999).

Mean concentrations of As in walruses from this study were higher in liver and kidney than in previous studies of Pacific walruses (reported in dw Taylor et al. 1989, Warburton and Seagars 1993, Seagars et al. 1994; Table 11 and reported in ww Taylor et al. 1989; Table 12).

The second element of concern, is Cd, which could be transported to the Arctic from industrial sources via the atmosphere and rivers (Macdonald et al. 2000), however elevated concentrations in Arctic marine mammal tissues are common and most likely from natural, geologic sources (Dietz et al. 1998). Concentrations of Cd in walrus tissues sampled at Saint Lawrence Island during 1981–1984 (Taylor et al. 1989) alarmed the Environmental Protection Agency, who prompted the State of Alaska, Division of Public Health to investigate people's exposure through diet. Mean concentrations of Cd in walrus kidney were measured at 173–205 µg/g dw and liver measured 20–42 µg/g dw. Walrus kidney was only eaten by a few people in small portions; however, liver was eaten more frequently and in larger portions. People known to consume walrus liver frequently were tested to find their blood and urine Cd levels were normal, well below concentrations associated with kidney damage, and no related illnesses were known to have occurred (Middaugh et al. 1986). Cadmium concentrations higher than the value known to cause kidney damage in terrestrial mammals and humans (i.e., 200 µg/g dw) have also been found in kidneys of ringed seals in Greenland causing concern (WHO 1992). It has become known that marine mammals contain high concentrations of Cd compared to terrestrial mammals and appear to tolerate such concentrations without kidney damage, possibly because they have evolved with naturally high concentrations in their diet (Deitz et al. 1998; Woshner et al. 2001a), and they may have mechanisms for detoxifying it. The metal-binding protein metallothionein has been documented in seals in association with Cd and other heavy metals and may combine to form nontoxic complexes (Mochizuki et al. 1985; Tohyama et al. 1986).

Mean concentrations of Cd in walruses from this study were lower in liver and kidney than in previous studies of Pacific walruses (reported as dw in Taylor et al. 1989, Warburton and Seagars 1993, Seagars et al. 1994; Table 11 and reported as ww in Taylor et al. 1989; Table 12). Cadmium concentrations in walruses from this study were also lower than for Atlantic walruses in liver, kidney, and muscle (Wagemann and Stewart 1994).

We did not find any other studies that analyzed walrus muscle for comparison but in our study, muscle was ~70 times lower than liver and 500 times lower than kidney. Female walruses had higher concentrations of Cd in liver, kidney, and muscle than males in this study (Table 7). Although we found female walruses had higher concentrations of Cd in liver and kidney than males (Table 7), Taylor et al. (1989) and Warburton and Seagars (1993) did not.

Ringed, bearded, spotted, and ribbon seals harvested in Alaska during 2003–2007 were analyzed using the same laboratory and methods. Bearded and ribbon seals had higher, but similar concentrations of Cd in liver than walruses followed by ringed seals; spotted seals had the lowest concentrations (Quakenbush and Citta 2008, 2009; Quakenbush et al. 2009, 2011a; ADF&G unpubl. data; Fig. 9). Bearded seals and walruses have similar diets of benthic invertebrates and thus may be expected to have similar Cd concentration. Ribbon seals, however, had the highest concentrations and they are thought to be more pelagic piscivores (Frost and Lowry 1980).

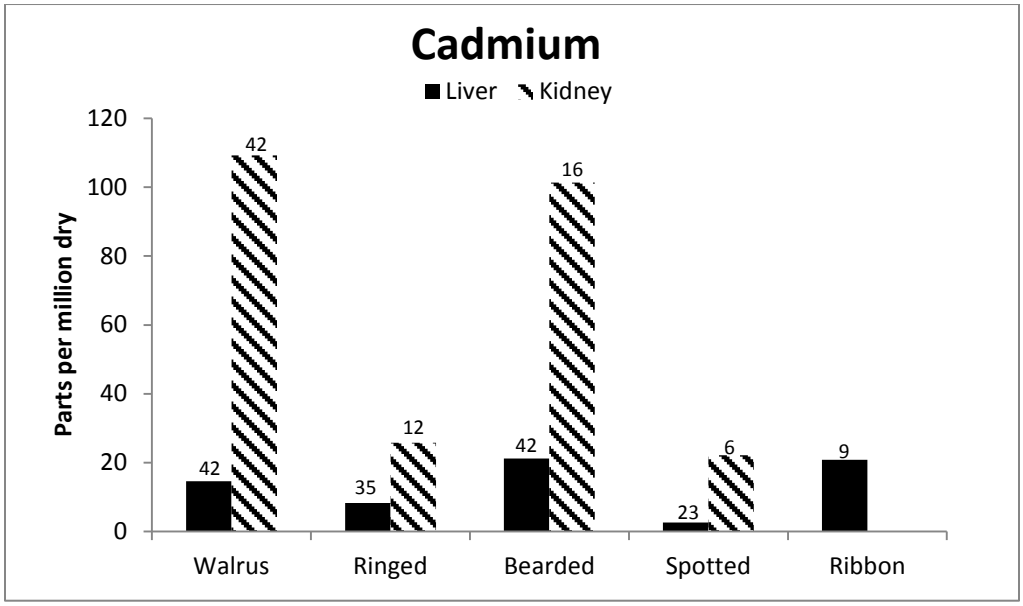

Figure 9. Comparison of cadmium concentrations (µg/g = ppm dry weight) in liver and kidney of walruses and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar. Only one ribbon seal kidney was analyzed and is not presented.

Lead, the third element of concern, is found naturally at commercial concentrations within walrus summer range and is extracted at Red Dog Mine north of Kotzebue Sound, a process that may increase its availability in the environment (O'Hara et al. 2003). Ammunition used for hunting contains Pb and could be ingested by walruses while feeding on the bottom. Elevated concentrations of Pb have been found in sediments and invertebrates near mine port sites or tailings in West Greenland and Baffin Island, Canada (Johansen et al. 1991, Larsen et al. 2001, Fallis 1982). Lead concentrations in walrus tissue from this study, however were low. Liver was highest and concentrations in muscle were BDL (Tables 4a and 4b). A study that analyzed liver tissue from bearded seals (also benthic feeders) harvested near the Red Dog Mine in northwestern Alaska also found low Pb concentration (Quakenbush and Citta 2009) and Pb in other seal species were also low in liver and kidney (Quakenbush and Citta 2008; Quakenbush et al. 2009, Quakenbush et al. 2011a,b; ADF&G unpubl. data). Females had higher concentrations of Pb than males in liver only in this study. Lead concentrations in walruses from this study were also lower than for Atlantic walruses in liver, kidney, and muscle (Wagemann and Stewart 1994).

Mean concentrations of Pb in walruses from this study were similar or lower in liver and kidney than in previous studies of Pacific walruses (reported in dw Taylor et al. 1989, Warburton and Seagars 1993, Seagars et al. 1994; Table 11 and reported in ww Taylor et al. 1989; Table 12). Lead concentrations in walruses from this study were also lower than for Atlantic walruses in liver, kidney, and muscle (Wagemann and Stewart 1994).

Mercury, the fourth element of concern, can be transported by air or water and follows a complicated cycle. Although efforts have been made to decrease global emissions of Hg, concentrations have been variable in marine biota with large geographic variability (Braune et al. 2015) that masks trends.

Mercury is chemically converted to MeHg, which can bioaccumulate, biomagnify, and is known to result in adverse health impacts including neurotoxicity (especially during *in utero* development), tissue damage, and decreased immune abilities especially in apex predators (Dietz et al. 2013, AMAP 2011). Although MeHg is of greatest concern, total Hg (THg) is most commonly analyzed and includes all chemical forms of Hg, which have different degrees of bioavailability. Particularly in liver and kidney, THg includes inorganic Hg that binds to Se and becomes biologically unavailable. This Hg-Se complex is stored in some tissues and concentrations increase with age. As such, THg in liver and kidney can be a useful measure of long-term exposure to Hg, but is limited for evaluating Hg toxicity or food safety. A comparison of THg concentrations among walrus tissues in this study showed liver to be four times higher than kidney and 37 times higher than muscle (Tables 4a and 4b). Females had higher concentrations of THg (not MeHg) than males in liver and kidney (Table 7).

In comparing THg in Pacific walruses through time, mean concentration in liver in the 1980s and 1991 was similar (3.39 ppm dw) to that in 2014–2016 (3.69 ppm dw) (Table 12), while mean concentrations in kidney decreased through time (0.95 to 0.83; Table 12). For comparisons that could only be made in ww concentrations, THg also decrease through time (Table 13).

In comparing THg in walruses from this study with Atlantic walruses in Canada (Wagemann and Stewart 1994), mean liver concentrations were similar to or lower (3.69 ± 5.30 vs 4.52 ± 3.60 ppm dw from Foxe Basin and 6.84 ± 5.48 from northern Quebec), lower in kidney (0.83 ± 0.55 vs 1.37 ± 0.53 from Foxe Basin) and lower in muscle (0.10 ±0.08 vs. 0.42±0.50 also from Foxe Basin).

Table 12. Comparison of mean concentrations of arsenic, cadmium, lead, and total mercury (µg/g = parts per million ***dry weight***) in walrus kidney (kid) and liver (liv) tissues between 1981 and 2014. This table was adapted from Warburton and Seagars (1993) including data from Taylor et al. (1989) converted to µg/g = parts per million dry weight by Warburton and Seagars 1993.

		Arsenic		Cadmium		Lead		Mercury		
Year	n	Kid	Liv	Kid	Liv	Kid	Liv	Kid	Liv	Source
1981		<0.01	0.03	204.9	41.2	<0.01	0.03	-	3.3	1
1982		<0.01	<0.01	173.2	22.0	0.9	0.7	-	0.7	1
1983		<0.01	0.01	180.3	20.1	0.09	0.1	-	5.7	1
1986		0.9	0.4	146.6	20.1	0.8	0.6	1	3.7	2
1988		1.2	0.5	166.3	29.4	1.3	0.7	1.1	4.6	2
1989		0.6	0.2	180.6	32.1	1.1	0.5	1.1	2.7	2
1991		1.6	1.2	122.5	19.9	0.3	0.4	0.6	3.0	3
Ave		***0.62***	***0.34***	***167.77***	***26.40***	***0.64***	***0.43***	***0.95***	***3.39***	
2012	14	1.61	0.88	120.71	17.07	0.08*	0.23	1.07	6.32	4
2013	14	1.12	0.79	101.63	11.71	0.1*	0.14	0.68	1.99	4
2014	14	1.41	1.03	105.16	15.12	0.04*	0.14	0.74	2.77	4
Ave		***1.38***	***0.90***	***109.17***	***14.63***	***0.07***	***0.17***	***0.83***	***3.69***	

1. Taylor et al. 1989; 2. Warburton and Seagars 1993; 3. Seagars et al. 1994; 4. This study
*For samples below detection, one half the detection limit was used.

Table 13. Comparison of mean concentrations of arsenic, cadmium, lead, and total mercury (µg/g = parts per million ***wet weight***) in walrus kidney and liver tissues from 1981–1984 and 2014–2015.

	Arsenic		Cadmium		Lead		Mercury		
Location	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Source
Gambell	–	0.01±0.03 (n=18)	54.17±22.24 (n=12)	11.18±9.92 (n=18)	0.05± 0.19 (n=12)	0.02±0.05 (n=18)	–	1.72±2.33 (n=8)	Taylor et al. 1989
Savoonga	–	0.02±0.04 (n=11)	–	14.41±12.92 (n=11)	–	0.02±0.05 (n=11)	–	0.90±0.98 (n=11)	Taylor et al. 1989
All	–	0.01±0.04 (n=57)	46.52±20.19 (n=42)	9.47±8.26 (n=65)	0.06±0.17 (n=39)	0.05±0.17 (n=57)	–	1.50±3.18 (n=62)	Taylor et al. 1989
Gambell and Savoonga	0.29±0.15 (n=42)	0.27±0.09 (n=42)	23.06±8.83 (n=42)	4.37±1.75 (n=42)	0.02±0.02 (n=42)	0.05±0.03 (n=42)	0.18±0.12 (n=42)	1.12±1.70 (n=42)	This study

In comparing THg in walruses with seals inhabiting the Bering and Chukchi seas, walruses had the lowest concentrations in liver and kidney (Dehn et al 2005; Quakenbush and Citta 2008, 2009; Quakenbush et al. 2009, 2011a,b; ADF&G unpubl) (Fig. 10).

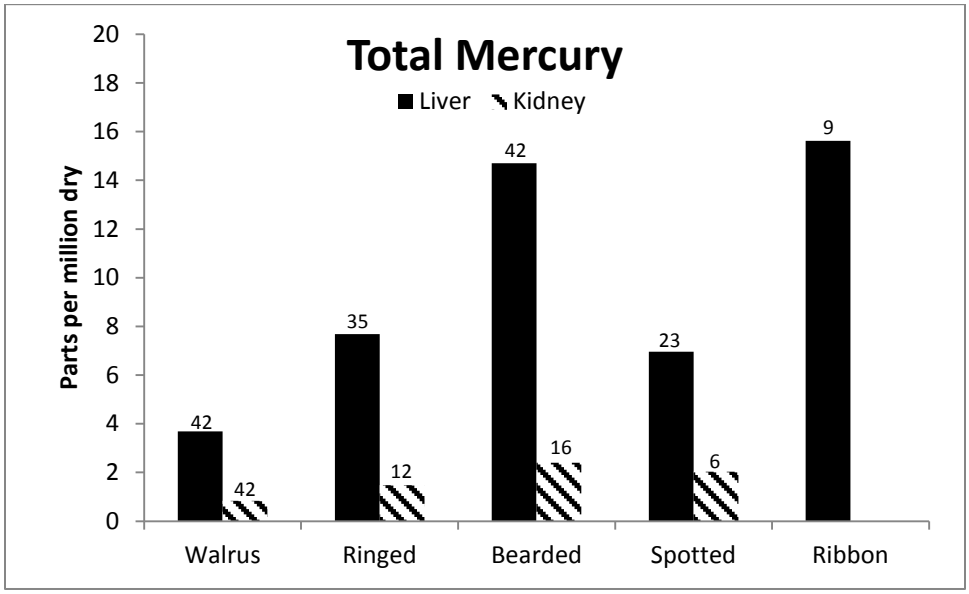

Figure 10. Comparison of total mercury concentrations (µg/g = ppm dry weight) in liver and kidney of walruses and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar.

In general, liver was higher than kidney for THg and MeHg, which was higher or similar to muscle. The mean percentage of MeHg (%MeHg) within THg however, is highest in muscle (~90%) followed by kidney and liver (~10%) each. The proportion of MeHg by tissue in ringed and bearded seals was similar to walruses, in that %MeHg was highest in seal muscle (77% ringed, 81.5% bearded) followed by seal kidney (14.8%, 4.1%), and liver (8.9%, 5.7%) (ADF&G unpubl. data).

We found no published studies reporting MeHg in Pacific walruses; however a poster presentation included MeHg for 51 walruses ≥ 1 years old and 24 fetuses and calves for liver and kidney only (Seagars et al. 1994). MeHg concentrations were higher in our study (liver 0.19 vs. 0.06 and kidney 0.09 vs 0.02 ppm dw), however methods from Seagars et al. (1994) were not available and the comparison may not be valid.

Concentrations of Se in tissues (indicated by molar ratios of Se:THg > 1) are thought to be beneficial and indicate an excess of Se available to bind to Hg to make it unavailable to form MeHg. Molar ratios were > 1 for all walrus tissues and highest in muscle where the proportion of MeHg was highest (Table 5b). Se was higher in females than males, but Hg was not (Table 7).

Overall trace element conclusions–A general comparison of mean concentrations through time shows that of the four elements of concern only As has increased. Methylmercury may be higher now, however the data are limited and the methods are not available to validate the comparison.

Cadmium, Pb, and THg are lower now (Tables 12 and 13). More studies are needed to understand at what concentrations and in what chemical forms trace elements of concern (i.e., As, Cd, Hg, Pb) cause health problems for walruses and the people who consume them. However, it is clear that apparently healthy, functioning walruses and other Arctic marine mammals commonly have concentrations in some tissues that would cause symptoms of toxicity for terrestrial mammals. There is evidence that marine mammals use proteins and other elements to bind with elements of concern to detoxify them. Thus, measuring the elements themselves in marine mammal tissues does not provide the information needed to evaluate health effects for marine mammals or people.

Organochlorines. Organochlorines, also called persistent organic pollutants, are man-made chemicals originating outside of the Arctic that accumulate in marine mammal tissues, especially blubber. Some compounds are expected to decrease due to discontinued use in the U.S. (e.g., DDT and PCB). This analysis of organochlorine contaminants in blubber, liver, kidney, and muscle of 42 walruses harvested in Alaska is the most extensive to date for Pacific walruses (Table 8).

Although organochlorines (OCs) can be measured at concentrations of ppb, we know little about what concentrations in what tissues might cause health problems for marine mammals or the people that consume them. We can however make general comparisons of concentrations measured in this study to those measured in Alaskan wildlife in the past, to walruses in Canada, and to other marine mammal species sharing the same waters (e.g., seals from the Bering and Chukchi seas). Direct comparisons are problematic due to differences in laboratory methods (including differences in minimum detection limits), number of compounds tested, number of compounds summed, and ways of reporting (e.g., wet weight to dry weight conversions, geometric means vs. arithmetic means, and how concentrations below detection limits were treated for statistical purposes). Comparisons are further complicated by the age and sex composition of the animals sampled; some compounds accumulate with age and are greater in males than females. Organochlorines are lipophilic and blubber had higher concentrations than other tissues for all compounds tested, usually by an order of magnitude.

Most forms of HCH were banned in the U.S. in 1970 for general agricultural use but Lindane is still used as a pesticide to protect seeds during planting and it is manufactured and in use in other countries. For ΣHCH, walruses in this study had lower concentrations in blubber than 14 Pacific walruses analyzed by Kucklick et al. (2006) and lower than Atlantic walruses in four locations of Arctic Canada (Muir et al. 1999). Walruses in this study had higher concentrations of ΣHCH than sympatric ringed and bearded seals and lower concentrations than spotted and ribbon seals (Fig. 11).

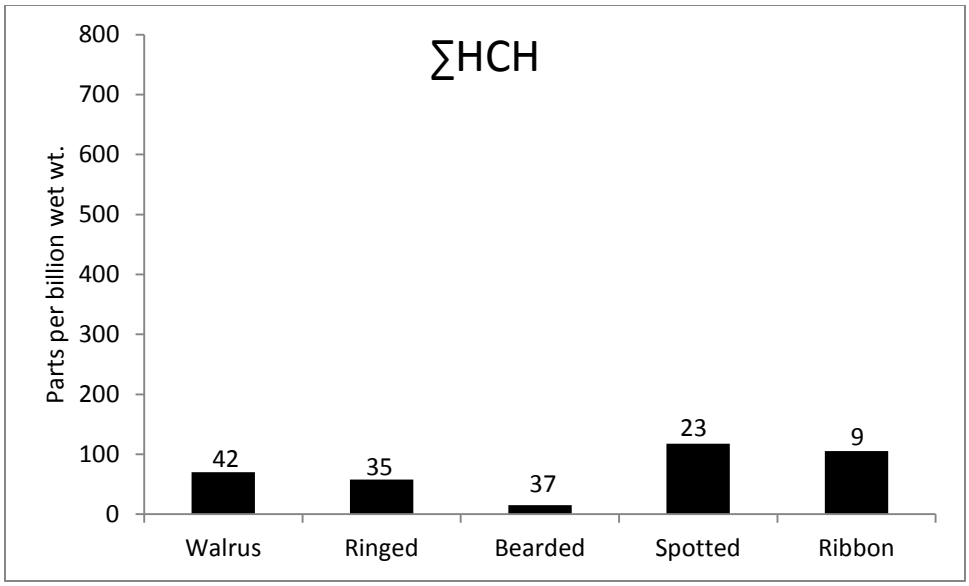

Figure 11. Comparison of the sum of alpha, beta, delta, and gamma hexachlorocyclohexane (ΣHCH) concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003-2007. Sample size appears above each bar.

Chlordane is also a pesticide but use in the U.S. ended in in 1988 (except to control fire ants in power transformers); it is still used in other countries. For ΣCHL, walruses in this study had lower concentrations in blubber than 14 Pacific walruses analyzed by Kucklick et al. (2006) and lower than Atlantic walruses in four locations of Arctic Canada (Muir et al. 1999). Walruses in this study had lower concentrations of ΣCHL than four arctic seal species (Fig. 12).

The well-known pesticide DDT, used for mosquito control, was banned in the U.S. in the 1970s and worldwide in 2001, except for limited use for malaria relief. For ΣDDT, walruses in this study had similar concentrations in blubber to 14 Pacific walruses analyzed by Kucklick et al. (2006) and lower concentrations than Atlantic walruses in four locations of Arctic Canada (Muir et al. 1999). Walruses in this study also had much lower concentrations of ΣDDT than four arctic seal species (Fig. 13) which is likely related to diet and location of feeding areas relative to DDT storage or use areas.

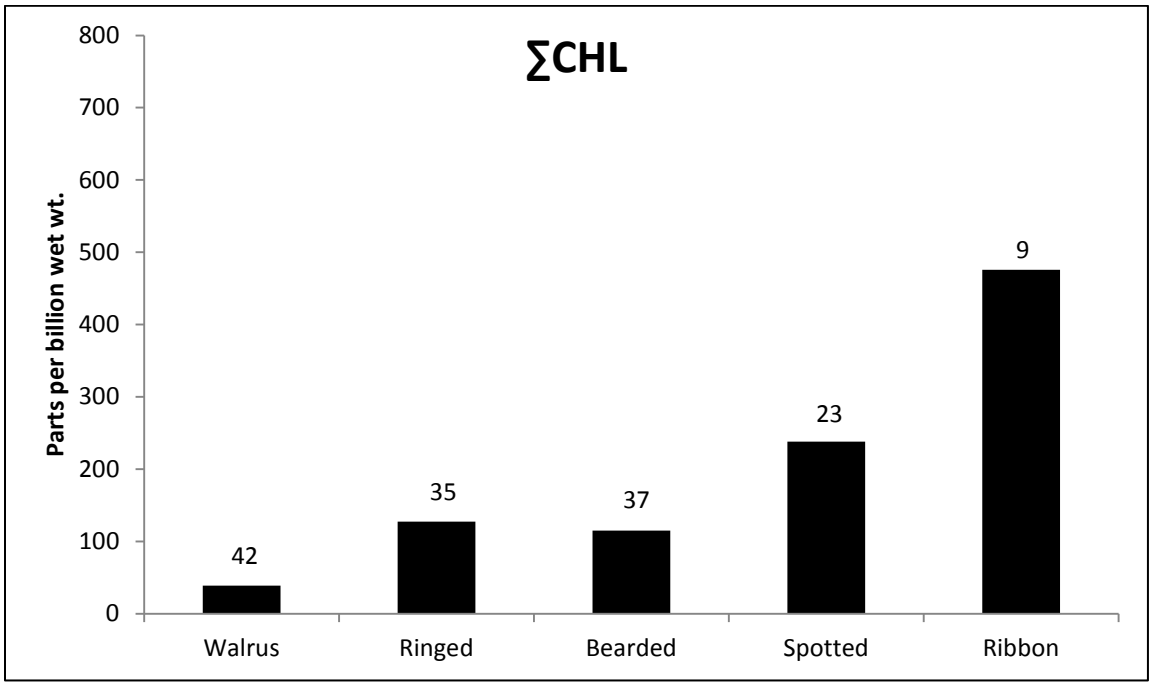

Figure 12. Comparison of the sum of six chlordane compounds concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003-2007. The number of samples analyzed appears above each bar.

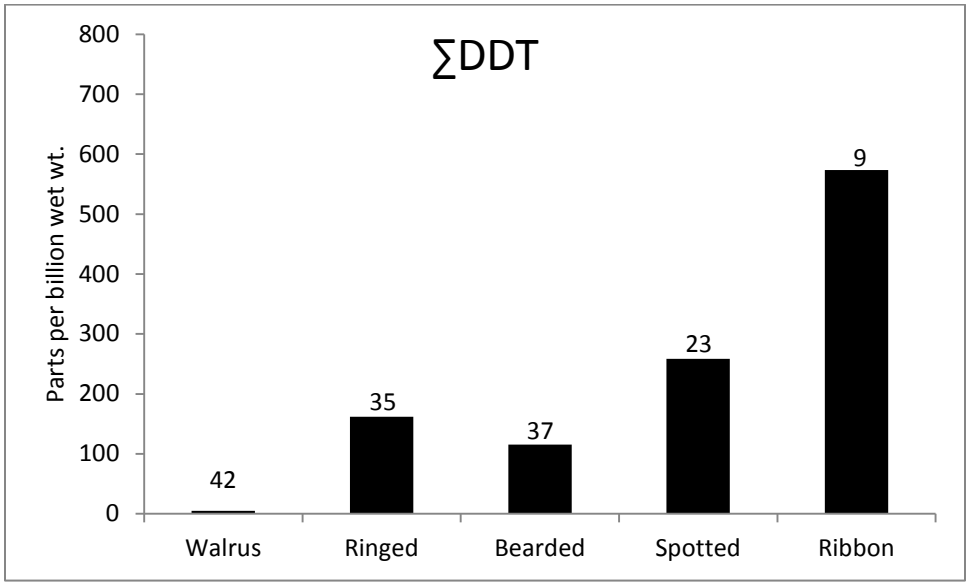

Figure 13. Comparison of the sum of seven dichlorodiphenyltrichloroethane (ΣDDT) concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar.

PCBs were widely used for insulating electrical transformers and although manufacturing, processing, distribution and use were banned in 1979 there are still some authorized uses of PCBs in the U.S. PCBs are especially difficult to compare across studies because many analyses include ~80 congeners that can be summed differently, therefore it is important to know what is being summed. For ΣPCB in this study we included all congeners analyzed (81–83 depending on year). The ones that were different by year were below detection levels. We also combined ten of the congeners used in other studies to form ΣPCB_{10} (i.e., 28, 31, 52, 101/90, 105, 118, 138/160, 153/132, 156/171/202, and 180) for comparison purposes (Muir et al. 2000, Kucklick et al. 2006). Concentrations in walruses were much lower than the four species of seals with which they are sympatric (Fig. 14). Walruses in this study had similar concentrations in blubber to 14 Pacific walruses analyzed by Kucklick et al. (2006). Taylor et al. (1989) had a much higher detection limit for PCBs (0.5 ppm), than Seagars and Garlich-Miller (2001; 0.05 ppm), which was much higher than this study (0.004 ppm) providing further evidence of the difficulty in comparing contaminant studies in general and over time.

Kucklick et al. (2006) also found organochlorine concentrations in Pacific walruses collected during 1993–1996 in the Bering and Chukchi seas to be much lower than ringed seals from the same region and suggested walruses might be better at metabolizing them than ringed seals. Norstrom and Muir (1994) attributed the same finding to walruses' lower trophic position. Of the four sympatric seal species, bearded seals have the most similar diet to walruses, eating more invertebrates and more benthic species than other seals, but also more fish than walruses (Lowry et al. 1980, Dehn et al. 2007, Quakenbush et al. 2011a). Bearded seals had the second lowest concentration of organochlorines of the four seal species and the most similar diet to walruses, which may indicate that trophic level is a factor in OC contaminant concentrations.

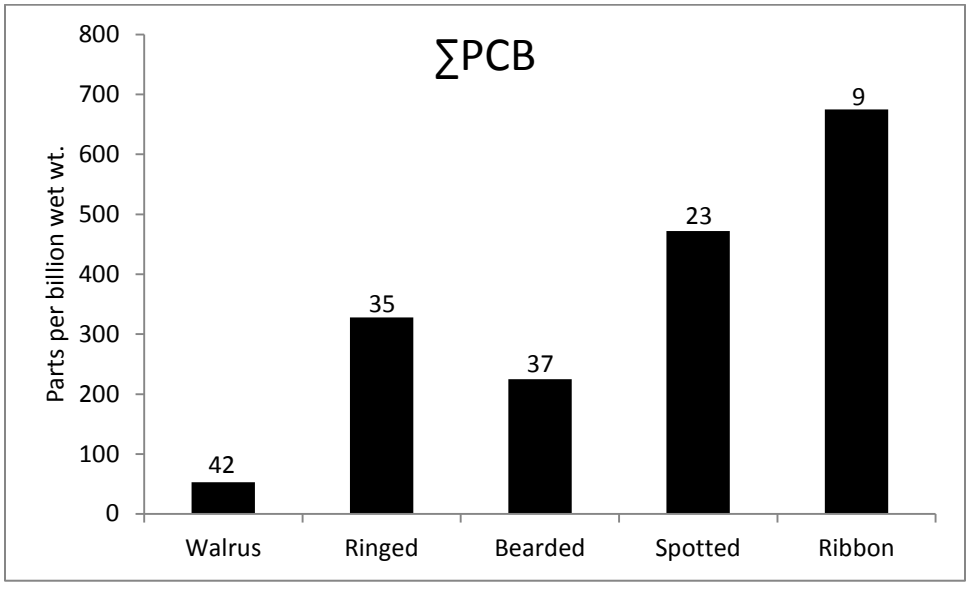

Figure 14. Comparison of the sum of all (81–83) polychlorinated biphenyls (PCB) concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar.

Dieldrin and oxychlordane concentrations were found to be lower in our study than in Taylor et al. (1989) and Kucklick et al. (2006). Mirex was lower in our study than in Kucklick et al. (2006). Taylor et al. (1989) only detected dieldrin and oxychlordane even though DDT, other CHLs, and PCBs were analyzed. It is likely these organochlorines were present, but high detection limits or other lab methods failed to detect them.

In this study, males had significantly higher mean concentrations of ΣHCH, ΣCHL, and ΣPCBs in blubber. There were no differences between sexes for ΣDDT. The difference between sexes is attributed to the ability of females to pass organochlorines on to offspring during fetal growth and lactation, while males accumulate them throughout their lifetime (Tanabe et al. 1994).

One male walrus harvested in 2012 had the highest concentrations of ΣHCH (in liver, kidney, and blubber), highest dieldrin (in liver and kidney), highest ΣPCB (in kidney and blubber), and highest ΣDDT (in kidney). This animal also had the highest MeHg concentrations in muscle, liver, and kidney. It was 18 years old and was rated as very healthy by the hunter. Concentrations of contaminants in this animal, well above others in this study, warranted further investigation. Higher trophic level predators have higher concentrations of contaminants and an analysis of stable isotope (nitrogen, $\delta^{15}N$ and carbon, $\delta^{13}C$) data for most of the walruses sampled (n=40) in this study suggest that this male was feeding differently (Figs. 15 and 16). One explanation could be that this animal was feeding on higher trophic level prey, possibly seals, which is known to occur in Atlantic (Murie et al. 1995) and Pacific walruses (Fay 1960, Lowry and Fay 1984, Fay et al. 1990,). Walruses that fed on seals in Hudson Bay had higher PCBs relative to other walruses (Muir et al. 1995).

Ringed seals have a $\delta^{15}N$ of 16.9±0.6‰ (Dehn et al. 2007) and $\delta^{15}N$ increases 3‰ for each change in trophic level (Peterson and Fry 1987). Therefore, we would expect a walrus that ate only ringed seals to have a $\delta^{15}N$ of around 19.9‰. Although this walrus did have the highest $\delta^{15}N$ value in muscle of all the walruses analyzed (14.12‰; Fig. 15) and was more than two standard deviations from the mean, its $\delta^{15}N$ value is lower than reported values for ice seals (Dehn. et al. 2007). It is possible that this walrus ate a combination of benthic prey and seals; however there are multiple combinations of non-seal prey that could have resulted in a $\delta^{15}N$ signal at this higher level. Some benthic invertebrates are higher trophically (e.g., predators like gastropods, shrimp, and crab) than others such as clams (Dehn et al. 2007, Iken et al. 2010, Seymour et al. 2014b). Therefore, it would be difficult to distinguish a walrus that occasionally ate seals from a walrus that preferred higher trophic level invertebrates such as gastropods.

Walruses are known to consume prey with a wide range of $\delta^{15}N$ values (Dehn et al. 2007; Sheffield and Grebmeier 2009; Iken et al, 2010). For example, the bivalve *Serripes groenlandicus* has a $\delta^{15}N$ value of 10.15±0.59‰ whereas gastropods (*Buccinum spp.*) have a $\delta^{15}N$ value of 15.58±0.39‰ (Iken et al. 2010).

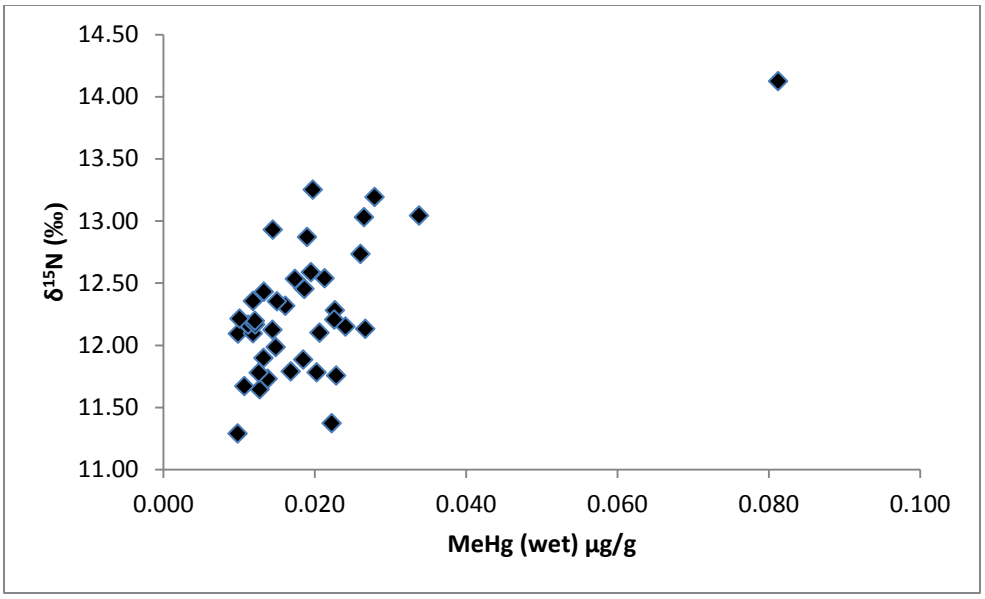

Figure 15. Stable nitrogen isotope values ($\delta^{15}N$) and MeHg in walrus muscle from 40 walruses collected at Gambell and Savoonga between 2012 and 2014.

The skin, blubber, muscle and viscera of seals have been found in some walrus stomachs (Lowry and Fay 1984; Fay et al. 1990) and blubber is known to have more depleted (lower) $\delta^{13}C$ values (Post et al. 2007). Therefore we would expect a seal-eating walrus to have a depleted carbon signature. This walrus, however, had one of the highest carbon values (-16.39‰; Fig. 16). Therefore, this walrus more likely consumed higher trophic level benthic species and not seals.

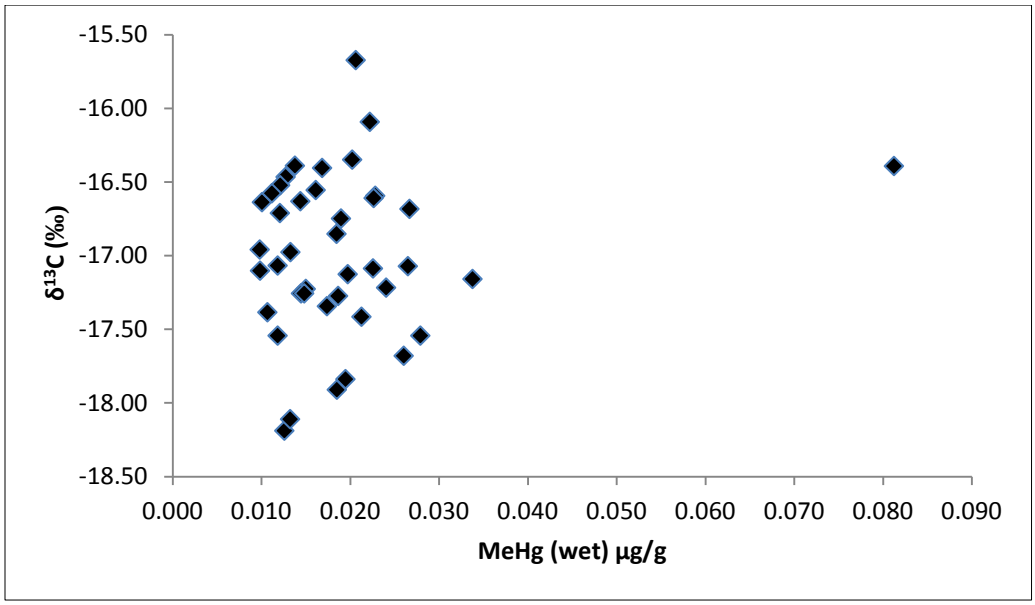

Figure 16. Stable carbon isotope values ($\delta^{13}C$) and MeHg in walrus muscle from 40 walruses collected at Gambell and Savoonga between 2012 and 2014.

Organochlorine concentrations in Pacific walruses harvested near Gambell and Savoonga were lower or similar to concentrations found in seals harvested in the Bering and Chukchi seas and to walruses in Canada. Although we expected that concentrations of some organochlorines would decline over time (e.g., PCBs may have declined in the environment and should decline in marine mammals) the differences in laboratory and analytical methods among studies confound temporal comparisons.

To our knowledge this is the first reporting of vitamin concentrations in Pacific walrus tissue. Although fat-soluble vitamins such as A (retinol) and E (α-Tocopherol) are known to be highest in blubber of marine mammals, we analyzed liver tissue at the recommendation of the laboratory for more consistent results. Although vitamin A is required for growth and maintenance functions little is known about normal concentrations and ranges in marine mammals including walruses. Retinol is of interest as a biomarker for organochlorine exposure as it has been shown to have a negative correlation with organochlorines (e.g., PCBs).

Our result showing higher vitamin A concentrations for males than females was also found in bowhead whales (Rosa et al. 2007), however concentrations in other marine mammals varied with reproductive status, season, and molt status. Non-pregnant female and male bowhead whales had the highest liver concentration, followed by subadults (both sexes), pregnant females, and then juveniles (both sexes). Whales sampled in the spring were higher than in the fall (Rosa et al. 2007), and molting ringed seals were higher than before molt (Routti et al. 2010). Vitamin A in liver of one Atlantic walrus (sex unknown) in Canada was reported as 42.8 ppm (Kuhnlein et al. 2006) which was lower than our lowest concentration (76.0 ppm for females; Fig. 5).

Vitamin E is important for reproductive, nervous, and immune system function (Kuhnlein et al. 2006). Consistent with our results, Rosa et al. (2007) found no difference between males and females in bowhead whales.

Conclusions

Walrus body condition was described by hunters as good. Diet is similar to previous studies. Concentrations of trace elements and organochlorine contaminants were similar to or lower than concentrations of ice seal species harvested in Alaska and the prevalence of diseases were also lower than that of seals that share the same habitats. Walruses are exposed to harmful algae blooms through diet and have the highest concentrations of marine mammals tested in Alaska. The overall sex ratio of the harvest was similar when Gambell and Savoonga harvests were combined across years. Pregnancy rates of harvested females were higher than theoretically possible for the population due to hunter selection and availability bias.

These results are especially valuable because they provide information that allows us to detect changes in parameters that are useful for monitoring population status when estimating population size and trend is not possible. Overall walruses appear to be in good body condition, are reproducing, have lower concentrations of contaminants than seals of the same region, and do not show prevalence for diseases of concern. Walruses are ingesting toxins from harmful algal blooms but no adverse effects have been documented.

Recommendations

Many more specific analyses can be made with the trace element and organochlorine data collected during this study, therefore we have made the raw contaminants data available for additional analyses and comparisons and for human health organizations to review for a human health and safety perspective, given that walruses are an important subsistence food for many Alaskans. Contaminants data summarized here can be found archived in the Pacific Walrus International Walrus Database managed by the U.S. Geological Survey (https://alaska.usgs.gov/science/biology/walrus/pwid/).

This study documented domoic acid and saxitoxin concentrations in urine and stomach and intestinal contents (including three genera of clams). Additional testing should be done to understand the inter-annual range and variability of these concentrations and at what levels walrus may become symptomatic. Human health organizations should consider monitoring clams from walrus stomachs to determine that they are within the safe range for the people who eat them.

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Literature Cited

AMAP. Arctic Monitoring and Assessment Program. 2011. Mercury in the Arctic. Oslo, Norway, 193 pp.

Arrigo, K. R., and G. L. van Dijken. 2015. Continued increases in Arctic Ocean primary production. Progress in Oceanography 136:60–70.

Azcue, J.M., and J. O. Nriagu. 1994. Arsenic: historical perspectives. Pages 1–15 *In:* Nriagu, J.O., ed. Arsenic in the Environment, Part 1: Cycling and characterization. John Wiley & Sons, New York, NY.

Becker, P. R., S. A. Wise, B. J. Koster, and R. Zeisler. 1991. Alaska Marine Mammal Tissue Archival Project: revised collection protocol. U.S. Dept. Commerce, National Institute of Standards and Technology Laboratory, Galthersburg, MD. 33 pp.

Bluhm, B. A., and R. Gradinger. 2008. Regional variability in food availability for arctic marine mammals. Ecological Applications 18(2) Supplement S77–S96.

Braune, B., J. Chételat, M. Amyot, T. Brown, M. Clayden, M. Evans, A. Fisk, A. Gaden, C. Girard, A. Hare, J. Kirk, I. Lehnherr, R. Letcher, L. Liseto, R. Macdonald, E. Mann, B. McMeans, D. Muir, N. O'Driscoll, A. Poulain, K. Reimer, and G. Stern. 2015. Mercury in the marine environment of the Canadian Arctic: review of recent findings. Science of the Total Environmnet 509–510:67–90.

Bukina, L. A., and A. I. Kolevatova. 2007. Role of walrus (*Odobenus rosmarus divergens*) in distribution of human trichinosis disease among indigenous people of Chukchi Peninsula. North Pacific Research Board Final Grant Report. NPRB Project 641.

Burns, J. J. 1981. Bearded seal (*Erignathus barbatus*). Pages 145–170 *in:* S. H. Ridgway and R. J. Harrison, eds. Handbook of Marine Mammals. Vol. 2 Seals. Academic Press, NY.

Correa, L., J. M. Castellini, L. T. Quakenbush, and T. M. O'Hara. 2015. Mercury and selenium concentrations in skeletal muscle, liver, and reagions of the heart and kidney in bearded saels from Alaska, USA. Enviromental Toxicoogy and Chemistry 34(10): 2403–2408.

Das, K., V. Debacker, S. Pillet, and J-M. Bouquegneau. 2003. Heavy metals in marine mammals. Pages 135–167 *in:* J. G.Vos, G. D. Bossart, M. Fournier, and T. J. O'Shea, eds. Toxicology of Marine Mammals. Taylor & Francis, London.

Dehn, L-A., G. G. Sheffield, E. H. Follmann, L. K. Duffy, D. L. Thomas, G. R. Bratton, R. J. Taylor, and T. M. O'Hara. 2005. Trace elements in tissues of phocid seals harvested in the Alaskan and Canadian Arctic: influence of age and feeding ecology. Canadian Journal of Zoology 83:726–746.

Dehn, L.-A., G. G. Sheffield, E. H. Follmann, L. K. Duffy, D. L. Thomas, and T. M. O'Hara. 2007. Feeding ecology of phocid seals and some walrus in the Alaskan and Canadian Arctic as determined by stomach contents and stable isotope analysis. Polar Biology 30:167–181.

Delaney, M. A., K. M. Colegrove, T. R. Spraker, R. L. Zuerner, R. L. Galloway, and F. M. D. Gulland. 2014. Isolation of *Leptospira* from a phocid: Acute renal failure and mortality from leptospirosis in rehabilitated northern elephant seals (*Mirounga angustirostris*), Califorina, USA. Journal of Wildlife Diseases, 50(3):621–627.

Dietz, R., J. Nørgaard, and J. C. Hansen. 1998. Have Arctic marine mammals adapted to high cadmium levels? Marine Pollution Bulletin. 36(6):490–492.

Dietz, R., C. Sonne, N. Basu, B. Braune, T. O'Hara, R. Letcher et al. 2013. What are the toxicological effects of mercury in Arctic biota? Science of the Total Environment 443:775–790.

Dubey, J. P., R. Zarnke, N. J. Thomas, S. K. Wong, W.Van Bonn, M. Briggs, J. W. Davis, R. Ewing, M. Mense, O.C.H. Kwok, S. Romand, and P. Thulliez. 2003. *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, and *Sarcocystic canis*-like infections in marine mammals. Veterinary Parasitology 116:275–296.

Earle, J. A. P., M. M. Melia, N. V. Doherty, O. Nielsen, and S. L. Cosby. 2011. Phocine distemper virus in seals, east coast, United States, 2006. Emerging Infectious Diseases 17(2):215–220.

Fay, F. H. 1960. Carnivorous walruses and some arctic zoonoses. Arctic 13:111–122.

Fay, F. H. 1990. Predation on a ringed seal, *Phoca hispida*, and a black guillemot, *Cepphus grille*, by a Pacific walrus, *Odobenus rosmarus divergens*. Marine Mammal Science 6(4):348–350.

Fay, F. H. 1982. Ecology and biology of the Pacific walrus, *Odobenus rosmarus divergens* Illiger. No. 74 USDI, FWS, North American Fauna. 279 pp.

Fay, F. H., Y. A. Bukhtiyarov, S. W. Stoker, and L. M. Shults. 1984. Foods of the Pacific walrus in winter and spring in the Bering Sea. Pages 81–88 *in:* F. H. Fay and G. A. Fedoseev, eds. Soviet-American cooperative research on marine mammals. Vol. 1-Pinnipeds. NOAA Technical Report No. 12.

Fay, F. H., B. P. Kelly, and J. L. Sease. 1989. Managing the exploitation of Pacific walruses: A tragedy of delayed response and poor communication. Marine Mammal Science 5:1–16.

Fay, F. H., J. L. Sease, and R. L. Merrick. 1990. Predation on a ringed seal, *Phoca hispida*, and a black guillemot, *Cepphus grylle*, by a Pacific walrus, *Odobenus rosmarus divergens*. Marine Mammal Science 6(3):348–350.

Fay, F. H., L. L. Eberhardt, B. P. Kelly, J. J. Burns, and L. T. Quakenbush. 1997. Status of the Pacific walrus population, 1950–1989. Marine Mammal Science 13:537–565.

Foster, G., A. P. MacMillan, J. Godfroid, F. Howie, H. M. Ross, A. Cloeckaert, R. J. Reid, S. Brew, and I. A. P. Patterson. 2002. A review of *Brucella* sp. infection of sea mammals with particular emphasis on isolates from Scotland. Veterinary Microbiology 90:563–580.

Francesconi, K.A., and J. S. Edmonds. 1993. Arsenic in the sea. 1993. Oceanography and Marine Biology Annual Review 31:111–151.

Francesconi, K.A., and J. S. Edmonds. 1997. Arsenic and marine organisms. Advances in Inorganic Chemistry 44:147–189.

Frost, K. J., and L. F. Lowry. 1980. Feeding of ribbon seals (*Phoca fasciata*) in the Bering Sea in spring. Canadian Journal of Zoology 58:1601–1607.

Garlich-Miller, J. L., R. E. A. Stewart, B. E. Stewart, and E. A. Hiltz. 1993. Comparison of mandibular with cemental growth-layer counts for ageing Atlantic walrus (*Odobenus rosmarus rosmarus*). Canadian Journal of Zoology 71:163–167.

Garlich-Miller, J. L., L. T. Quakenbush, and J. F. Bromaghin. 2006. Trends in age structure and productivity of Pacific walruses harvested in the Bering Strait region of Alaska, 1952–2002. Marine Mammal Science 22:880–896.

Gerber, J. A., J. Roletto, L. E. Morgan, D. M. Smith, and L. J. Gage. 1993. Findings in pinnipeds stranded along the central and northern California coast, 1984–1990. Journal of Wildlife Diseases 29(3)423–433.

Gibson, A. K., S. Raverty, D. M. Lambourn, J. Huggins, S. L. Magargal and M. E. Grigg, 2011. Polyparasitism is associated with increased disease severity in *Toxoplasma gondii*-infected marine sentinel species. PLoS Neglected Tropical Disease 5(5):e1142.

Grebmeier, J. M., J. E. Overland, S. E. Moore, E. B. Farley, E. C. Carmack, L. W. Cooper, K. E. Frey, J. H. Helle, F. A. McLaughlin, and S. L. McNutt. 2006. A major ecosystem shift in the northern Bering Sea. Science 311:1461–1463.

Gulland, F. M., M. Koski, L. J. Lowenstine, A. Colagross, L. Morgan, and T. Sparker. 1996. Leptospirosis in Califorina sea lions (*Zalophus californianus*) stranded along the central Californina coast, 1981–1994. 1996. Journal of Wildlife Disease 32(4):572–580.

Gulland, F. M., M. Haulena, D. Fauquier, G. Langlois, M. E., Lander, T. Zabka, and R. Duerr. 2002. Domoic acid toxicity in Californian sea lions (*Zalophus californianus*): clinical signs, treatment and survival. Veterinary Record 150(15):475–480.

Haman, K. H., A. R. Sweeny, S. A. Raverty, P-Y. Daoust, R. Cawthorn, N. Sundar, D. Wadowska, O. Nielsen, K. Burek Huntington, L. Quakenbush, M. Hammill, H. Fenton,

A. W. Trites, and M. E. Grigg. 2015. A novel coccidian parasite, *Sarcocystis pinnipedi*, caused significant mortality in grey seals (*Halichoerus grypus*) in the western north Atlantic. 21st Biennial Conference on the Biology of Marine Mammals, 13–18 December, San Francisco, CA (Abstract).

Iken, K., B. Bluhm, and K. Dunton. 2010. Benthic food-web structure under differing water mass properties in the southern Chukchi Sea. Deep-Sea Research II 57:71–85.

Johansen, P., M. M Hansen, G. Asmund, and P. B. Neilsen. 1991. Marine organisms as indicators of heavy metal pollution—experience from 16 years of monitoring at a lead zinc mine in Greenland. Chemical Ecology 5(1–2):35–55.

Kennedy, S. 1998. Morbillivirus infections in aquatic mammals. Journal of Comparative Physiology 119:201–225.

Kennedy, S., T. Kuiken, P. D. Jepson, R. Deaville, M. Forsyth, T. Barrett, M. W. G. van de Bildt, A. D. M. E. Osterhaus, T. Eybatov, C. Duck, A. Kydyrmanov, I. Mitrofanov, and S. Wilson. 2000. Mass die-off of Caspian seals caused by canine distemper virus. Emerging Infectious Diseases 6:637–645.

Knott, K. K., D. Boyd, G. M. Ylitalo, and T. M. O'Hara. 2011. Concentrations of mercury and polychlorinated biphenyls in blood of southern Beaufort Sea polar bears (Ursus maritimus) during spring: variations with lipids and stable isotope (N15, C13) values. Canadian Journal of Zoology 89(11):999–1012.

Koeman, J. H., W. S. M. van de Ven, J. J. M. de Goeij, P. S. Tjioe, and J. L. van Haaften. 1975. Mercury and selenium in marine mammals and birds. Science of the Total Environment 3:279–287.

Kozlov, D.P., 1966. Occurrence of trichinosis in the walrus on USSR territory. Tr. Gel'mintol. Lab. 19:86–89.

Kubota, R., T. Kunito, and S. Tanabe. 2001. Arsenic accumulation in the liver tissue of marine mammals. Environmental Pollution 115:303–312.

Kuhnlein, H. V., V. Barthet, A. Farren, E. Falahi, D. Leggee, O. Receveur, and P. Berti. 2006. Vitamins A, D, and E in Canadian Arctic traditional food and adult diets. Journal of Food Composition and Analysis 19:495–506.

Kucklick, J. R., M. M. Krahn, P. R. Becker, B. J. Porter, M. M. Schantz, G. S. York, T. M. O'Hara, and T. A. Wise. 2006. Persistent organic pollutants in Alaskan ringed seal (*Phoca hispida*) and walrus (*Odobenus rosmarus*) blubber. Journal of Environmental Monitoring 8:848–854.

Kunito, T., R. Kubota, J. Fujihara, T. Agusa, and S. Tanabe. 2008. Arsenic in marine mammals, seabirds, and sea turtles. Pages 31–69 *In:* D. M. Whitacre , ed. Reviews of environmental contaminations and toxicology. Springer, New York, NY, USA.

Larsen, T. S., J. A. Kristensen, G. Asmund, and P. Bjerregarrd. 2001. Lead and zinc in sediments and biota from Maarmorilik, West Greenland: an assessment of the environmental impact of mining wastes on an Arctic fjord system. Environmental Pollution 114(2):275–283.

Larsen, A. K., I. H. Nymo, B. Briquemont, K. K. Sørensen, and J. Godfroid. 2013. Entrance and survival of *Brucella pinnipedialis* hooded seal strain in human macrophages and epithelial cells. PLoS ONE 8(12):e84861. doi:10.1371/journal.pone.0084861

Loffler, S. G., V. Rago, M. Martínez, M. Uhart, M. Florin-Christenesen, G. Romero, and B. Brihuega. 2015. Isolation of a seawater tolerant *Leptospira* spp. from a southern right whale (*Eubalaena australis*). PLoS ONE 10(12) e0144974. doi:10.1371/journal. pone.0144974

Lowry, L. F., K. J. Frost, and J. J. Burns. 1980. Feeding of bearded seals in the Bering and Chukchi Seas and trophic interaction with Pacific walruses. Arctic 33:330–342.

Lowry, L. F., and F. H. Fay. 1984. Seal eating by walruses in the Bering and Chukchi Seas. Polar Biology 3:11–18.

Macdonald, R., L. Barrie, T. Bidleman, M. Diamond, D. Gregor, and R. Semkin et al. 2000. Sources, occurrence and pathways of contaminants in the Arctic. Science of the Total Environment 254:93–234.

Method 1630. EPA-821-R-01-020. 2001. Methyl mercury in water by distillation, aqueous ethlyation, purge and trap, and CVAFS. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, D.C.

Middaugh, J. P. 1986. Cadmium in walrus — St. Lawrence Island. State of Alaska Epidemiology Bulletin No. 22.

Miller, W. G., L. G. Adams, T. A. Ficht, N. F. Cheville, J. P. Payeur, D. R. Harley, S. M. Carol House, and S. H. Ridgway. 1999. *Brucella*-induced abortions and infection in bottlenose dolphins (*Tursiops truncatus*). Journal of Zoo and Wildlife Medicine 30(1):100–110.

Mochizuki, Y., K.T. Suzuke, H. Sunaga, T. Kobayashi , and R. Doi. 1985. Separation and characterization of metallothionein in two species of seals by high performance liquid chromatography–atomic absorption spectrophotometry. Comparative Biochemistry and Physiology C 82:249–254.

Moore, S. E., J. M. Grebmeier, and J. R. Davies. 2003. Gray whale distribution relative to forage habitat in the northern Bering Sea: current conditions and retrospective summary. Canadian Journal of Zoology 81:734–742.

Moses, S. K., A. V. Whiting, G. R. Bratton, R. J. Taylor, and T. M. O'Hara, 2009. Inorganic nutrients and contaminants in spotted seals (*Phoca largha*) and sheefish (*Stenodus leucicthys*) of NW Alaska: linking the health of arctic wildlife and subsistence users. International Journal of Circumpolar Health 68(1):53–74.

Mueter, F. J. and M. A. Litzow. 2008. Sea ice retreat alters the biogeography of the Bering Sea Continental Shelf. Ecological Applications 18**:** 309–320.

Muir, D. C. G., M. D. Segstro, K. A. Hobson, C. A. Ford, R. E. A. Stewart, and S. Olpinski. 1995. Can seal eating explain elevated levels of PCBs and organochlorine pesticides in walrus blubber from eastern Hudson Bay (Canada)? Environmental Pollution 90(3):335–348.

Muir, D., B. Braune, B. DeMarch, R. Norstrom, R. Wagemann, L. Lockhart, B. Hargrave, D. Bright, R. Addison, J. Payne, and K. Reimer. 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. Science of the Total Environment 230:83–144.

Muir, D., F. Riget, M. Cleemann, J. Skaare, L. Kleivane, H. Nakata, R. Dietz, T. Severinsen and S. Tanabe. 2000. Circumpolar trends of PCBs and organochlorine pesticides in the Arctic marine environment inferred from levels in ringed seals. Environmental Science and Technology 34(12):2431–2438.

Norstrom, R. J. and D. C. G. Muir. 1994. Chlorinated hydrocarbon contaminants in arctic marine mammals. Science of the Total Environment 154:107–128.

O'Hara, T. M., J. C. George, J. Blake, K. Burek, G. Carroll, J. Dau, L. Bennett, C. P. McCoy, P. Gerard, and V. Woshner. 2003. Investigation of heavy metals in a large mortality event in caribou of northern Alaska. Arctic 56(2):125–135.

O'Shea, T.J. 1999. Environmental contaminants and marine mammals. Pages 485–564 *In*: J. E. Reynolds, III, and S. A. Rommel, eds. Biology of Marine Mammals. Smithsonian Institution Press, Washington, D.C.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Ponce R. A., G. M. Egeland, J. P. Middaugh, and P. R. Becker. 1997. Twenty years of trace metals analyses of marine mammals: evaluation and summation of data from Alaska and other Arctic regions. State of Alaska Epidemiology Bulletin 1(3):1–15.

Post, D. M., C. A. Layman, D. A. Arrington, G. Takimoto, J. Quattrochi, and C. G. Montana. 2007. Getting to the fat of the matter: models, methods, and assumptions for dealing with lipids in stable isotope analysis. Oecologia 152:179–189.

Quakenbush, L. T. and J. J. Citta. 2008. Biology of the ribbon seal in Alaska. Unpublished report to NMFS. 45 pp.

Quakenbush, L. and J. J. Citta. 2009. Trace element concentrations in bearded seals (*Erignathus barbatus*) near Red Dog Mine compared to other locations in Alaska. Journal of Marine Biology. doi:10.1155/2009/275040.

Quakenbush, L. T., J. J. Citta, and J. A. Crawford. 2009. Biology of the spotted seal (*Phoca largha*) in Alaska from 1962–2008. Final Report to National Marine Fisheries Service. 66 pp.

Quakenbush, L. T., J. J. Citta, and J. A. Crawford. 2011a. Biology of the ringed seal (*Phoca hispida*) in Alaska, 1960–2010. Final Report to National Marine Fisheries Service. 72 pp.

Quakenbush, L. T., J. J. Citta, and J. A. Crawford. 2011b. Biology of the bearded seal (*Erignathus barbatus*) in Alaska, 1961–2009. Final Report to National Marine Fisheries Service. 71 pp.

R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. http://www.R-project.org/.

Rhyan J. C., T. Gidlewski, D. R. Ewalt, S. G. Hennager, D. M. Lambourne, and S. C. Olsen. 2001. Seroconversion and abortion in cattle experimentally infected with *Brucella* sp. isolated from a Pacific harbor seal (*Phoca vitulina richardsi*). Journal of Veterinary Diagnostic Investigation 13:379–382.

Rosa C., J. E. Blake, L. Mazzaro, P. Hoekstra, G. M. Ylitalo, and T. M. O'Hara. 2007. Vitamin A and E tissue distribution with comparisons to organochlorine concentrations in the serum, blubber and liver of the bowhead whale (*Balaena mysticetus*). Comparative Biochemistry and Physiology, Part B 148:454–462.

Routti, H., B. M. Jenssen, C. Lydersen, C. Bäckman, A. Arukwe, M.Nyman, K. M. Kovacs, and G.W. Gabrielsen. 2010. Hormone, vitamin and contaminant status during the moulting/fastin period in ringed seals (*Pusa* [*Phoca*] *hispida*) from Svalbard. Comparative Biochemistry and Physiology, Part A 155:70–76.

Rust, L., F. Gulland, E. Frame, and K. Lefebvre. 2014. Domoic acid in milk of free living Californa marine mammals indicates lactational exposure occurs. Marine Mammal Science 30(3):1272–1278. DOI: 10.1111/mms.12117.

Seagars, D. J., C. E. Bowlby, D. Burn, and J. Garlich-Miller. 1994. Metal concentrations in liver and kidney tissues of Pacific warlus: 1991 Zaslonovo Bering Seae research cruise samples. Unpublished poster presented at 45th Arctic Science Conference. Anchorage, Alaska and Vladivostok, Russia, August 25–September 2.

Seagars, D. J., and J. Garlich-Miller. 2001. Organochlorine compounds and aliphatic hydrocarbons in Pacific walrus blubber. Marine pollution Bulletin 43:122–131.

Seymour, J., L. Horstmann-Dehn, C. Rosa, J. A. Lopez. 2014a. Occurrence and genotypic analysis of *Trichinella* species in Alaska marine-associated mammals of the Bering and Chukchi seas. Veterinary Parasitology 200:153–164.

Seymour, J., L. Horstmann-Dehn, and M. J. Wooller. 2014b. Proportion of higher trophic-level prey in the diet of Pacific walruses (*Odobenus rosmarus divergens*). Polar Biology 37:597–609.

Schell, D. M., B. A. Barnett, and K. A. Vinette. 1998. Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi, and Beaufort seas. Marine Ecology Progress Series 162:11–23.

Sheffield, G., and J. M. Grebmeier. 2009. Pacific walrus (*Odobenus rosmarus divergens*): Differential prey digestion and diet. Marine Mammal Science 25(4): 761–777. DOI: 10.1111/j.1748-7692.2009.00316.x.

Speckman S. G., V. I. Chernook, D. M. Burn, M. S. Udevitz, A. A. Kochnev, A. Vasilev, C. V. Jay, A. Lisovsky, A. S. Fishbach, and R. B. Benter. 2011. Results and evaluation of survey to estimate Pacific walrus population size, 2006. Marine Mammal Science 27(3):514–553. DOI: 10.1111/j.1748-7692.2010.00419.x.

Tanabe, S., J. K. Sung, D. Y. Choi, N. Baba. M. Kyota, K. Yoshida, and R. Tatsukawa. 1994. Persistent organochlorine residues in northern fur seal from Pacific coast of Japan since 1971. Environmental Pollution 85:305–314.

Thatcher, C.D., J. B. Meldrum, S. E. Wikse, W. D. Whittier. 1985. Arsenic toxicosis and suspected chromium toxicosis in a herd of cattle. Journal of the American Veterinary Medical Association 187(2):179–182.

Taylor, D. L., S. Schleibe, and H. Metsker. 1989. Contaminants in blubber, liver, and kidney tissues of Pacific walruses. Marine Pollution Bulletin 20(9):465–468.

Tohyama, C., S. I. Himeno, C. Watanabe, T. Suzuki, and M. Morita. 1986. The relationship of the increased level of metallothionein with heavy metals in the tissue of the harbor seal (*Phoca vitulina*). Exotoxicology and Environmental Safety 12:85–94.

Wagemann, R., and D. C. G. Muir. 1984. Concentrations of heavy metals and organochlorine concentrations in marine mammals of northern waters: overview and evaluation. Canadian Technical Report Fisheries and Aquatic Sciences 1279:v + 97 pp.

Wagemann, R., and R. E. A. Stewart. 1994. Concentrations of heavy metals and selenium in tissues and some foods of walrus (*Odobenus rosmarus rosmarus*) from eastern Canadian Arctic and sub-Arctic, and associations between metals, age, and gender. Canadian Journal of Fisheries and Aquatic Sciences 51:426–436.

Wang, S. W., S. M. Budge, R. R. Gradinger, K. Iken, and M. J. Wooller. 2014. Fatty acid and stable isotope characteristics of sea ice and pelagic particulate organic matter in the Bering Sea: tools for estimating sea ice algal contribution of Arctic food web production. Oecologia 174(3):699–712. Doi:10.1007/s00442-013-2832-3

Warburton, J. and D. J. Seagars. 1993. Heavy metal concentrations in liver and kidney tissues of Pacific walrus: continuation of a baseline study. Technical Report R7/MMM 93-1. U.S. Fish and Wildlife Service, Anchorage, Alaska, 23 pp.

Wekell, J. J. Hurst, and K. A. Lefebvre. 2004. The origin of the regulatory limits for PSP and ASP toxins in shellfish. Journal of Shellfish Research 23(3): 927–930.

Welfinger-Smith, G., J. L. Minholz, S. Byrne, V. Waghyi, J. Gologergen, J. Kava, M. Apatiki, E. Ungott, P. K. Miller, J. G. Arnason, and D. O. Carpenter. 2011. Journal of Toxicology and Environmental Health, Part A 74:1195–1214.

WHO. 1992. Cadmium. Environmental Health Criteria. World Health Organization, Geneva. Vol. 134.

Woshner, V. M., T. M. O'Hara, G. R. Bratton, R. S. Suydam, and V. R. Beasley. 2001*a*. Concentrations and interactions of selected essential and non-essential elements in bowhead and beluga whales of Arctic Alaska. Journal of Wildlife Diseases 37:693–710.

Woshner, V. M., T. M. O'Hara, G. R. Bratton, and V. R. Beasley. 2001*b*. Concentrations and interactions of selected essential and non-essential elements in ringed seals and polar bears of Arctic Alaska. Journal of Wildlife Diseases 37:711–721.

Zarnke, R. L., T. C. Harder, H. W. Vos, J. M. Ver Hoef, and A. D. M. E. Osterhaus. 1997. Serologic survey for phocid herpesvirus-1 and -2 in marine mammals from Alaska and Russia. Journal of Wildlife Diseases 33:459–465.

Zarnke, R. L., J. T. Saliki, A. P. Macmillan, S. D. Brew, C. E. Dawson, J. M. Ver Hoef, K. J. Frost, and R. J. Small. 2006. Serologic survey for *Brucella* spp., phocid herpesvirus-1, phocid herpesvirus-2, and phocine distemper virus in harbor seals from Alaska, 1976–1999. Journal of Wildlife Diseases 42:290–300.