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Lori Polasek
Marine Mammal Program

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Final Report

I. Project Identifiers

A. Award Number: NA16NMF4390029
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C. Co-Investigator(s): Lori Quakenbush
D. Project Title: Biological Monitoring of Ice Seals in Alaska to Determine Health and Status of Populations—Diet, Disease, Contaminants, Reproduction, Body Condition, Growth, and Age at Maturity
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II. Project Summary

Bearded (*Erignathus barbatus*), spotted (*Phoca largha*), ribbon (*Histriophoca fasciata*), and ringed (*Pusa hispida*) seals are the species of Alaska's seals collectively called ice seals because of their association with sea ice and their dependence on it for resting, pupping, and molting. Ice seals are important components of the ecosystems of the Bering, Chukchi, and Beaufort seas and they are important to the subsistence-based culture of Alaska Natives for food and raw materials. In Alaska, the subsistence harvest of marine mammals, including ice seals, has provided important information regarding population status and health since the 1960s. Decreasing sea ice is expected to affect ice seal populations by reducing the amount and time that sea ice is available for resting, pupping, pup rearing, and molting. This project continues the long-term sampling of the subsistence harvest to monitor parameters related to ice seal population status and health in the Bering and Chukchi seas. This project sampled bearded, spotted, and ribbon seals only; ringed seals were sampled and reported under a concurrent NOAA Species Recovery Grant (Award No. NA16NMF4720079). The purpose of this project was to address critical data gaps in understanding how seals are responding to changes in sea ice, infectious diseases, and contaminants and how quickly these changes could alter population dynamics.

The Alaska Department of Fish and Game (ADF&G) works with villages in the Bering and Chukchi seas (e.g., Hooper Bay, Gambell, Shishmaref, Point Hope, and Utqiaġvik) to sample the subsistence ice seal harvest for parameters related to population status and health. We collect measurements (length, girth, blubber thickness), and tissues (teeth, whiskers, claws, blood, stomach, intestine, liver, kidney muscle, blubber, female reproductive tracts) to address infectious disease exposure, contaminants, diet, body condition, pregnancy rate, growth rate, age at maturity, and proportion of pups in the harvest. In addition to providing seal samples from the harvest, hunters provided local knowledge about seal condition, availability, behavior, health, and whether their hunting practices have changed

relative to ice and weather conditions or changes in seal distribution. This project provides essential information on the health and status of ice seals and allows us to monitor, document, and evaluate changes in population status, availability to subsistence hunters, contaminants, and other health factors.

During this project period of 2016–2020, we collected morphometric data and tissue samples from 1,618 seals harvested for subsistence by Alaska Natives (539 bearded, 1,067 spotted, and 12 ribbon seals) to evaluate the health and status of each species. We found that (1) there was no increase in the prevalence of helminth parasites and no new parasite species, (2) harmful algal bloom (HAB) toxins were present in all species and for bearded seals prevalence of domoic acid had increased in stomach contents to 100% between 2012 and 2019 in the Bering Sea, (3) we expanded the understanding of a pinniped specific disease, *Brucella pinnipedialis*, relative to ice seals and found exposure to *Coxiella burnetii* in bearded and ribbon seals, (4) we tested tissues for contaminants and have accumulated a dataset that will allow a comprehensive analysis of elements, organochlorines, and other contaminants to be compared over time, (5) the diet of bearded seals included 44 major prey types (11 fish, 33 invertebrates) and spotted seals consumed fewer Arctic cod, *Boreogadus saida*, during 2016–2020 than during 2000–2015, (6) we analyzed length at age data to detect birth years when conditions were good or poor; except for bearded seal pups born in 2018, recent years have not been poor for bearded or spotted seal growth, (7) using blubber thickness as an index for body condition, no years were below average for bearded seals during this project period, however 2017 and 2018 were below average for spotted seals, but by 2019 spotted seals were above average body condition again, and (8) pregnancy rates for bearded and spotted seals were higher after 2010 than during earlier periods and age at maturity was lower in the 2000s for bearded and spotted seals than in the 1960s and 1970s indicating higher productivity in recent years. Overall, these indices to seal population health and status are positive and do not show a sustained negative response to recent decreases in sea ice or increases in the length of the open-water season. The most pressing health concern we identified is the increase of HABs which should continue to be monitored.

III. **Purpose of Project**

Little is known about the biology and ecology of ice seals and how changes in sea ice may affect populations or how quickly. Ice seals are widely distributed in remote, ice-covered waters making marine mammal abundance surveys dangerous, difficult, and expensive. Therefore, population estimates that can be used to detect population trends are not currently available for ice seals. However, we can evaluate indices related to population abundance, health, and availability to subsistence hunters to monitor the status of these populations. Tracking these indices is of elevated importance for monitoring ice seal responses to rapid changes in sea ice and related environmental conditions.

By collecting and analyzing biological samples and harvest information from subsistence-harvested seals at selected locations annually, we can assess the health and status of each species. Indices that can be evaluated include: sex and age of seals harvested, age at first reproduction, pregnancy rate, length at age, body condition, diet, disease exposure, and parasite and contaminant load. The Arctic marine ecosystem is changing, and data collected by this monitoring program provide a means to detect and monitor the effects of such changes on ice seals. This project provided the only long-term data available for ice seals in Alaska and results allow NMFS to evaluate how ice seals are responding to changes in the environment, the effect responses may have on populations, and how long it may take to see population level effects so that management actions can be considered promptly. Objectives were:

1. Collect morphometric data and samples from bearded, spotted, and ribbon seals during spring and fall subsistence harvests using methods comparable to past data collections and enter data into our database to include in annual reports, the final report, and manuscripts.

2. Analyze samples collected during this project period to evaluate health and status of each species.

2a. Analyze blood for disease exposure and evaluate relative to overall seal health including reproduction and survival.

2b. Select tissues for contaminants testing and send to contract laboratory for analysis. Analyze contaminants data collected previously and evaluate relative to overall seal health including reproduction and survival.

2c. Analyze stomach contents for diet, measure fish otoliths to determine size of fish eaten.

2d. Analyze body length and age to determine growth.

2e. Analyze blubber thickness for body condition.

2f. Analyze female reproductive tracts and age for pregnancy and age of maturity.

2g. Determine proportion of pups in the harvest as an index of pup survival to weaning.

3. Collect local knowledge about seals and seal hunting during this project period to best interpret results of sample analysis.

4. Submit a contaminants manuscript and a parasite manuscript to peer-reviewed scientific journals using samples and data collected prior to and during this project period.

IV. Approach

A.

Methods. We collected morphometric information (length, girth, blubber thickness) and biological samples (e.g., teeth, stomach, liver, kidney, blubber, muscle, female reproductive tracts, whiskers, and claws) from seals harvested for subsistence in Bering and Chukchi Sea villages. We used commercial laboratories to section and age teeth, to analyze tissues for contaminant concentrations, and to screen blood sera for disease exposure. We sorted prey items from stomachs and identified prey to the lowest taxonomic level in house and by consulting other experts. We also used the annual proportion of pups in the sample as an index of the survival of pups through the weaning period. We distributed questionnaires to hunters to collect local knowledge and evaluate hunter bias in the samples. Our analytical methods are included below with our results.

B.

Partners and collaborators. This project was supported by participating villages through their tribal councils, the Ice Seal Committee (ISC), the North Slope Borough (NSB), the University of Alaska Museum (UAM) and many researchers and students with which we share samples and data to maximize the use of the samples and what we can learn from them. In most locations, residents are trained to collect information and samples. The primary villages that have participated since the early 2000s include Point Hope, Shishmaref, Gambell, and Hooper Bay. These villages are important because of their geographic locations, harvest levels, the availability of historical data for retrospective analyses, and their interest and willingness to participate. Utqiaġvik, Wainwright, and Point Lay, also contribute samples through the NSB. We process these samples and incorporate data into our database; we also provide data specific to the contributed specimens back to the organization that provided them. This is an effective way to increase sample sizes and geographical range without increasing our cost of collection and we can provide information to the organizations for their specific uses. We developed a strong collaboration with the NSB regarding seal sampling and data sharing. We contributed samples and data to many researchers and students (see Section VC).

We worked with Dr. Heather Walden of the Department of Infectious Diseases and Pathology at the College of Veterinary Medicine at the University of Florida on parasite (helminth) identification and a manuscript.

We worked with Dr. Kathi Lefebvre, Northwest Fisheries Science Center, NOAA Fisheries on HABs detection, sampling protocols, two manuscripts and two abstracts.

V. Results, Evaluation and Conclusions

A.

Objective 1: **Collect morphometric data and tissue samples from bearded, spotted, and ribbon seals.** During this 2016–2020 project period, we collected measurements and samples from 1,618 seals (539 bearded, 1,067 spotted, and 12 ribbon seals) from Utqiaġvik, Wainwright, Point Lay, Point Hope, Shishmaref, Nome, Saint Michael, Gambell, and Hooper Bay (Table 1). An analysis of the age structure of the sampled harvest (2000–2018) found that the average age for bearded seals was 4.8 years (SE= 0.17) and for spotted seals it was 2.9 years (SE= 0.09). Maximum age of bearded and spotted seals was 40 and 44 years, respectively; in contrast, the oldest ribbon seal was 25 years old (Adam et al. 2020, see Section VI). This objective was fully achieved.

Objective 2: **Analyze samples to evaluate the health and status of bearded, spotted and ribbon seals harvested for subsistence.**

2a. Analyze blood for disease exposure and evaluate relative to overall seal health including reproduction and survival.
Disease Screening – Serum was collected from 50 bearded and one spotted seal during this project period and archived at ADF&G for future disease screening. Whole blood was collected on filter paper for 27 bearded and 28 spotted seals and contributed to a pilot project with the University of Alaska Fairbanks (UAF), Marine Ecotoxicology and Trophic Assessment Laboratory to determine if disease exposure can be detected from blood collected this way. This collection method would greatly simplify blood collection in remote locations because it would not require a centrifuge or freezing. Blood on filter paper can be used to quantify mercury in whole blood (Hansen et al. 2014) and for carbon and nitrogen isotope values (O'Hara et al. 2018). None of sera collected from the filter paper, however, resulted in positive readings when tested for *Coxiella burnetii,* therefore, more samples will need to be tested. Preliminary data from this project were presented as a poster at the Biennial Conference on the Biology of Marine Mammals in Halifax, Nova Scotia, in October 2017 (Castellini et al. 2017, see Section VI).

Previously archived serum samples from 16 bearded and two ribbon seals were tested for *Coxiella burnetii* using indirect fluorescent antibody testing; titers ≥1:128 were considered positive. *Coxiella burnetii* is a zoonotic bacterium that can cause reproductive failure in marine mammals (Minor et al. 2013) and Q fever in humans (Kersh et

al. 2020). Ruminants are the primary reservoir and although *C. burnetii* has been documented in Alaska Native people that live near fur seal rookeries on the Pribilof islands (Kersh et al. 2020), it is not known if the *C. burnetti* strains that infect marine mammals can be transmitted to humans (Kersh et al. 2020). In humans, phase II titers are usually higher during acute infections whereas phase I are higher during chronic infections. Three of the bearded seals (19%) were positive for *C. burnetii* antibodies; of those one tested positive for phase I antibodies, one for phase II, and one was positive for both. One of the ribbon seals (50%) also tested positive for phase I antibodies.

Screening for *Brucella* in phocid seals is problematic because standard tests are too generic and do not target marine mammal specific *Brucella* species. Terrestrial species of *Brucella* are zoonotic and known to cause reproductive failure and other symptoms. In ice seals, however, little pathology has been associated with seals that test positive and no humans have been reported with symptoms even though many people process and handle raw tissues and oil frequently. Because of the lack of symptoms in both seals and humans, we worked with ADF&G veterinarian Dr. Kimberlee Beckmen, a group of virologists from the Arctic University of Norway in Tromsø, Norway, and SAC Consulting Veterinary Services in the United Kingdom, to investigate *Brucella* in seals. We used archived sera from spotted and ribbon seals to better understand the prevalence and effects of the marine species, *Brucella pinnipedialis*, including that *B. pinnipedialis* effects otariids very differently than phocids (Nymo et al. 2018, see Section VI). Archived bearded seal sera were also used to support that *B. pinnipedialis* is not associated with pathology in seals and not zoonotic, although further testing should be conducted to confirm (Foster et al. 2018; see Section VI).

Nasal swabs from 43 bearded and two ribbon seals were used in a study to identify phocine distemper virus (PDV) in pinnipeds in the North Pacific Ocean. All samples we contributed were negative for the virus (Van Wormer et al. 2019, see Section VI).

Parasites – The species and prevalence of parasites were predicted to change with the warming climate (Burek et al. 2008), therefore during this project period we analyzed tissues (stomach, intestine, heart, liver, lung, gall bladder) collected during 2006–2015 from 141 seals (including 75 bearded, 14 spotted, and nine ribbon) for internal helminth parasites to evaluate changes in helminth load and species composition in ice seals. The parasites we found are common in phocids, many are considered non-pathogenic while others such as lungworms and heart worms have the potential to cause illness and mortality in their hosts. Although none of the helminths found were

new to the Bering-Chukchi region, this study found the first host record of the lungworm *Parafilaroides (Filaroides) gymnurus* in a ribbon seal. This is also the first report of the lungworm *Otostongylus circumlitus* in a ribbon seal and *P. (F.) gymnurus* in bearded seals from the Bering-Chukchi region (previously identified in the Sea of Okhotsk). We found a lower prevalence of the cestode genus *Pyramicocephalus* in bearded seals (3%) than reported previously for the species *Pyramicocephalus phocarum* (44–100%) in the Bering-Chukchi region. The acanthocephalan genus *Bolbosoma* was not found in our study but the genus was previously identified in spotted and ribbon seals. Therefore, as of 2015, no new parasite species were identified, and the prevalence of endemic parasites has not increased. These data were presented as a poster at the Alaska Marine Science Symposium, AMSS (Bryan et al. 2020, See Section VI) and published in the Journal of Wildlife Diseases (Walden et al. 2020, see Section VI).

We worked with Dr. Heather Walden of the Department of Infectious Diseases and Pathology at the College of Veterinary Medicine at the University of Florida on the manuscript. Representative (voucher) helminth specimens were submitted to the University of Florida Museum of Natural History, Nematoda UF 22-68, Platyhelminthes UF 1062-1137, and Rotifera (Acanthocephala) UF 3-34.

Harmful Algal Bloom (HAB) Toxins – We tested for the presence of neurotoxins (domoic acid [DA] and saxitoxin [STX]) produced by HABs which are known to be present and are expected to increase in the Arctic as sea ice decreases and waters warm. In warmer waters some marine algae can multiply quickly (i.e., "bloom") and produce toxins that accumulate in clams and some fish, that are then transferred to marine mammals that eat those clams and fish. During this project period we tested 228 bearded, 94 spotted, and 20 ribbon seals, collected during 2007–2019 for DA and STX and analyzed additional data and found that for bearded seals 157 of 344 (46%) had detectable concentrations of DA and 96 of 404 (24%) had detectable concentrations of STX. For spotted seals, 14 of 268 (5%) had detectable concentrations of DA and 9 of 257 (4%) had detectable concentrations of STX. For ribbon seals, only 1 of 28 (4%) had DA and none of 28 had STX. Of all the ice seal species, including ringed seals, bearded seals had the highest prevalence of both toxins, however, ringed seals had the highest maximum concentration of DA at 1,740 ng/mL, whereas the maximum found in bearded seals was 1,353 ng/mL. We also found that the prevalence of DA in bearded seal stomach contents from the Bering Sea has increased over time from 0% in 2012 to 100% in 2019. This is consistent with warmer waters in the Bering Sea.

These data are included in our follow-up manuscript to Lefebvre et al. (2016, See Section VI), in Hendrix et al. (*In prep*.) that will address DA and STX concentrations for bearded, spotted, ribbon, and ringed seals in the Bering and Chukchi seas during 2005–2019. We also compared toxin concentrations in stomach contents with colon content and found that toxins are more concentrated in the colon than in stomach, therefore comparing individuals across sample matrices is not appropriate. Because of these results, we have changed our sampling protocol to collect samples for HABs analysis from the colon instead of the stomach.

Although prevalence and some concentrations of DA and STX are higher than expected for seals in these northern seas, no evidence of behavioral or pathological affects have been found.

We worked with Dr. Kathi Lefebvre, Northwest Fisheries Science Center, NOAA Fisheries on HABs issues and produced one published paper (Lefebvre et al. 2016, See Section VI), two abstracts (Lefebvre et al. 2017 and Lefebvre et al. 2020, See Section VI) and have a manuscript in preparation (Hendrix et al. *In prep*., see Section VI).

This sub-objective was achieved. We analyzed blood sera and explored other potentially more effective ways (e.g., filter paper, nasal swabs, and PCR) to better detect disease and determine the effect of disease on seal health. We expanded the understanding of a pinniped specific *Brucella pinnipedialis* relative to ice seals and found exposure to *Coxiella burnetii* in bearded and ribbon seals. In addition to detecting disease using blood we expanded our disease analyses using other methods and addressed parasites and HABs.

2b. Select tissues for contaminants testing and send to contract laboratory for analysis. Analyze contaminants data collected previously and evaluate relative to overall seal health including reproduction and survival.

Contaminants – This project allowed us to accumulate sample sizes for many contaminants large enough to determine contaminant concentrations in multiple tissues of bearded, spotted, and ribbon seals. Because of this, we will be able to explore the influence of tissue type, sex, age, and reproductive status on contaminant concentration and to make comprehensive comparisons between two periods 2003–2007 and 2011–2016 to determine recent trends.

Concentrations of elemental contaminants (mercury, cadmium, lead, arsenic, and vanadium) from 65 bearded, 39 spotted, and 18 ribbon seals have been determined in laboratories and are available to be

analyzed (Table 2). Tissues tested include liver, kidney, and muscle; arsenic was also tested in blubber. We also determined concentrations of the most toxic form of mercury, methylmercury (MeHg), in liver, kidney, and muscle (Table 2). MeHg is known to combine with selenium to form a non-toxic compound SeHg. Concentrations of mercury, methylmercury, and selenium concentrations in liver, kidney, and muscle for bearded, spotted, and ribbon seals collected during 2011–2016 are now available for a comprehensive analysis. Data are also available to compare changes in the concentrations of total mercury and selenium collected during two periods (2003–2007 and 2011–2016).

Selenium is one of 14 other elements, some of which are essential elements (e.g. iron, calcium, magnesium) that were also tested. Little is known about normal values of these elements in ice seals, therefore providing their average and range can be useful in comparing healthy to stranded or sick seals, such as during an Unusual Mortality Event.

Organochlorine contaminant (OCs) concentrations from 57 bearded, 37 spotted, and 20 ribbon seals collected during 2003–2007 and 2011–2016 have been determined in laboratories and are available to be compared by sex, age, tissue type, reproductive status, and time (Table 3). OCs are man-made compounds, some of which are no longer manufactured in the U.S. (e.g., PCBs and DDT) and are expected to be decreasing in the marine environment.

Concentrations of polybrominated diphenyl ether compounds (PBDEs) were determined in blubber samples from 14 bearded, 8 spotted, and 7 ribbon seals and perflourinated contaminants (PFCs) were determined in liver from 13 bearded seals (Table 3) during this study period to compare with concentrations from 2003 for PBDEs (Quakenbush 2007) and from 2003–2007 for PFCs (Quakenbush and Citta 2008a). These two types of contaminants are more recent man-made additions to the environment and may have been increasing when we published our papers in 2007 and 2008, a comparison with a more recent period will provide the current trend.

During this project period we were able to accumulate the data needed to analyze concentrations of essential elements, OCs, PBDEs, and PFCs to evaluate contaminants relative to overall seal health including reproduction and survival. We also used tissue concentrations of elemental contaminants and OCs from ice seals collected during 2003–2007 and compared them to results from walrus tissues collected near Gambell and Savoonga during 2012–2014 to evaluate contaminants in Alaskan pinnipeds and to look at potential dietary pathways by comparing concentrations by species. These data were presented on a

poster (Bryan et al. 2017, see Section VI) and in a report (Quakenbush et al. 2016, see Section VI).

This project funding and timeframe, however, were not adequate for completing a comprehensive analysis that would allow us to compare our contaminant results to the literature to evaluate the health status of seals harvested in Alaska relative to other Arctic regions. We are planning to pursue additional funding to complete this analysis, provide results to human health organizations (e.g., the State of Alaska Health and Human Services, Alaska Native Health Consortium, and the Arctic Council's Arctic Monitoring and Assessment Program [AMAP]) for assessment and possible development of human consumption guidelines. We will also provide results in a common-language user-friendly newsletter summary to the Ice Seal Committee, Tribal Councils, and marine mammal subsistence communities and make the data publicly available so it can be included in future analyses of contaminants.

This sub-objective has been partially achieved. Tissues to be analyzed for contaminants were selected and tested by a contract laboratory in adequate sample sizes for analysis. However, because laboratory analysis is expensive, and many samples were needed we did not have the funding to analyze the results with data collected previously to evaluate contaminant relationship with overall seal health including reproduction and survival.

2c. Analyze stomach contents for diet, measure fish otoliths to determine size of fish eaten.

Diet – Stomachs from 1,187 seals (464 bearded, 709 spotted, and 14 ribbon seals) were processed during the project period, 2016–2020. Many of the stomachs we processed were empty (including 52 bearded, 238 spotted, and 11 ribbon seals) this is probably because prey are usually digested within 24 hours of consumption. Prey items were sorted into major groups and identified to the lowest taxonomic level possible. We identify many prey items in our lab but rely on William Walker for uncommon or eroded fish otoliths and cephalopod beaks; uncommon and difficult invertebrates are identified with the assistance of NRF Taxonomic Services and UAF's Institute of Marine Science. Our previous analyses of ice seal diets have identified differences between seasons (open-water, June–October and ice-covered, November–May) and age classes (non-pups [≥1 year old] and pups). Therefore, when sample sizes allowed, we summarized seal diets by season and age class and identified changes in diet between the project period (2016–2020) and years prior (2000–2015).

Bearded seal diet was the most diverse; 44 major prey groups had a frequency of occurrence (FO) ≥ 20% (11 fish and 33 invertebrate groups). Overall, non-pup bearded seals consumed fish less frequently during 2016–2020, especially sculpins (*Gymnocanthus* sp. and *Myoxocephalus* sp.) during both seasons and flatfish (*Limanda* sp.) during the open-water season (Table 4). However, during the ice-covered season, non-pup seals consumed Pacific sand lance (*Ammodytes hexapterus*) and saffron cod (*Eleginus gracilis)* more frequently during 2016–2020. Changes in the consumption of invertebrates between periods was not consistent. Non-pups consumed annelids, polychaetas, and mollusks less frequently during 2016–2020, however, pups consumed these groups more frequently. Pups consumed amphipods, especially Gammarideans, more frequently during 2016–2020. Overall, shrimp consumption did not change much between periods, however, non-pup bearded seals consumed *Argis* sp. more frequently during both seasons during 2016–2020, as did pups during the ice-covered season. There were as also changes in the amount of crab consumed during the ice-covered season during 2016–2019; pup bearded seals consumed more *Chionoecetes* sp. whereas non-pups consumed less of *Chionoecetes* sp. and crab of the *Telmessus* genus were consumed more often by non-pup bearded seals during 2016–2019.

Spotted seals are generally considered piscivorous. Indeed, fish were consumed more frequently than invertebrates during both periods and seasons. Arctic cod (*Boreogadus saida*), an important prey species for spotted seals, were consumed less frequently during 2016–2020, especially by non-pups during the ice-covered season. Both age classes consumed invertebrates during the open-water season roughly 25% more frequently during 2016–2020 than during the earlier period (Table 5).

Ribbon seals are rarely harvested and when they are, their stomachs are often empty, therefore we had few stomachs and were not able to assess changes in ribbon seal diet. During 2016–2020, only three stomachs had contents and only one of these had fish. During the 2000s, ribbon seals consumed fish, especially gadids, more frequently than invertebrates. Of invertebrates, crustaceans were most frequently consumed (Table 6).

Otolith length can be used to determine the sizes of fish eaten by seals relative to seal sex, age, and harvest location. We measured 3,465 otoliths found in the stomachs of bearded seals, 3,024 otoliths from spotted seals, and 5 from ribbon seals during 2016–2020. We photographed each measured otolith and archived the photographs (Fig. 1).

Otolith length is also useful to determine if the size of species-specific fish consumed by seals has changed over time. A preliminary analysis of the length of Arctic and saffron cod otoliths found in ringed seal stomachs (conducted under a Species Recovery Grant) identified that average length of Arctic cod otoliths did not differ from 2011 to 2018, however, average length of saffron cod otoliths increased significantly from 5.6 mm in 2011 to 7.9 mm in 2018 (Biderman et al. 2020, see Section VI). Spotted seals also eat a lot of cod; as such, a similar analysis of cod eaten by spotted seals will be valuable to better understand seal prey size preference.

This sub-objective has been achieved. We analyzed stomach contents for diet from 464 bearded, 709 spotted, and 14 ribbon seals during this project period, and we measured a total of 6,494 otoliths for use in estimating fish size.

2d. Analyze body length and age to determine growth.

Morphometrics – We collected body length measurements of seals to assess growth for comparisons by period. Seals grow faster when they are young and grow longer when conditions are good during their first year after birth.

We examined residuals of growth (i.e., length given age) by period to determine if seals were on average longer or shorter during the project period (2016–2020) compared to prior years (2000–2015). Seals were harvested by 11 villages; pups were analyzed separately from non-pups (≥1 year of age). We used R software (function: 'glm') to calculate residuals of growth and compare growth among birth years. We used age at harvest to calculate birth year. Growth within the first year of age is essentially linear; therefore, for pups we fit a linear model to length at age in months, assuming all seals were born on 1 April (thus age would be 1 April to month of harvest). For pups, residual growth is the difference between an individual's length and the fitted regression line at a given age. Growth after the first year of age was clearly non-linear. To calculate residual growth of non-pups, we calculated the mean length at each age in years and then subtracted the mean length from the length of each seal within the same age class. We pooled seals ≥10 years of age, because seals have generally reached their asymptotic length by that age (McLaren, 1958a, 1958b; Burns, 1981; Quakenbush et al., 2011a, 2011b). We then linked the residual growth of each seal with its year of birth. Linking residual growth with birth year assumes the length of a seal is more dependent upon events that occur earlier in life rather than later in life. For example, we are assuming that a year with poor foraging conditions or a shortened nursing period will have lasting effects on individuals and will affect pups and one-year-olds more than eight- or nine-year-olds.

This is reasonable because seals attain approximately 50% of their body length within approximately the first three years of life. Finally, for each birth year, we plotted the residual growth and looked for years, or strings of years associated with seals that were long (or short), given their age at harvest. These morphometric analyses of body length were included in Crawford et al. (2015).

Pooling samples collected since 2000, we assessed residual length measurements and paired them with ages of 3,861 seals (867 bearded and 2,994 spotted). Too few ribbon seals were sampled to assess residual growth.

With few exceptions, the residual growth of bearded seals did not vary from average in most birth years (Fig. 2). There is some evidence that non-pup bearded seals were somewhat longer than average during 2001 and 2015–2017 and shorter than average in 1999, 2000, 2012 and 2018. Pups were also shorter than average in 2018.

The residual growth of non-pup spotted seals did not vary from average in most birth years (Fig. 3a). Non-pups were shorter than average in 1998, 2000, 2017, and 2018. Pups were longer than average during 2008–2010, and shorter than average during 2014 and 2015, but longer than average again in 2016 and 2017 (Fig. 3b).

This sub-objective was achieved. We used length at age data to detect birth years when conditions were good and poor. These data indicate that except for bearded seal pups born in 2018 and spotted seals pups born in 2014 and 2015, conditions during recent years have not restricted bearded or spotted seal growth.

2e. Analyze blubber thickness for body condition.

Morphometrics – We collected blubber thickness measurements to monitor body condition (see Crawford et al. 2015, Quakenbush and Citta 2008b, Quakenbush et al. 2009, Quakenbush et al. 2011a and b). We compared sternal blubber thickness for bearded seals harvested during 1973–2019, spotted seals during 1968–2019, and ribbon seals during 1967–2016. We modeled sternal blubber thickness by month accounting for age class, sex, standard length (cm) and period as determined by model selection. Random effects were harvest location and year for all models at first, but then year was removed if not needed. Twenty-one models were compared that included various combinations of month x age class interaction, period, sex, and standard length. The top model was chosen by AIC weight. If a variable was determined insignificant in the final model, then samples without that variable information were added back into the final

dataset. The final model was used to determine mean blubber thickness by month and 95% confidence intervals.

Average blubber thickness varied seasonally. The maximum blubber thickness for bearded seals occurred in April for adults (5+ yrs old) and January for subadults (1–4 yrs old, Fig. 4). No bearded seal samples were collected in February or December when blubber is expected to be the thickest. By comparison, the maximum blubber thickness for spotted seals was February for adults (4+ yrs old) and April for subadults (1–3 yrs old, Fig. 5), however, no subadult spotted seals were collected in January. Maximum blubber thickness for ribbon seals was February for adults (4+ yrs old) and April for subadults, when considering months with >1 subadult sample (1–3 yrs old, Fig. 6).

We also looked at changes in blubber thickness over time and analyzed years where more than 10 samples were available for bearded seals, 15 for spotted seals, and 3 for ribbon seals. We used residuals from the final models to look at interannual variation (i.e., which years were below or above average). If year was a random effect in the final model, it was removed, and the model was run again for this step. By looking at the residuals of each final model, we are assessing the leftover variability in sternal blubber thickness after accounting for significant factors. Modeling residuals against year shows how much data deviated from overall mean (determined by each final model) in each year. Years were only included that contained sufficient sample size, which varied by species (see above).

We found that sternal blubber thickness for all bearded seals was notably below average in 2011 around the time of the UME but returned to average by 2012 (Fig. 7). By comparison, spotted seal blubber thickness was below average in 2001 and 2018, and above average in 2000 and 2007 (Fig. 8). Few data are available for ribbon seals. Of the nine years available, 1968 and 2002 were below average and 2016 was above average (Fig. 9).

This sub-objective was achieved. We were able to use blubber thickness as an index to body condition. Body condition varied by month for both adults and subadults, and by controlling for month and age class we detected harvest years where body condition was below average and above average. During this project period, no years were below average for bearded seals, however 2017 and 2018 were below average for spotted seals. By 2019, however, spotted seals were above average body condition again.

2f. Analyze female reproductive tracts and age for pregnancy and

age of maturity.
Productivity – We received, processed, and examined female reproductive tracts for reproductive status and condition from 131 bearded, 225 spotted, and 3 ribbon seals during this project period. We compared these data to data collected in the 1960s, 1970s 1980s, 2000s, and 2010–2015 to evaluate pregnancy rate and age of maturity. We defined pregnancy rate as the proportion of mature females with a corpora lutea in the year of harvest. However, if a corpora lutea was present but no fetus was evident by Nov. 1, the seal was considered not pregnant. Age of maturity was estimated as the age at which 50% of females had ovulated at least once (DeMaster, 1978) and data were analyzed using a probit regression.

We found that pregnancy rate for bearded seals was relatively high for all periods; it was lowest in the 1960s (88%) and highest from 2010–2015 (99%) (Fig. 10). During this project period (2016–2019) bearded seal pregnancy rate was 95%. Pregnancy rate for spotted seals was more varied; it was lowest in the 2000s (76%) and highest during this project period (95%) (Fig. 10). Fewer reproductive tracts were collected for ribbon seals; however, we were able to calculate pregnancy rate for the 2000s at 92% and the 2010s at 100% (Fig. 10).

Annual pregnancy rates varied over time for bearded and spotted seals (Figs. 11 and 12). During 2007–2019 (years with a sample size of ≥7), the annual pregnancy rate for bearded seals has ranged from 88% to 100% (Fig. 11). During 2008–2019 (years with a sample size of ≥7), the annual pregnancy rate for spotted seals has ranged from 70% to 100% (Fig. 12).

The average age of maturity for bearded seals was highest in the 1960s (4.0 years old) and was lowest during 2010–2015 (2.6 years old); it was 3.0 years old during this project period (Fig. 13). The average age of maturity for spotted seals was highest in the 1970s (4.1 years old) and also was lowest during 2010–2015 (3.0 years old); it was 3.6 years old during this project period (Fig. 13). Age at maturity could not be calculated for ribbon seals because of the low sample size. As of 2019, we did not detect lower pregnancy rates or older maturation in bearded or spotted seals as was predicted with declining sea ice and record low winter sea ice extent in the Bering Sea in 2017 and 2018.

We presented four posters on the reproductive status of ice seals (Bryan et al. 2018, 2019, Adam et al. 2019, and Quakenbush et al. 2020, see Section VI).

This sub-objective was achieved. We determined pregnancy rates and ages of maturity as an index to productivity. Pregnancy rates were

higher for bearded and spotted seals since 2010 than during earlier periods. Age at maturity was lower for bearded and spotted seals in the 2000s than in the 1960s and 1970s. Younger ages of maturity suggest that conditions are good for growth and the females are able to grow quickly and begin to reproduce younger.

2g. Determine proportion of pups in the sampled harvest as an index of pup survival to weaning.
The spring seal harvest occurs after pups are weaned, therefore the proportion of pups in the sampled harvest provides a measure of pups that have survived past weaning. A comparison of these proportions can indicate poor years for pup production. By monitoring this proportion annually, we can detect strings of years that may indicate a trend.

The proportion of bearded seal pups in the sampled harvest has increased over time. It was lowest during the 1960s (18.5%) and highest during this project period (66.2%) (Fig. 14a). Annually, bearded seal pups have contributed more than 25% of the harvest of bearded seals since 2006 (Fig. 14b). In contrast, the proportion of spotted seal pups in the sampled harvest was highest during the 1960s (57.2%), lowest during the 1970s (36.0%) and stable across the remaining periods (41.8–45%) (Fig. 15a). Annually, spotted seal pups have contributed more than 25% of the harvest since 2006 (Fig. 15b).

Although the higher proportion of bearded seal pups in the harvest may be due to increased productivity and pup survival, other factors could be responsible. It is possible that recent environmental changes could have altered the timing of hunting or changed the migratory patterns of pups, increasing their availability when hunting occurs. Regardless, this pattern suggests pups are available and surviving past weaning during the recent period despite earlier ice break-up. These data were presented on five posters during this project period (Bryan et al. 2018, 2019; Adam et al. 2019, 2020; and Quakenbush et al. 2020, see Section VI).

This sub-objective was achieved. We were able to determine the proportion of pups in the sampled harvest and use it as an index of pup survival to weaning. The results suggest pups are being produced and surviving past weaning.

Objective 3: **Collect local knowledge about seals and seal hunting for interpretation of sample results.** Hunter preferences or timing of harvest can bias our sampling results. To understand the bias and accurately interpret the results of harvest sampling to the population level, we work with the hunters to understand their hunting practices. Hunter questionnaires and interviews were used to understand how

hunter behavior affects what seals are harvested and how harvested seals represent the population. We collected 98 hunter questionnaires during the project period from Point Hope, Shishmaref, Gambell, and Savoonga. In general, surveys conducted between 2013–2019 indicated there was no change in hunter preferences or the timing of the harvest. When hunters did indicate changes in timing of the harvest, they usually stated that the hunting season is shorter because the ice leaves earlier, there is more wind, and that the seals arrive earlier. Although some hunters noted changes in the seals conditions such as hair loss, sores, and thin blubber and yellow blubber for bearded seals, many people also said the seals are healthy.

B. **Modifications.** We modified the original goal of producing a contaminants manuscript. A contaminants manuscript was submitted but rejected by the journal due to inadequate sample sizes. Contaminants analyses are expensive, and many samples are needed to evaluate differences among species (sex, age, and reproductive status) and tissue type (liver, kidney, muscle, blubber). Therefore we focused on increasing sample sizes to produce a robust contaminant data set so that a comprehensive comparison of species and tissues between time periods that included data collected and analyzed during this project and previous and concurrent (ringed seal Species Recovery Grant) projects could be conducted in the future.

During our disease investigations using blood serum, we found that traditional serology methods which test for exposure to diseases were not useful in determining illness due to active disease infections. Therefore, we stopped analyzing sera and began exploring the use of PCR to better evaluate disease directly.

C. **Additional work conducted.** During this award period, we contributed samples and data to other researchers with objectives that were outside of the specific objectives of this project but advanced our understanding of ice seals and their health and status. These projects included:

1) Contributing claws to a project that used hormones in claws to compare reproduction, stress, and diet of female bearded and ringed seals in the Bering and Chukchi seas, Alaska, between 1953–1968 and 1998–2014 (Crain et al. *In press*, also Karpovich et al. 2020 for methods, see Section VI) and contributing spotted seal whiskers to examine temporal changes in cortisol concentrations and stable isotopes in sections of whiskers (Karpovich et al. 2018, see section VI).
2) Contributing bearded seal tissue for isotope analysis to determine resource partitioning between Pacific walruses and bearded seals in the Alaska Arctic and sub-arctic (Oxtoby et al. 2017, see Section VI).

3) Contributing seal blubber thickness data for an analysis of how polar bear prey condition and atmospheric circulation patterns influence polar bear body condition, recruitment, and feeding ecology in the Chukchi Sea (Rode et al. *In prep*., see Section VI).
4) Contributing seal blubber for analysis and development of a new approach to Chukchi Sea polar bear diet estimation by simultaneous modeling of protein and adipose assimilation (Stricker et al. *In prep*., see Section VI).
5) Contributing samples of *longissimus dorsi* muscle collected from 41 bearded, 12 spotted seals (and 10 ringed seals collected under a different grant) to determine the oxygen storage capacities and physiological limits to diving for ice seals. The study found that (1) spotted and ringed seal myoglobin content is similar to other phocids, whereas bearded seal myoglobin content is more similar to that of walruses, (2) myoglobin content and acid buffering capacity increased with age for all three seal species, and (3) spotted and ringed seals had a relatively even mix of fast- and slow-twitch fibers whereas bearded seals had higher proportions of fast-twitch fibers (68%). These different muscle fiber characteristics likely affect diving and foraging ability (Hermann-Sorensen et al. 2018, Tengler et al. 2018, Tengler et al. 2019, and Tengler et al. 2020, see Section VI). We provided samples and information about seal biology and behavior to Mariah Tengler and Dr. Nicole Thometz at the University of San Francisco for this study, Mariah is currently finishing a Master's thesis using these data.
6) Contributing otoliths from seal stomachs to estimate sizes of fish consumed by ice seals using otolith length – fish length relationships (Walker and Norcross 2016, and Walker 2017; see Section VI).
7) Contributing diet data for bearded seals from the Chukchi Sea for an analysis to model bearded seal movements relative to benthic communities and environmental characteristics (Cameron et al. 2017, see Section VI)
8) Contributing bearded tissue samples previously collected to Southwest Fisheries Science Center as a source of DNA for genetic studies. A poster about genetics and stock structure for ringed and bearded seals was presented at the Alaska Marine Science Symposium in 2017 (Lang et al. 2017, see Section VI)

Future work. We are pursuing funding to analyze the contaminants data and prepare several manuscripts to accomplish the original stated project goal. We anticipate several manuscripts including 1) elemental contaminants, 2) organochlorine compounds (aka OCs, POPs), 3) PBDEs, and 4) PFCs.

Conclusions. Except for producing a contaminants manuscript, all other objectives were fully achieved. During this project period of 2016–2020, we

collected morphometric data and tissue samples from 1,618 seals harvested by Alaska Natives for subsistence (539 bearded, 1,067 spotted, and 12 ribbon seals) to evaluate the health and status of each species. We found that (1) there was no increase in the prevalence of helminth parasites and no new parasite species, (2) harmful algal bloom toxins were present in all species and for bearded seals prevalence of domoic acid had increased in stomach contents to 100% between 2012 and 2019 in the Bering Sea, (3) we expanded the understanding of a pinniped specific disease, *Brucella pinnipedialis*, relative to ice seals and found exposure to *Coxiella burnetii* in bearded and ribbon seals, (4) we tested tissues for contaminants and have accumulated a dataset that will allow a comprehensive analysis of elements, organochlorines, and other contaminants to be compared over time, (5) the diet of bearded seals included 44 major prey types (11 fish, 33 invertebrates) and spotted seals consumed fewer Arctic cod, *Boreogadus saida*, during 2016–2020 than during 2000–2015, (6) we analyzed length at age data to detect birth years when conditions were good and poor; except for bearded seal pups born in 2018, recent years have not been poor for bearded or spotted seal growth, (7) using blubber thickness as an index for body condition, no years were below average for bearded seals during this project period, however 2017 and 2018 were below average for spotted seals, but by 2019 spotted seals were above average body condition again, and (8) pregnancy rates for bearded and spotted seals were higher after 2010 than during earlier periods and age at maturity was lower in the 2000s for bearded and spotted seals than in the 1960s and 1970s, indicating higher productivity in recent years. Overall, these indices to seal population health and status are positive and do not show a sustained negative response to recent decreases in sea ice or increases in the length of the open-water season. The most pressing health concern we identified is the increase of HABs, which should continue to be monitored.

VI. **Products and Publications**

Products and publications are attached in the order that they appear below. Publications that are *In press* and *In prep*. are not attached.

Publications

Crain, D., S. Karpovich, L. Quakenbush, and L. Polasek. *In press*. Using claws to compare reproduction, stress, and diet of female bearded and ringed seals in the Bering and Chukchi seas, Alaska, between 1953–1968 and 1998–2014. Conservation Physiology.

Foster, G., I.H. Nymo, K.M. Kovacs, K.B. Beckmen, A.C. Brownlow, J.L. Baily, M.P. Dagleish, J. Muchowski, L.L. Perrett, M. Tryland, C. Lydersen, J. Godfroid, B. McGovern, and A.M. Whatmore. 2018. First isolation of *Brucella pinnipedialis* and detection of *Brucella* antibodies from bearded seals *Erignathus barbatus*. Diseases of Aquatic Organisms 128:13–20.

Hendrix, A.M., K.A. Lefebvre, L. Quakenbush, A. Bryan, R. Stimmelmayr, G. Sheffield, G. Wisswaesser, M.L. Willis, E.K. Bowers, P. Kendrick, E. Frame, T. Burbacher, and D. J. Marcinek. *In prep*. Ice seals as sentinels for algal toxin presence in Arctic food webs. Harmful Algae.

Karpovich, S.A., L.A. Horstmann, and L.K. Polasek LK. 2020. Validation of a novel method to create temporal records of hormone concentrations from the claws of ringed and bearded seals. Conservation Physiology doi:10.1093/conphys/coaa073.

Karpovich, S.A., J.P. Skinner, L.A. Kapronczai, J.A. Smith, and D.M. Janz. 2018. Examination of relationships between stable isotopes and cortisol concentrations along the length of phocid whiskers. Marine Mammal Science doi:10.1111/mms.12546

Lefebvre, K., L. Quakenbush, K. Burek Huntington, G. Sheffield, R. Stimmelmayr, A. Bryan, et al. 2016. Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment. Harmful Algae 55:13–24. Doi:10.1016/j.hal/2016.01.007

Nymo, I.H., R. Rødven, K. Beckmen, A.K. Larsen, M. Tryland, L. Quakenbush, and J. Godfroid. 2018. Brucella antibodies in Alaskan true seals and eared seals-two different stories. Frontiers in Veterinary Science. 5(8):1–11. doi: 10.3389/fvets.2018.00008

Oxtoby, L.E., L. Horstmann, S.M. Budge., D.M. O'Brien, S.W. Wang, T. Schollmeier, and M. J. Wooller. 2017. Resource partitioning between Pacific walruses and bearded seals in the Alaska Arctic and sub-arctic. Oecologia. 184 (2): 385–398. DOI 10.1007/s00442-017-3883-7

Rode, K.D., E.V. Regehr, J.F. Bromaghin, R.R. Wilson, M. St. Martin, J.A. Crawford, and L. Quakenbush. *In prep.* Prey condition and atmospheric circulation patterns influence polar bear body condition, recruitment, and feeding ecology in the Chukchi Sea.

Stricker, C.A., K.D. Rode, B.D. Taras, J.F. Bromaghin, L. Horstmann, E.V. Regehr, M.St. Martin, L. Quakenbush, and R.R. Wilson. *In prep*. A novel approach to predator diet estimation through simultaneous modeling of protein and adipose assimilation applied to contemporary Chukchi Sea polar bears.

VanWormer, E., J.A.K. Mazet, A. Hall, V.A. Gill, P.L. Boveng, J.M. London, T. Gelatt, B.S. Fadely, M. E. Lander, J. Sterling, V. N. Burkanov, R. R. Ream, P. M. Brock, L. D. Rea, B. R. Smith, A. Jeffers, M. Henstock, M. J. Rehberg, K. A. Burek-Huntington, S.L. Cosby, J.A. Hammond, T. Goldstein. 2019. Viral emergence in marine mammals in the North Pacific may be linked to Arctic sea ice reduction. Scientific Reports. 9:15569. doi.org/10.1038/s41598-019-51699-4

Walden, H.S., A.L. Bryan, A. McIntosh, P. Tuomi, A. Hoover-Miller, R. Stimmelmayr, and L. Quakenbush. *In press.* Helminth fauna of ice seals in the Alaskan Bering and Chukchi Seas, 2006–15. Journal of Wildlife Diseases

Products: Abstracts, Posters, Reports

Adam, R., A. Bryan, L. Quakenbush, J. Crawford, and L. Biderman. 2019. Bearded seal productivity in Alaska using harvest-based monitoring, 1960s, 1970s, 2000s, and 2010s. Alaska Marine Science Symposium, 28 January–1 February 2019, Anchorage AK. (Poster)

Adam, R., A. Bryan, L. Quakenbush, J. Crawford, and L. Biderman. 2020. Age structure of subsistence harvested ice seals in Alaska 2000–2018. Alaska Marine Science Symposium, 27–30 January, Anchorage AK. (Poster)

Ballard, E.J., L.M. Barrett, J.L. Dearolf, N.M. Thometz, A. Bryan, C. Reichmuth. 2019. Hybrid fibers in the bearded seal (*Erignathus barbatus*) longissimus dorsi muscle. Society for Integrative and Comparative Biology Conference, 3–7 January 2019, Tampa Bay, FL. (Poster)

Biderman, L., A. Bryan, J. Crawford, J. Citta, and L. Quakenbush. 2020. Occurrence of Arctic and saffron cod in the diet of ringed seals at Shishmaref, 1975–2018. Alaska Marine Science Symposium, 27–30 January, Anchorage AK. (Poster)

Bryan, A.L., L. Quakenbush, J.A. Snyder, H. Kiyuklook, S. Anningayou, and M.A. Nelson. 2017. Final results from hunter-assisted sampling of walruses near Saint Lawrence Island, Alaska, 2012–2014 and 2016. Alaska Marine Science Symposium, 23–27 January, Anchorage, AK (Abstract).

Bryan, A.L., L. Quakenbush, J. Crawford, L. Biderman, and R. Adam. 2018. Spotted seal productivity in Alaska using harvest-based monitoring, 1960s, 1970s, and 2000s. Alaska Marine Science Symposium, 22–26 January 2018, Anchorage AK. (Poster)

Bryan, A., L. Quakenbush, J. Crawford, L. Biderman, and R. Adam. 2019. Ringed, bearded, and spotted seal productivity in Alaska using harvest-based monitoring, 1960s–1980s and 2000–2018. World Marine Mammal Conference. December 9–12, Barcelona, Spain. (Poster)

Bryan, A.L., H.S. Walden, A. McIntosh, P. Tuomi, A. Hoover-Miller, R. Stimmelmayr, and L.T. Quakenbush. 2020. Helminth fauna of ice seals in the Alaskan Bering and Chukchi Seas, 2006–2015. Alaska Marine Science Symposium, 27–30 January, Anchorage AK. (Poster)

Castellini, J.M., T.M. O'Hara, F. Gulland, R.S. Wells, L.D. Rea, L. Quakenbush, and J. Berner. 2017. Use of Nobuto^{TM} filter papers for concurrent analysis of contaminants, nutrients, carbon (C) and Nitrogen (N) stable isotopes, and

serology. 22nd Biennial Society for Marine Mammalogy Conference, 22–27 October 2017, Halifax, Nova Scotia, CA. (Poster)

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Hermann-Sorensen, H., B. Ruscher-Hill, M. Tengler, A. Bryan, C. Reichmuth, N. Thometz. 2018. Aerobic and anaerobic properties of bearded seal locomotor muscle. Society for Integrative and Comparative Biology Conference, 3–7 January 2018, San Francisco, CA. (Poster)

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Tengler, M., A. Bryan, C. Reichmuth, and N.M. Thometz. 2019. Physiology of locomotor muscle in ringed, bearded, and spotted seals. Society for Integrative and Comparative Biology Conference, 3–7 January 2020, Austin, TX. (Abstract)

Tengler, M., A. Bryan, C. Reichmuth, J.L. Dearolf, N.M. Thometz. 2020. Muscle physiology of ice-associated Alaskan seals. Alaska Marine Science Symposium, 27–30 January, Anchorage AK. (Poster)

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Key Words - Bearded seal, spotted seal, ribbon seal, ice seals, Bering Sea, Chukchi Sea, disease, parasites, harmful algal toxins, contaminants, productivity, body condition, health indices

Table 1. Seal samples received by village and species, 2016–2020.

	Barrow	Wainwright	Point Lay	Point Hope	Shishmaref	Nome	St. Michael	Gambell	Hooper Bay	Total
Bearded	45	0	0	182	43	0	1	266	2	**539**
Spotted	2	1	1	5	637	1	1	417	2	**1,067**
Ribbon	0	0	0	0	5	1	0	6	0	**12**
Totals	**47**	**1**	**1**	**187**	**685**	**2**	**2**	**689**	**4**	**1,618**

Table 2. Sample sizes of trace metals for bearded, spotted, and ribbon seals by analysis, tissue, and period.

	Bearded seal		Spotted seal		Ribbon seal	
Analysis-tissue	2003-07	2011-16	2003-07	2011-16	2003-07	2011-16
Trace metals-liver	41	24	23	16	9	9
Trace metals-kidney	16	14	6	9	1	6
Trace metals-muscle	4	14	0	9	0	6
As-blubber	0	24	0	9	0	6
MeHg-liver	0	14	0	9	0	6
MeHg-kidney	0	14	0	9	0	6
MeHg-muscle	0	14	0	9	0	6

Table 3. Sample sizes of organochlorine contaminants (OCs), PBDE's, and PFC for bearded, spotted, and ribbon seals by contaminant, tissue, species, and period.

	Bearded seal		Spotted seal		Ribbon seal	
	2003-07	2011-16	2003-07	2011-16	2003-07	2011-16
OCs-blubber	33	24	20	17	9	11
PBDEs-blubber	5	14	3	8	6	7
PFCs-liver	17	13	9	0	8	0

Table 4. Frequency of occurrence (%FO) of common prey items identified in the stomach contents of bearded seals harvested for subsistence in the Bering and Chukchi seas, 2000–2020. Common prey items were identified in ≥ 20% of seal stomachs. The open-water period included samples collected from June to October and the ice-covered period from November to May. Seals of age 0 were classified as pups, all seals ≥ 1 year old were classified as non-pups. Decreases of 10% in %FO for each prey item from 2000–2015 to 2016–2020 are highlighted with light-red, 25% are dark red; increases of % FO of 10% are highlighted light-blue, 25% are dark blue.

Season	Open-water				Ice-covered			
Age class	Non-pup		Pup		Non-pup		Pup	
Period	2000–2015	2016–2020	2000–2015	2016–2020	2000–2015	2016–2020	2000–2015	2016–2020
n	315	41	199	34	83	54	72	36
Fish	87.6%	75.6%	82.4%	82.4%	84.3%	83.3%	72.2%	86.1%
Ammodytes hexapterus	15.9%	9.8%	5.5%	2.9%	16.9%	42.6%	5.6%	27.8%
Gadidae	57.1%	41.5%	38.2%	17.6%	47.0%	42.6%	31.9%	44.4%
Boreogadus saida	44.1%	24.4%	15.6%	2.9%	42.2%	31.5%	25.0%	16.7%
Eleginus gracilis	30.8%	22.0%	25.6%	8.8%	7.2%	24.1%	12.5%	19.4%
Cottidae	68.6%	46.3%	61.8%	67.6%	69.9%	53.7%	51.4%	58.3%
Gymnocanthus sp	45.1%	7.3%	19.6%	11.8%	42.2%	16.7%	23.6%	33.3%
Myoxocephalus sp	47.0%	22.0%	45.7%	47.1%	49.4%	18.5%	31.9%	36.1%
Stichaeidae	29.8%	9.8%	13.6%	2.9%	22.9%	13.0%	20.8%	11.1%
Pleuronectidae	48.3%	24.4%	59.3%	76.5%	36.1%	42.6%	29.2%	52.8%
Limanda sp	35.6%	2.4%	46.7%	38.2%	9.6%	0.0%	25.0%	16.7%
Limanda aspera	15.2%	2.4%	36.7%	38.2%	6.0%	0.0%	16.7%	16.7%
Invertebrates	95.6%	92.7%	95.5%	100.0%	97.6%	90.7%	98.6%	100.0%
Sponges	11.7%	26.8%	3.0%	11.8%	27.7%	18.5%	6.9%	5.6%
Annelids	35.6%	22.0%	26.1%	38.2%	38.6%	25.9%	36.1%	50.0%
Polychaeta	35.6%	19.5%	26.1%	38.2%	38.6%	25.9%	34.7%	50.0%
Polynoidae	29.5%	7.3%	14.6%	11.8%	14.5%	13.0%	16.7%	25.0%
Echiuridae	55.2%	46.3%	28.6%	5.9%	37.3%	20.4%	26.4%	30.6%
Mollusca	64.4%	51.2%	52.3%	64.7%	65.1%	51.9%	61.1%	69.4%
Gastropoda	25.4%	22.0%	15.6%	17.6%	30.1%	24.1%	26.4%	33.3%
Bivalvia	50.2%	41.5%	40.2%	55.9%	47.0%	38.9%	34.7%	41.7%
Cephalopoda	19.0%	0.0%	5.0%	0.0%	13.3%	1.9%	12.5%	0.0%
Crustaceans	89.5%	85.4%	93.0%	91.2%	89.2%	83.3%	93.1%	97.2%
Isopoda	8.6%	7.3%	14.6%	11.8%	9.6%	3.7%	12.5%	8.3%
Amphipoda	28.3%	26.8%	19.6%	47.1%	26.5%	25.9%	31.9%	52.8%
Gammaridea	27.0%	22.0%	16.1%	41.2%	20.5%	22.2%	31.9%	47.2%
Uristidae	12.4%	4.9%	6.5%	14.7%	7.2%	1.9%	19.4%	27.8%
Anonyx sp	11.4%	4.9%	5.5%	14.7%	6.0%	1.9%	12.5%	27.8%
Decapod	87.0%	85.4%	89.4%	79.4%	85.5%	81.5%	91.7%	97.2%

Shrimp	83.8%	75.6%	83.4%	70.6%	69.9%	72.2%	79.2%	88.9%
Hippolytidae	20.0%	17.1%	11.1%	5.9%	21.7%	14.8%	25.0%	41.7%
Pandalidae	11.1%	7.3%	7.5%	2.9%	21.7%	18.5%	13.9%	16.7%
Crangonidae	79.0%	73.2%	73.9%	61.8%	48.2%	57.4%	61.1%	80.6%
Crangon sp	43.5%	43.9%	53.8%	44.1%	19.3%	22.2%	30.6%	30.6%
Crangon alaskensis	30.8%	34.1%	42.2%	41.2%	4.8%	16.7%	20.8%	27.8%
Sclerocrangon boreas	37.8%	31.7%	14.6%	8.8%	13.3%	14.8%	11.1%	25.0%
Argis sp	54.0%	68.3%	20.1%	17.6%	28.9%	50.0%	26.4%	55.6%
Argis lar	50.8%	68.3%	16.1%	11.8%	25.3%	44.4%	20.8%	36.1%
Crab	67.9%	58.5%	35.7%	29.4%	74.7%	72.2%	65.3%	83.3%
Anomura	14.9%	24.4%	5.0%	2.9%	3.6%	11.1%	9.7%	22.2%
Brachyura	63.2%	51.2%	24.1%	23.5%	68.7%	72.2%	48.6%	63.9%
Telmessus sp	30.5%	31.7%	8.5%	11.8%	3.6%	33.3%	6.9%	11.1%
Telmessus cheiragonus	29.8%	31.7%	7.0%	11.8%	1.2%	31.5%	6.9%	11.1%
Oregoniidae	42.2%	34.1%	15.1%	11.8%	63.9%	48.1%	37.5%	55.6%
Hyas sp	22.2%	14.6%	8.5%	2.9%	33.7%	24.1%	25.0%	30.6%
Chionoecetes sp	25.4%	19.5%	7.0%	2.9%	50.6%	37.0%	18.1%	38.9%

Table 5. Frequency of occurrence (%FO) of common prey items identified in the stomach contents of spotted seals harvested for subsistence in the Bering and Chukchi seas, 2000–2020. Common prey items were identified in ≥ 20% of seal stomachs. The open-water period included samples collected from June to October and the ice-covered period from November to May. Seals of age 0 were classified as pups, all seals ≥ 1 year old were classified as non-pups. Decreases of 10% in %FO for each prey item from 2000–2015 to 2016–2020 are highlighted with light-red, 25% are dark red; increases of % FO of 10% are highlighted light-blue, 25% are dark blue.

Season	Open-water				Ice-covered			
Age class	Non-pup		Pup		Non-pup		Pup	
Period	2000–2015	2016–2020	2000–2015	2016–2020	2000–2015	2016–2020	2000–2015	2016–2020
n	448	102	274	57	65	44	46	19
Fish	96.4%	95.1%	96.0%	91.2%	100.0%	95.5%	87.0%	89.5%
Clupea pallasi	58.9%	36.3%	37.2%	14.0%	12.3%	27.3%	10.9%	0.0%
Osmerus mordax	23.2%	27.5%	26.3%	10.5%	29.2%	27.3%	19.6%	10.5%
Mallotus villosus	6.3%	17.6%	9.1%	17.5%	16.9%	18.2%	17.4%	57.9%
Gadidae	40.6%	52.9%	46.0%	49.1%	76.9%	70.5%	60.9%	52.6%
Boreogadus saida	17.2%	5.9%	17.5%	14.0%	55.4%	18.2%	39.1%	15.8%
Eleginus gracilis	29.9%	38.2%	36.5%	24.6%	50.8%	54.5%	39.1%	31.6%
Invertebrates	27.9%	53.9%	26.3%	49.1%	41.5%	50.0%	41.3%	52.6%
Crustaceans	24.6%	41.2%	21.9%	38.6%	36.9%	40.9%	39.1%	47.4%
Amphipoda	10.9%	15.7%	10.6%	15.8%	12.3%	22.7%	15.2%	36.8%
Decapod	19.2%	24.5%	15.0%	26.3%	32.3%	22.7%	26.1%	10.5%
Shrimp	17.6%	23.5%	14.6%	26.3%	30.8%	22.7%	26.1%	10.5%
Crangonidae	10.3%	15.7%	7.7%	15.8%	13.8%	20.5%	8.7%	5.3%

Table 6. Frequency of occurrence (%FO) of common prey items identified in the stomach contents of ribbon seals harvested for subsistence in the Bering and Chukchi seas, 2000–2020. Common prey items were identified in ≥ 20% of seal stomachs. Few ribbon seals are harvested for subsistence. Therefore, sample sizes were too low to make comparisons between seasons or age classes of ribbon seals.

Period	2000–2015	2016–2020
n	22	3
Fish	81.8%	33.3%
Gadidae	63.6%	0.0%
Boreogadus saida	54.5%	0.0%
Eleginus gracilis	31.8%	0.0%
Invertebrates	54.5%	66.7%
Mollusca	9.1%	33.3%
Crustaceans	50.0%	33.3%
Amphipoda	13.6%	33.3%
Decapod	31.8%	0.0%
Shrimp	31.8%	0.0%

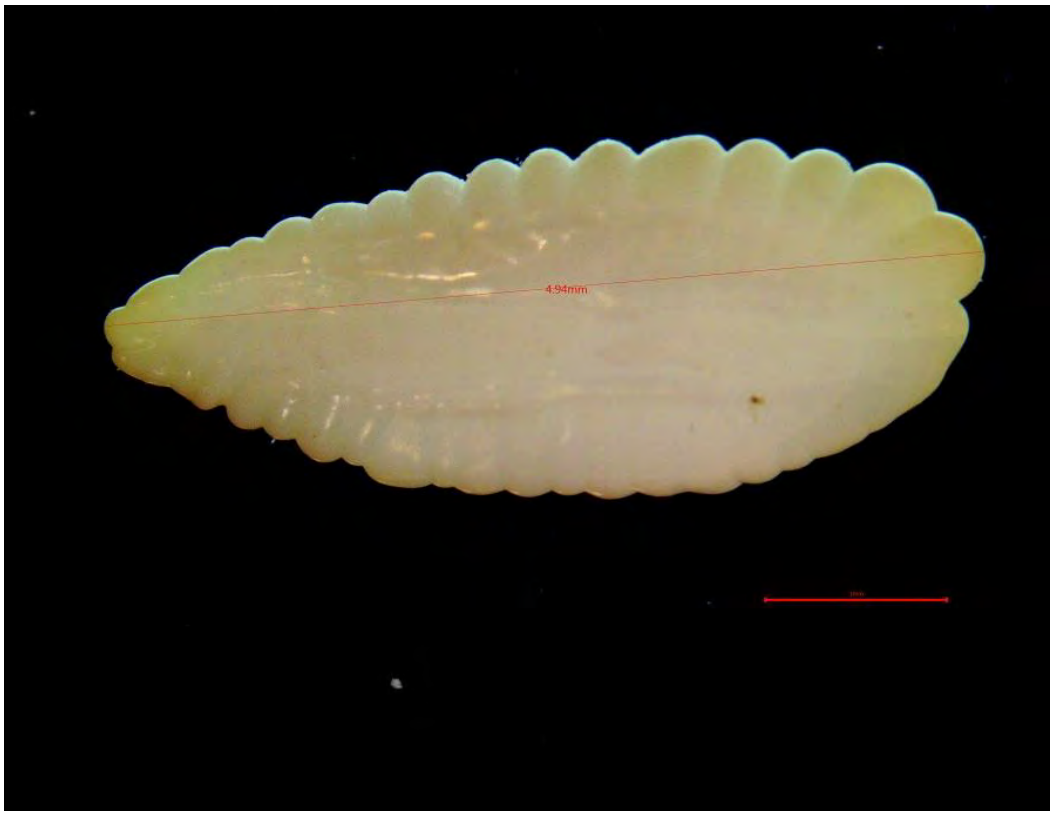

Figure 1. Saffron cod otolith from a bearded seal stomach. Otolith length is measured electronically from rostrum to postrostrum in mm using MU1000 AmScope digital camera and software attached to a Leica M125 stereo microscope. This image is 20X magnification. The red line below the otolith represents 1 mm. These photos are archived at ADF&G.

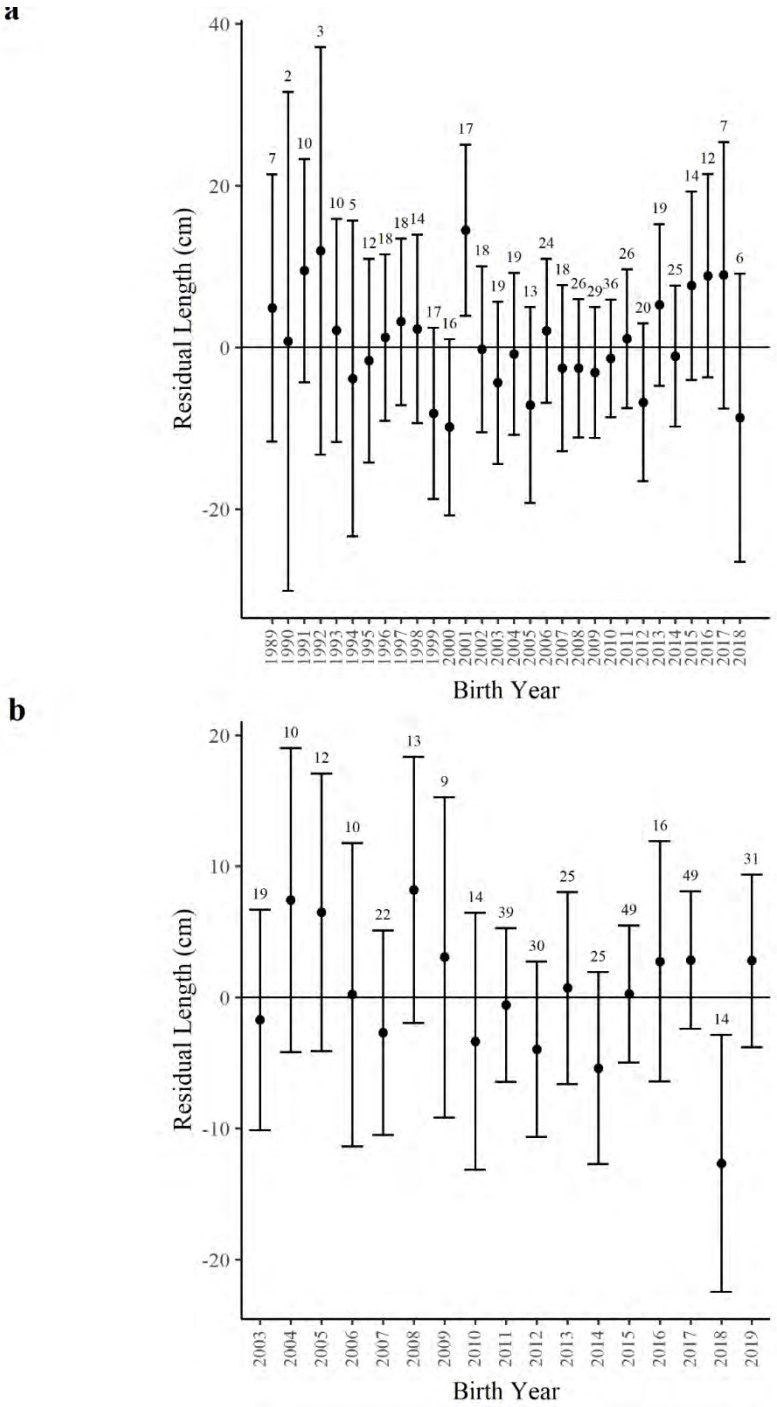

Figure 2. The mean residual length of (a) non-pup (≥1 year of age; n = 480) and (b) pup (n = 387) bearded seals, plotted by birth year. Seals were harvested for subsistence in Alaska (1998–2019). Residual lengths for each seal were calculated from difference between the measured standard length for each seal and the mean length for seals of the same age. The number of seals analyzed for each birth year is listed above the 95% confidence intervals. The first year plotted is the first birth year with ≥7 seals analyzed. Negative residuals indicate that seals were shorter than average, for their age and positive residuals indicate seals were longer than average for their age.

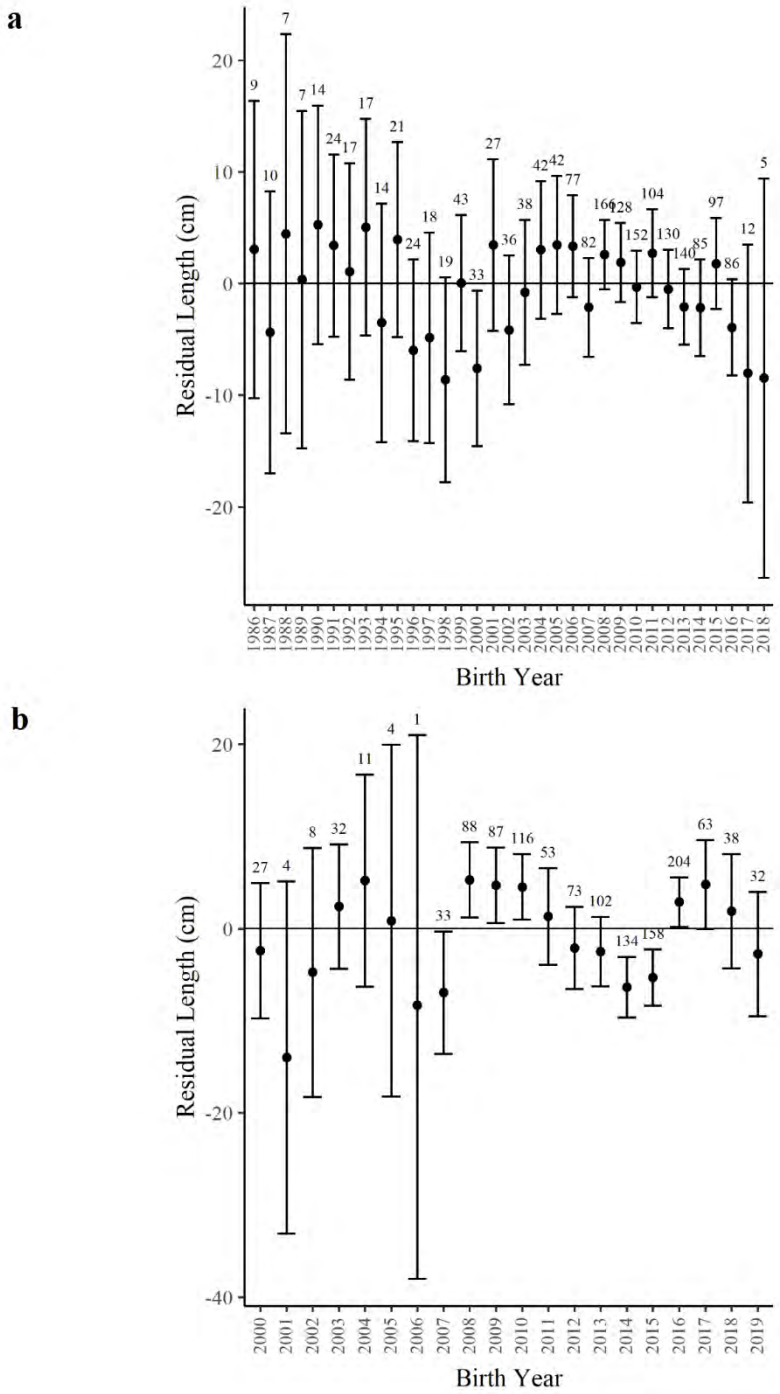

Figure 3. The mean residual length of (a) non-pup (≥1 year of age; n = 1,726) and (b) pup (n = 1,268) spotted seals, plotted by birth year. Seals were harvested for subsistence in Alaska (1998–2019). Residual lengths for each seal were calculated from difference between the measured standard length for each seal and the mean length for seals of the same age. The number of seals analyzed for each birth year is listed above the 95% confidence intervals. The first year plotted is the first birth year with ≥7 seals analyzed. Negative residuals indicate that seals were shorter than average, for their age. Positive residuals indicate that seals were longer than average for their age.

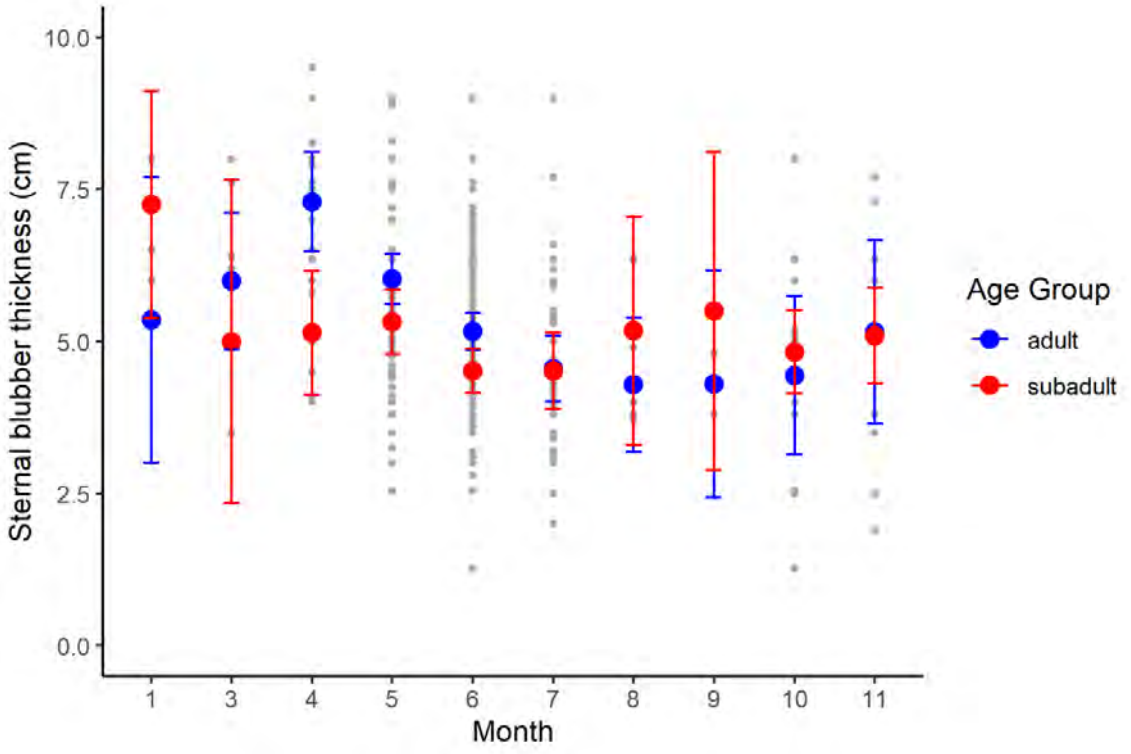

Figure 4. Mean monthly sternal blubber thickness for subadult (red) and adult (blue) bearded seals, 1973–2019. Subadults were classified as 1–4 years old and adults were 5 years of age and older. The error bars represent the 95% confidence interval. There were no data for February or December. The top model for monthly sternal blubber thickness only included the month by age group interaction ($F = 4.03$, d.f. = 10, 396.07, $p < 0.001$), which meant a dataset with 482 observations was used. Year was a random effect and all diagnostic plots showed it was a good fit.

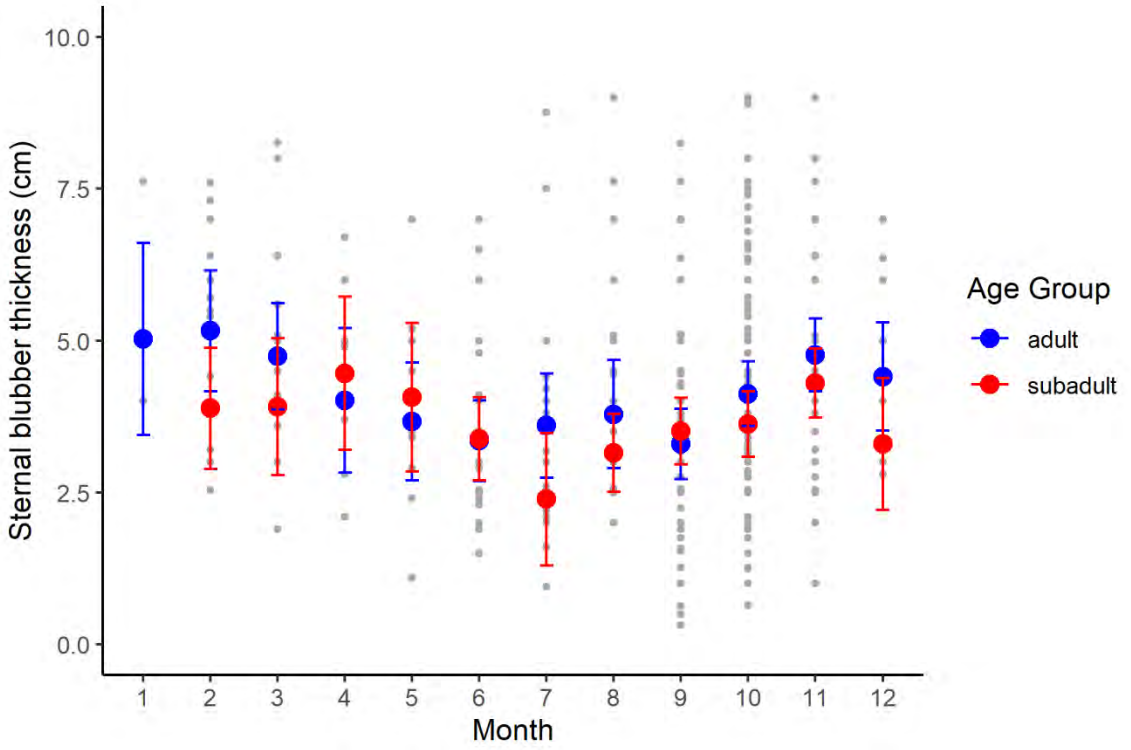

Figure 5. Mean sternal blubber thickness by month for subadult (red) and adult (blue) spotted seals, 1968–2019. Subadults were classified as 1–3 years old and adults were 4 years of age and older. The error bars represent the 95% confidence interval. The top model for monthly sternal blubber thickness included all variables: month by age group interaction ($F = 3.43$, d.f. = 11, 1058.44, $p < 0.001$), sex ($F = 13.54$, d.f. = 1, 1135.48, $p < 0.001$), period ($F = 6.19$, d.f. = 1, 147.45, $p = 0.01$) and standard length ($F = 54.45$, d.f. = 1, 1129.19, $p < 0.001$), which meant a dataset with 1167 observations was used. Year was a not a random effect and all diagnostic plots showed it was a good fit.

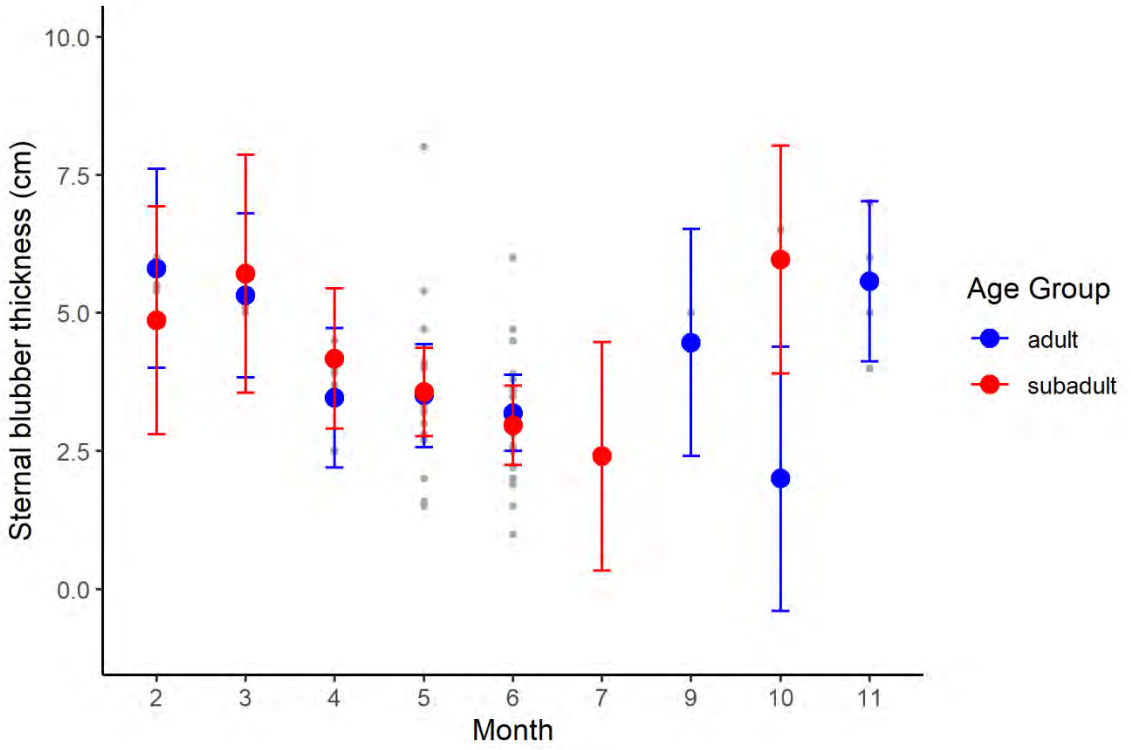

Figure 6. Mean sternal blubber thickness by month for subadult (red) and adult (blue) ribbon seals, 1967–2016. Subadults were classified as 1–3 years old and adults were 4 years of age and older. The error bars represent the 95% confidence interval. There were no data for January or December. The top model for monthly sternal blubber thickness only included the month by age group interaction ($F = 2.19$, d.f. = 6, 65.85, $p = 0.05$), which meant a dataset with 96 observations was used. Year was not a random effect and all diagnostic plots showed the model was a good fit.

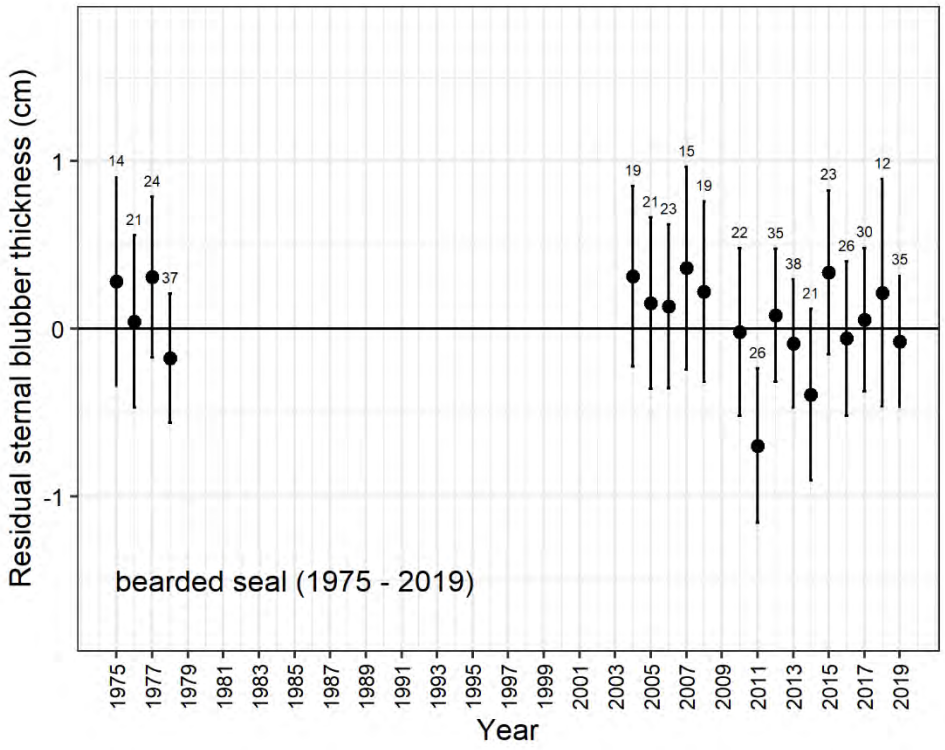

Figure 7. Mean residual sternal blubber thickness for bearded seals by year of harvest after accounting for month and age class. The number of seals analyzed each year is listed above the 95% confidence intervals. Only years with greater than 10 samples were included in the analysis.

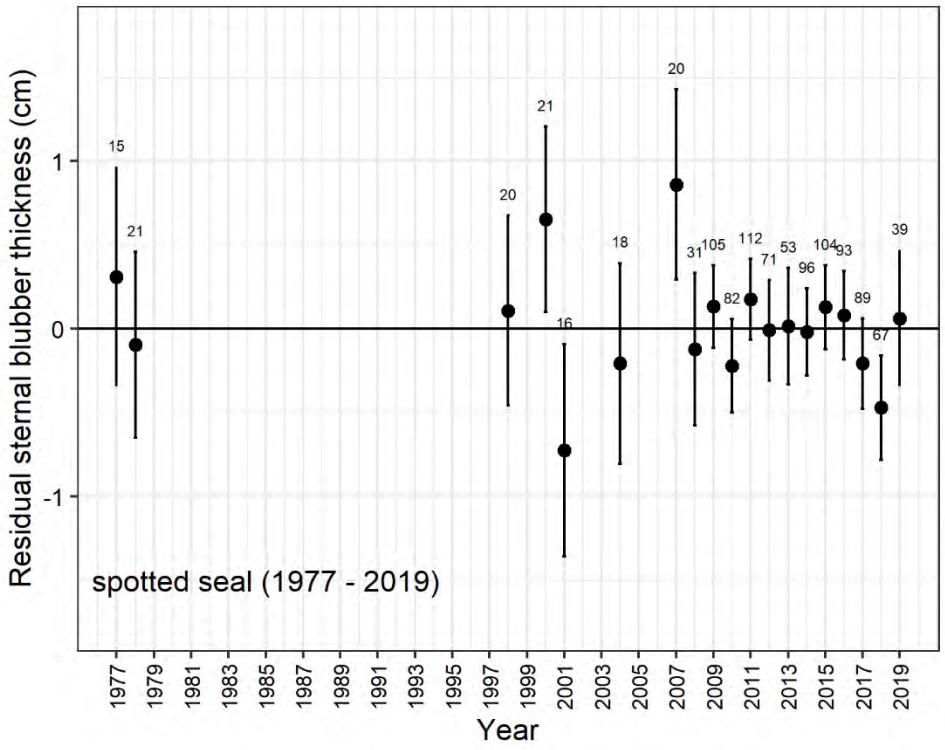

Figure 8. Mean residual sternal blubber thickness for spotted seals by year of harvest after accounting for month, age class, sex, and standard length. The number of seals analyzed each year is listed above the 95% confidence intervals. Only years with greater than 15 samples were included in the analysis.

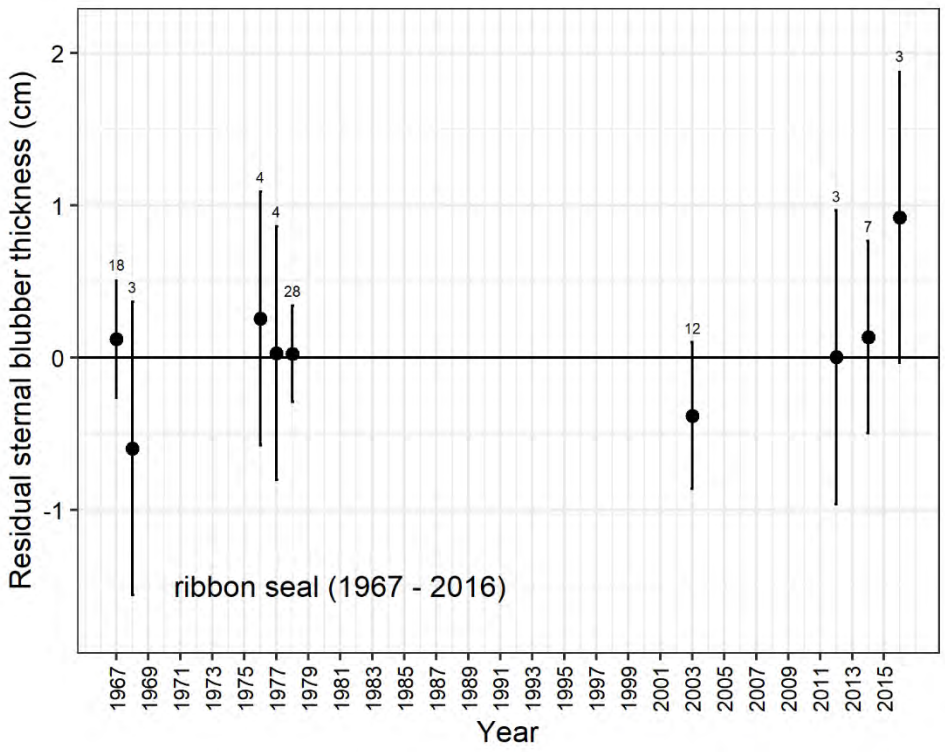

Figure 9. Mean residual sternal blubber thickness for ribbon seals by year of harvest after accounting for month, age class, sex, and standard length. The number of seals analyzed each year is listed above the 95% confidence intervals. Only years with greater than 3 samples were included in the analysis.

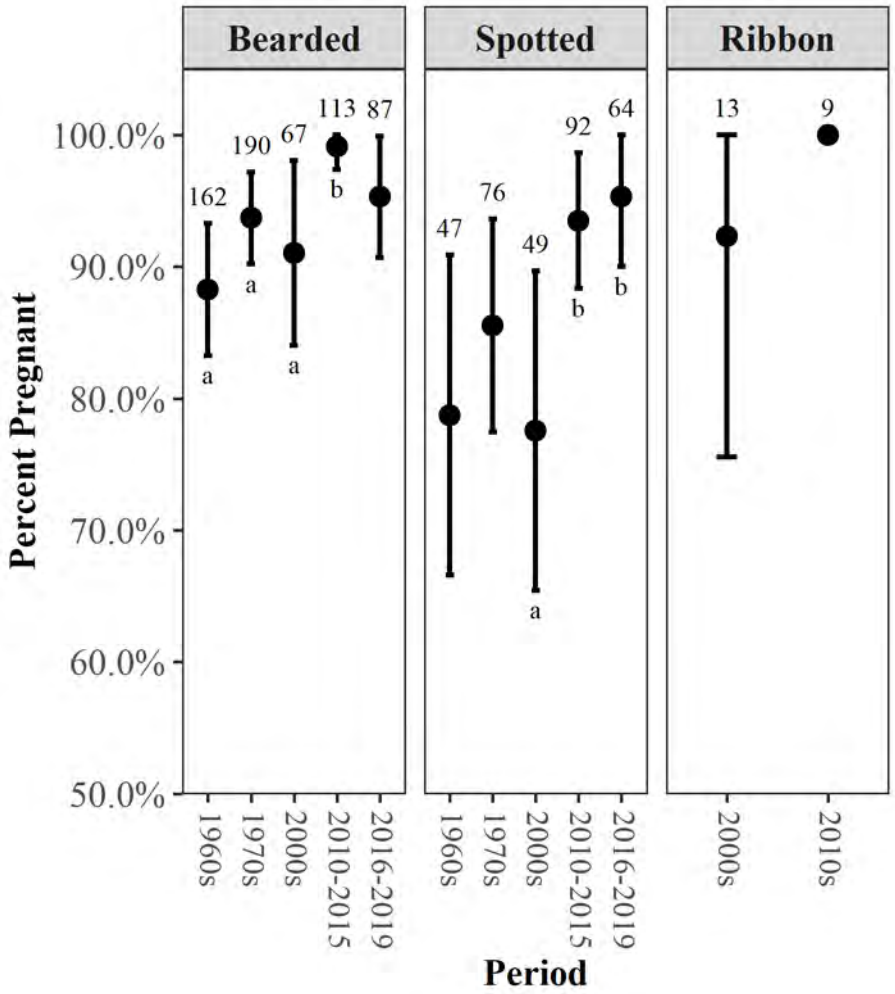

Figure 10. Pregnancy rates for mature bearded, spotted, and ribbon seals summarized by period. Seals were harvested for subsistence at 11 villages in Alaska along the Bering, Chukchi, and Beaufort sea coasts (1963–2019). The number of seals analyzed each period is listed above the 95% confidence intervals, different letters listed below indicate significant differences ($p < 0.05$). No letter indicates the period is not significantly different from any others.

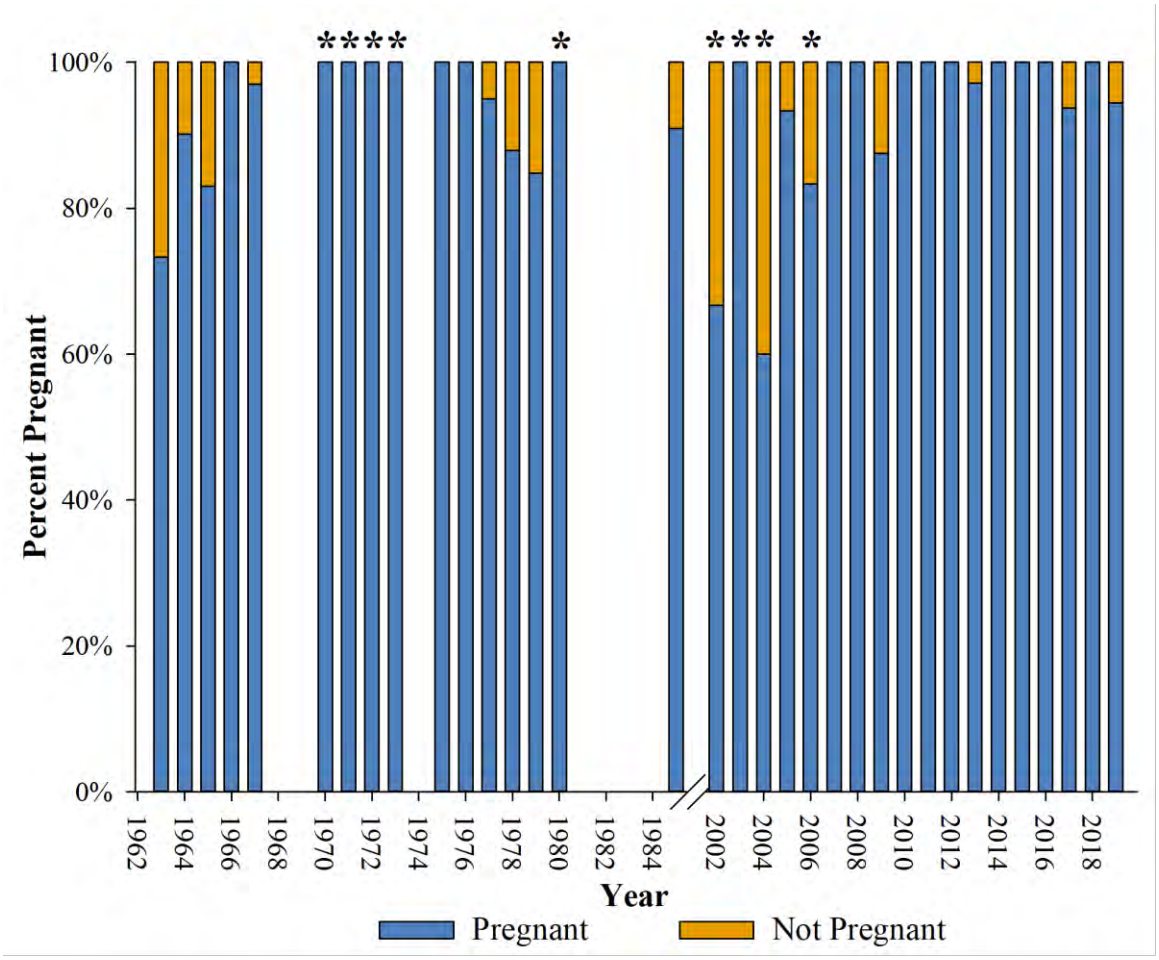

Figure 11. Annual pregnancy rate for mature bearded seals harvested for subsistence at 11 villages along the Bering, Chukchi, and Beaufort sea coasts of Alaska (1963–2019). Asterisks ("*") designate years with samples sizes < 7 seals.

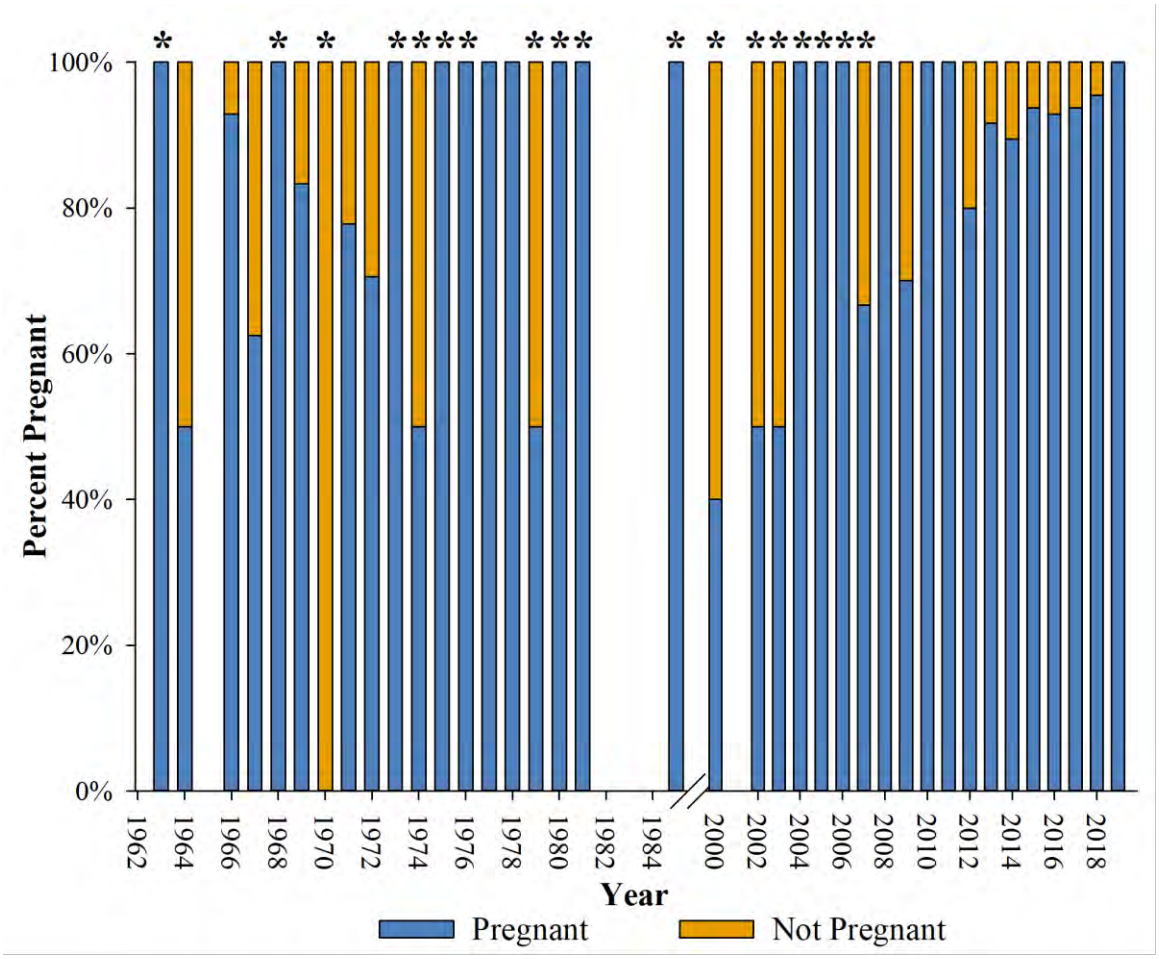

Figure 12. Annual pregnancy rate for mature spotted seals harvested for subsistence at 11 villages along the Bering, Chukchi, and Beaufort sea coasts of Alaska (1963–2019). Asterisks ("*") designate years with samples sizes < 7 seals.

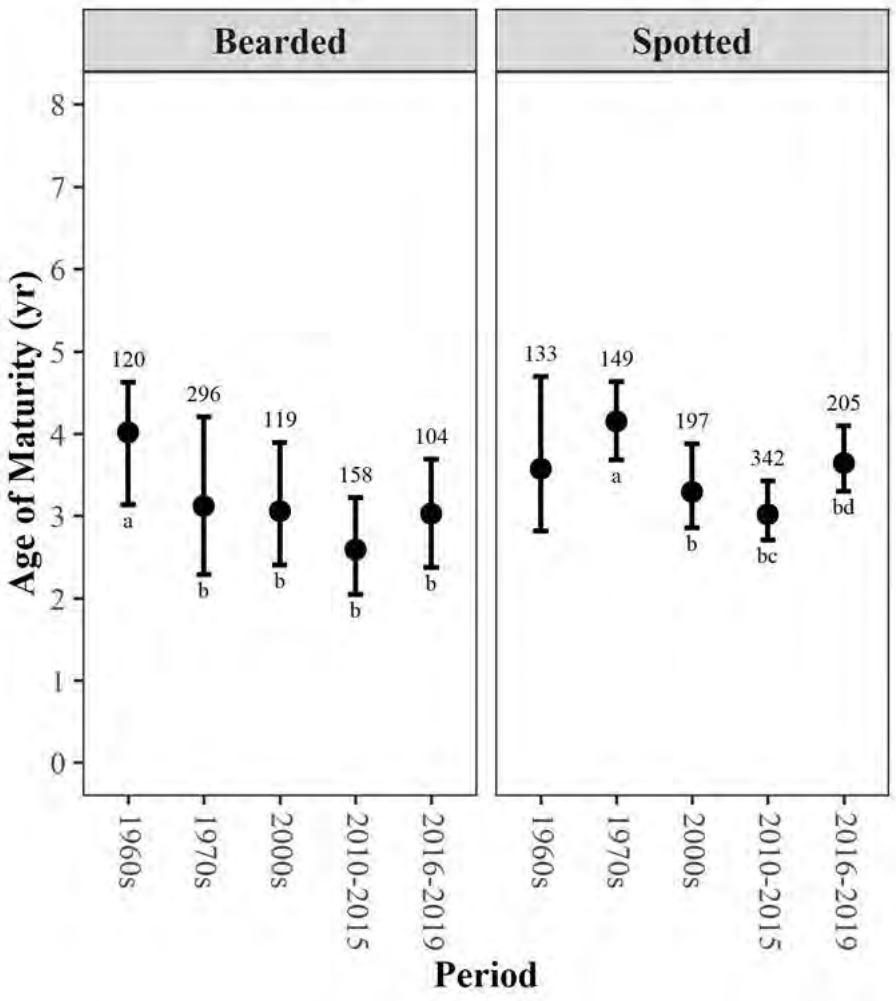

Figure 13. Mean age of maturity for bearded and spotted seals summarized by period. Seals were harvested for subsistence at 11 villages along the Bering, Chukchi, and Beaufort sea coasts of Alaska (1963–2019). The number of seals analyzed each period is listed above the 95% confidence intervals, different letters listed below indicate significant differences ($p < 0.05$). No letter indicates the period is not significantly different from any others.

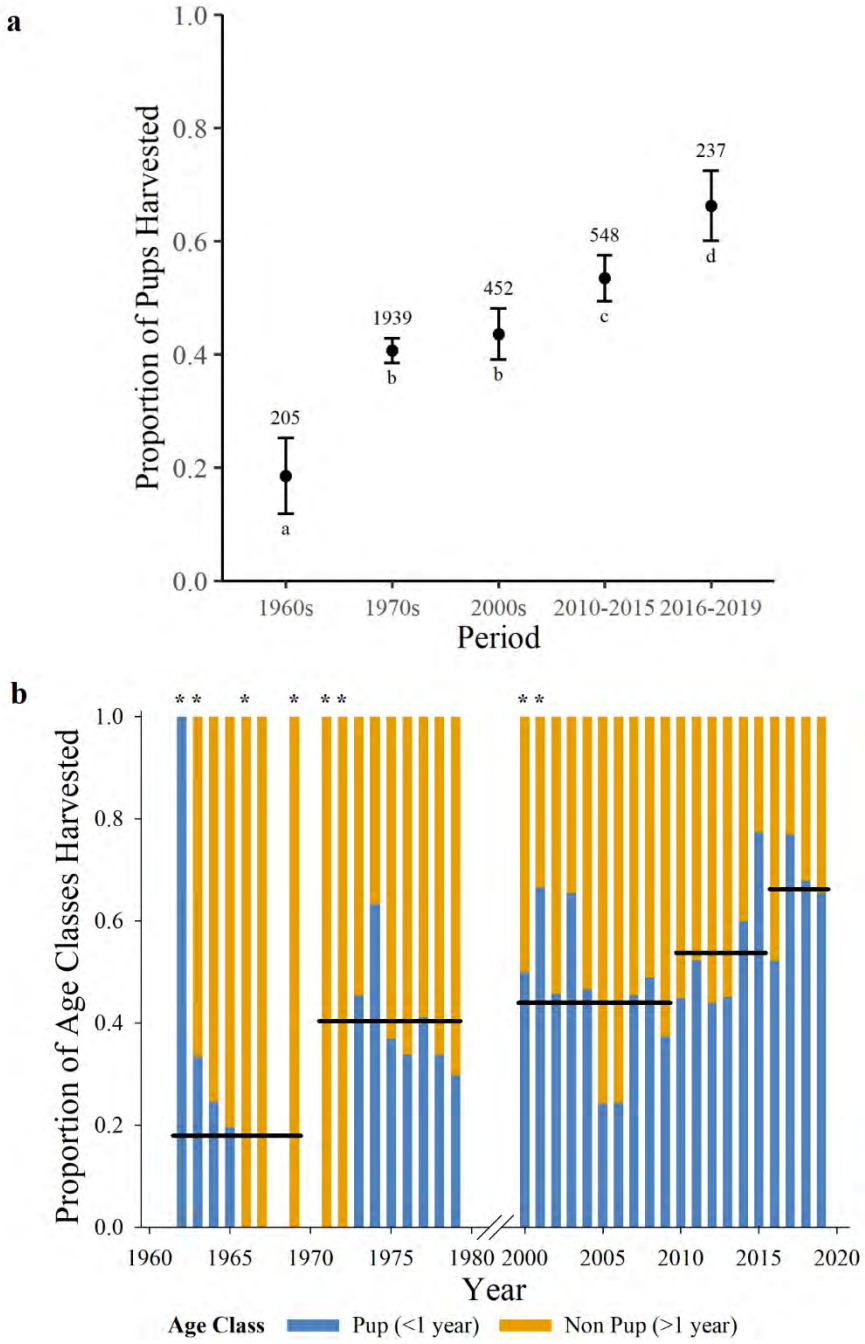

Figure 14. (a) The mean proportion of bearded seal pups harvested for subsistence at 11 villages along the Bering, Chukchi, and Beaufort sea coasts of Alaska, prior to (1960s, 1970s, 2000s, and 2010–2015) and during the project period (2016–2019). The number of seals analyzed each period is listed above the 95% confidence intervals. Letters listed below designate significant differences ($p < 0.05$). (b) The annual proportion of age classes of bearded seals harvested. Asterisks ("*") designate years with samples sizes < 10 seals. All other years included ≥ 25 seals. Bold black lines identify the mean proportion of pups harvested by period (1960s, 1970s, 2000s, 2010–2015, and the project period 2016–2019).

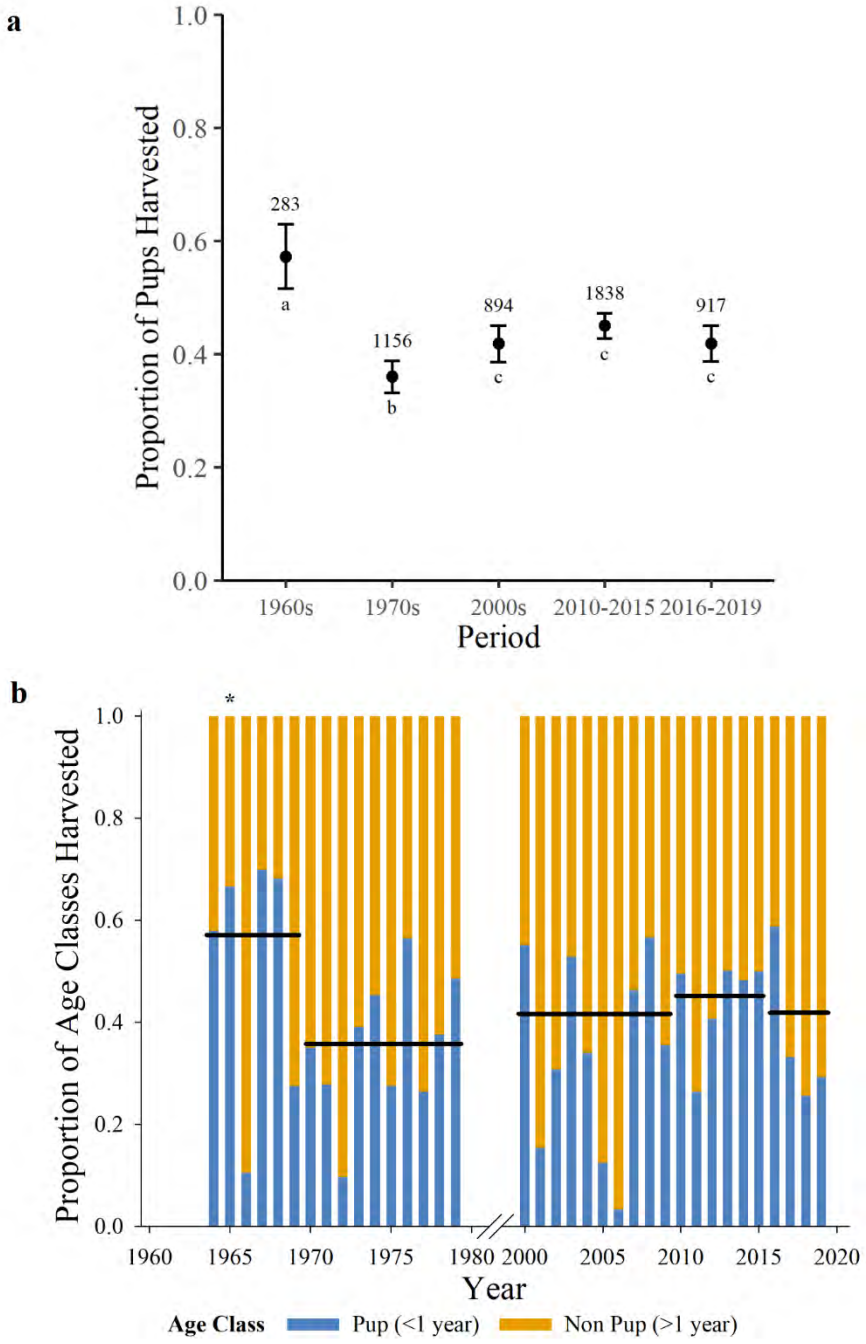

Figure 15. (a) The mean proportion of spotted seal pups harvested for subsistence at 12 villages along the Bering, Chukchi, and Beaufort sea coasts of Alaska, prior to (1960s, 1970s, 2000s, and 2010–2015) and during the project period (2016–2019). The number of seals analyzed each period is listed above the 95% confidence intervals. Letters listed below designate significant differences ($p < 0.05$). (b) The annual proportion of age classes of spotted seals harvested. Asterisks ("*") designate the year with samples sizes < 10 seals. All other years included ≥ 20 seals. Bold black lines identify the mean proportion of pups harvested by period (1960s, 1970s, 2000s, 2010–2015, and the project period 2016–2019).

Appendix B.

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DISEASES OF AQUATIC ORGANISMS
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First isolation of *Brucella pinnipedialis* and detection of *Brucella* antibodies from bearded seals *Erignathus barbatus*

Geoffrey Foster[1,*], Ingebjørg H. Nymo[2,7], Kit M. Kovacs[3], Kimberlee B. Beckmen[4], Andrew C. Brownlow[1], Johanna L. Baily[5,8], Mark P. Dagleish[5], Jakub Muchowski[6], Lorraine L. Perrett[6], Morten Tryland[2], Christian Lydersen[3], Jacques Godfroid[2], Barry McGovern[1,9], Adrian M. Whatmore[6]

[1]SAC Consulting Veterinary Services, Drummondhill, Stratherrick Road, Inverness IV2 4JZ, UK
[2]Arctic Infection Biology, Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Postboks 6050 Langnes, 9037 Tromsø, Norway
[3]Norwegian Polar Institute, Fram Centre, 9296 Tromsø, Norway
[4]Alaska Department of Fish and Game, 1300 College Road, Fairbanks, AK 99701-1551, USA
[5]Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, UK
[6]APHA Weybridge, Woodham Lane, Addlestone, Surrey KT15 3NB, UK
[7]*Present address:* Norwegian Veterinary Institute, Stakkevollveien 23, 9010 Tromsø, Norway
[8]*Present address:* Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, UK
[9]*Present address:* Cetacean Ecology and Acoustics Laboratory, School of Veterinary Science, University of Queensland, QLD 4343, Australia

ABSTRACT: *Brucella* species infecting marine mammals was first reported in 1994 and in the years since has been documented in various species of pinnipeds and cetaceans. While these reports have included species that inhabit Arctic waters, the few available studies on bearded seals *Erignathus barbatus* have failed to detect *Brucella* infection to date. We report the first isolation of *Brucella pinnipedialis* from a bearded seal. The isolate was recovered from the mesenteric lymph node of a bearded seal that stranded in Scotland and typed as ST24, a sequence type associated typically with pinnipeds. Furthermore, serological studies of free-ranging bearded seals in their native waters detected antibodies to *Brucella* in seals from the Chukchi Sea (1990–2011; 19%) and Svalbard (1995–2007; 8%), whereas no antibodies were detected in bearded seals from the Bering Sea or Bering Strait or from captive bearded seals.

KEY WORDS: Antibodies · Bearded seal · *Brucella pinnipedialis* · Isolation · Multilocus sequence typing · MLST

INTRODUCTION

The isolation of *Brucella* from marine mammals was first reported in 1994 from 4 free-ranging harbour seals *Phoca vitulina*, 2 harbour porpoises *Phocoena phocoena* and a common dolphin *Delphinus delphis*, all inhabiting Scottish coastal waters (Ross et al. 1994), and from an aborted foetus born to a captive bottlenose dolphin *Tursiops truncatus* in the USA (Ewalt et al. 1994). Since these initial reports, *Brucella* infection has become recognised in cetaceans and pinnipeds inhabiting many of the world's oceans (Foster et al. 2002, Nymo et al. 2011), and 2 species, *Brucella ceti* and *B. pinnipedialis*, have been

*Corresponding author: geoffrey.foster@sac.co.uk

described for isolates with cetaceans and seals as preferred hosts, respectively (Foster et al. 2007). These species are genetically distinct from *Brucella* associated with terrestrial mammals (Whatmore et al. 2016).

With respect to Scottish coastal waters, *B. pinnipedialis* has been recovered from the other resident seal species, grey seals *Halichoerus grypus*, as well as from hooded seals *Cystophora cristata*, which are occasional visitors to the region (Foster et al. 1996, 2002). The isolation of *B. pinnipedialis* has also been reported from hooded seals in their native Arctic waters and from harbour and grey seals elsewhere in Europe (Nymo et al. 2011). Further afield, *B. pinnipedialis* has been cultured from other pinniped species including Pacific harbour seal *Phoca vitulina richardsi* (Garner et al. 1997), ringed seal *Pusa hispida*, harp seal *Pagophilus groenlandica* (Forbes et al. 2000) and California sea lion *Zalophus californianus* (Goldstein et al. 2009). Serological studies provide further presumptive evidence that *Brucella* infections are widespread among other pinniped species, including some resident in the Southern Hemisphere (Nymo et al. 2011). Taken together, culture and serological evidence (Foster et al. 2002, Nymo et al. 2011) indicate that *Brucella* is endemic in many of the marine mammals that inhabit the world's open oceans and seas. Seropositive animals, however, can be due to immunological cross-reactions, in particular *Yersinia enterocolitica* serotype O9, although this strain has not been recovered from marine mammals to date (G. Foster pers. obs.) or an organism from a different genus; thus, the isolation of *Brucella* by cultural methods remains the gold standard of definitive proof of infection in different hosts and discrete populations of marine mammals.

There have been few reports on studies of *Brucella* infection in bearded seals *Erignathus barbatus* to date, but where performed, no evidence of exposure was found (Tryland et al. 1999, Calle et al. 2008). Bearded seals are members of the Phocidae family and represent the only species within the genus *Erignathus*. They have a patchy circumpolar distribution throughout the Arctic and Subarctic between 45° and 85° N. Two subspecies are recognised, *E. barbatus barbatus*, which ranges from the central Canadian Arctic eastwards to the central Eurasian Arctic, and *E. barbatus nauticus*, which ranges from the central Canadian Arctic westwards to the Laptev Sea, Russia. The availability of sea ice to breed, moult and rest on in shallow water areas is thought to be an important factor governing the distribution of this benthic-feeding seal (Kovacs 2016). In a review of their extralimital records, bearded seals have been reported from the Netherlands, France and Spain in the eastern Atlantic and the island of Rügen in the Baltic Sea (van Bree 2000). Sightings in the UK are rare, with most modern reports occurring around the Scottish coast, including the Shetland and Orkney islands and single sightings from the Isle of Mull, Aberdeenshire and Fife (JNCC/Defra 2013).

This paper documents the first recovery and characterisation of *B. pinnipedialis* from a bearded seal. The results of a serological study of free-living bearded seals in Arctic waters and captive members of the species kept at the Polaria aquarium in Tromsø, Norway, are also presented.

MATERIALS AND METHODS

Bearded seal necropsy

In early February 2012, a stranded bearded seal (M61/12) was reported to the Scottish Marine Animal Strandings Scheme. The juvenile male animal had stranded dead at Annachie Lagoon, St. Fergus, on the Aberdeenshire coast of the northeastern Scottish mainland (57° 34′ 10.74″ N, 1° 49′ 22.02″ W) and represented the first report of a stranded bearded seal in Scotland since records began in 1992. The carcase was transported to SAC Consulting Veterinary Services, Inverness, for a post-mortem examination performed according to a standard protocol (Dierauf 1994). Samples of brain, lung, liver, spleen, kidney, mesenteric lymph node, urinary bladder and small intestine were cultured on Columbia sheep blood agar (CSBA) (Oxoid) and Farrell's medium (FM) (Farrell 1974) and incubated at 37°C in air with 5% added CO_2 as described previously (Foster et al. 2002). Plates were examined for growth daily for 4 d and at frequent intervals thereafter up to 14 d. Isolates with colonial appearance typical of *Brucella* were tested initially for Gram reaction, cellular morphology, acid fastness with the modified Ziehl-Neelsen stain, agglutination with *Brucella abortus* antiserum (Remel) and ability to grow in air without added CO_2. Further testing included urea hydrolysis, H_2S production, inhibition by basic fuchsin at 1:50 000 and 1:100 000, agglutination with monospecific antisera A and M and lysis by phages TB, Wb, BK2, Fi, Iz and R/C all at routine testing dose. Multilocus sequence typing (MLST) using a 9 locus scheme was performed as described previously (Whatmore

et al. 2007). Tissue samples for histological examination (whole brain, trigeminal ganglion, skin, thyroid gland, adrenal gland, urinary bladder, spleen, lung, kidney, heart and pancreas) were collected, trimmed and processed routinely through graded alcohols and embedded in paraffin wax prior to sectioning (5 µm), mounting on glass microscope slides and staining with haematoxylin and eosin. Blood was collected from the left ventricle of the heart using a sterile needle into heparinised and plain 6 ml vacutainers (BD) for serology, and urine analysis was performed using the Combur 9 test (Roche).

Serology

The Alaska Department of Fish and Game ice seal program recovered serum from heart blood samples from subsistence-harvested bearded seals. Seals were shot on sea ice by Alaska Native hunters, as allowed under the Marine Mammal Protection Act of 1972, in the Chukchi Sea and Bering Strait off the northern and northwestern coasts of Alaska during May, June, July and October. In addition, 17 seals were sampled immediately post mortem during 1978–1979 scientific collections conducted April to June by the Outer Continental Shelf Environmental Assessment Program during NOAA cruises in the Bering Sea under National Marine Fisheries permit no. 194. Samples from bearded seals from Svalbard were obtained from both dead and live animals. Sixteen animals were shot on the ice as part of Norwegian scientific catches to address stocks and diets for different seal species and their role in the marine ecosystems and interactions with fisheries (1992–1995). Blood was collected on the ice when cutting the main blood vessels to the foreflippers during standard bleeding-out procedures of seals (Tryland et al. 1999). From live bearded seals (pups; 1995–2007), blood was obtained from the extradural intra-vertebral vein using an 80 mm needle (14 gauge, 2.1 mm) mounted on a 50 ml syringe. Blood was transferred into blood-collecting tubes (Venoject, Terumo), and serum was prepared (3000 × *g*, 15 min) and stored at −20°C until analysis. Sex and age category (pup: <1 yr, juvenile: <3 yr, adult: >3 yr) were known for some or all of the seals at each location (Table 1).

Furthermore, blood samples were obtained from 5 bearded seals kept in captivity at the Polaria aquarium in Tromsø. Blood was drawn from the plantar venous plexus of the hind flippers, using a 0.8 × 50 mm needle and blood collecting tubes (Venoject). Serum was prepared by centrifugation at 3000 × *g* for 15 min and stored at −20°C until analysis. These animals, initially captured in the wild in Svalbard, had been kept in captivity since they were approximately 5 wk of age; the seals interact extensively with humans through training and feeding (Stokke 2010). They were 9 to 10 yr of age at the time of sampling and had been trained to tolerate handling and blood sampling (Table 1).

Serum samples (n = 205) were analyzed for anti-*Brucella* antibodies with a protein A/G indirect enzyme-linked immunosorbent assay as described previously (Nymo et al. 2013a). The mean optical density (OD) of duplicate wells was expressed as a percentage of the reactivity of a seal positive control: (OD sample/OD positive control) × 100 = percent positivity (%P). The cut-off was 73.6 %P.

Statistical analysis

All statistical analysis was performed in JMP 11 Pro (SAS Institute).

Table 1. Bearded seals investigated for anti-*Brucella* antibodies with a protein A/G indirect enzyme-linked immunosorbent assay. Sampling location, sampling years, sex and age category are given. Data are number of seropositives from total tested for each category, with percentages in parentheses. Pup: <1 yr; juvenile: <3 yr; adult: >3 yr; (–) no data

Location	Years	Sex			Age category				
		Female	Male	Unknown	Pup	Juvenile	Adult	Unknown	Total
Bering Sea	1978–2005	0/8 (0)	0/15 (0)	0/1 (0)	0/3 (0)	0/15 (0)	0/5 (0)	0/1 (0)	0/24 (0)
Bering Strait	2001–2005	0/6 (0)	0/7 (0)	0/1 (0)	0/6 (0)	0/1 (0)	0/6 (0)	0/1 (0)	0/14 (0)
Chukchi Sea	1990–2011	10/49 (20)	4/31 (13)	2/6 (33)	0/7 (0)	3/12 (25)	5/24 (21)	8/43 (19)	16/86 (19)
Svalbard	1995–2008	4/29 (14)	2/47 (4)	–	5/67 (7)	–	1/9 (11)	–	6/76 (8)
Captive	2006–2008	0/3 (0)	0/2 (0)	–	–	–	0/5 (0)	–	0/5 (0)
Total	1978–2011	14/95 (15)	6/102 (6)	2/8 (25)	5/83 (6)	3/28 (11)	6/49 (12)	8/45 (18)	22/205 (11)

RESULTS

Bearded seal necropsy

The carcase of M61/12 was fresh and had been chilled, but not frozen, prior to necropsy 2 d after notification. The animal was 149 cm in total length, 79 cm in girth behind the front flippers and in moderate to poor body condition, with a mid-sternal blubber thickness of 16 mm.

Hair loss ranged from complete over the ventral surface of the animal to partial coverage over the flank, with bilateral symmetrical zones of alopecia, back to almost complete hair loss over the perilumbar region. The head and neck exhibited almost full coverage, excepting significant alopecia periocularly and over the dorsal muzzle. In addition, the foreflippers exhibited partial alopecia over the carpal and phylangeal regions. No regions showed evidence for hair regrowth.

The oesophagus and stomach contained a notable amount of sand, and marine debris comprising a fragment of worn black plastic sheeting 45 mm long and a single round pebble 1 cm in diameter were recovered from the stomach. No prey items were found. Thyroid glands were grossly unremarkable. The lungs and cerebral vessels were markedly congested, and the bladder mucosa was grossly reddened; the urine was turbid and dark red in colour, and a high level of haemoglobin (ca 50 erythrocytes per µl) was detected with the Combur 9 test. The brain showed diffuse dilation of cerebral vessels, but the cerebrospinal fluid was unremarkable.

Bacteriology

Small numbers of colonies typical of *Brucella* were recovered from the mesenteric lymph node on CSBA and FM after 4 d. In addition, *Vibrio alginolyticus* was recovered from multiple tissues. Cells of suspect *Brucella* colonies were tiny Gram-negative coccobacilli, which were acid fast when tested in the modified Ziehl-Neelsen stain. Agglutination was obtained in slide tests with *Brucella abortus* antiserum. The strain required CO_2 for growth and was urease positive, H_2S negative and A dominant. Growth was inhibited by basic fuchsin at 1:50 000 and 1:100 000, and cultures were completely lysed by Tb phage and partially lysed by Wb, BK2 and Iz, with no lytic effect with Fi and R/C. The strain was identified by MLST as *B. pinnipedialis* sequence type (ST) 24.

Histopathology

The most significant histological change in M61/12 consisted of moderate multifocal granulomatous and eosinophilic meningoencephalitis within the brain, often centred on degenerate or intact nematode parasite larvae, with perivascular cuffing and multifocal haemorrhages. The nematode larvae were not identified, but gross morphology of worms seen in the stomach was consistent with anisakid nematodes *Pseudoterranova bulbosa* or *Contracaecum osculatum*. It is plausible that the granulomatous foci in the brain were the result of aberrant tissue migration of L4 larval stages from these species. Mild multifocal histiocytic and eosinophilic pneumonia (likely parasitic) was also noted along with moderate splenic histiocytosis with mild lymphodepletion. The skin lesions consisted of mild epidermal hyperplasia with follicular atrophy with no evidence of vasculitis or dermal necrosis. Moderate to marked thyroid follicular hyperplasia and moderate to marked bilateral adrenocortical hyperplasia were present. The most significant lesions, and likely cause of death, were multiple granulomatous foci in many regions of the brain consistent with migrating nematode larvae. Overall, the seal appeared to have indications of chronic morbidity and malnutrition/pica which, given the extralimital nature of this case, could be due to pathogen exposure and/or inadequate feeding capacity.

Serology

Antibodies to *Brucella* were detected in 22 of 200 (11%) serum samples collected from wild bearded seals in Alaska and Svalbard (Table 1). Sixteen of the seropositive seals came from 86 (19%) animals that were subsistence harvested in the Chukchi Sea between 1990 and 2011: 1 juvenile female, 2 juvenile males, 4 adult females, 1 adult male, 5 females of unknown age, 1 male of unknown age and 2 animals of unknown sex and age (Table 1). The other seropositive bearded seals, 6 of 76 (8%), were all captured in the Svalbard archipelago during the period 1995 to 2007. The positive animals were 3 female and 2 male pups and the mother of one of the female seropositive pups. It is not known whether the mothers of the other seropositive pups were among the animals sampled. Antibodies to *Brucella* were not detected from any of the 38 bearded seals subsistence harvested in the Bering Strait or collected in the Bering Sea or from the 5 animals kept in captivity

at Polaria (Table 1). *Brucella* antibodies were detected in the blood collected from the necropsied animal (M61/12).

The average %P of the seropositive seals was 87.7 %P (SD 9.4), and the average %P of the seronegative seals was 25.9 %P (SD 21.6). The material included 6 mother–pup pairs from Svalbard sampled between 6 and 25 May 1995. One mother–pup pair was seropositive (mother: 93.2 %P, pup: 88.8 %P). The remaining pairs had %P values below the cut-off; however, the %P of the pup could be predicted from the %P of the mother with the following formula: pup = −75.8 + (1.7 × mother), $r^2 = 0.84$.

DISCUSSION

This study documents for the first time the recovery of *Brucella* from a bearded seal as well as the first serological evidence of *Brucella* exposure in this host. Antibodies were detected in sera from 2 of the 4 groups of free-ranging bearded seals sampled, the Chukchi Sea (19 %) and the Svalbard archipelago (8 %); however, they were not detected from 38 bearded seals from the Bering Strait region or the Bering Sea (Table 1). A previous small-scale study also failed to detect *Brucella* antibodies from 6 bearded seals taken during a subsistence hunt at St. Lawrence Island in the Bering Sea (Calle et al. 2008), so evidence of exposure to *Brucella* in this region remains lacking (Fig. 1). The Pacific bearded seals are not distinct populations; they move from the Bering Sea through the Bering Strait with the advancing and retreating ice edges. The detection of seropositive bearded seals from the Chukchi Sea therefore may be significant for *Erignathus barbatus nauticus* across their entire area. Another serological study for *Brucella* in bearded seals did not detect antibodies from 2 locations in the North Atlantic, while antibodies were detected in the other 3 sympatric species sampled, hooded, harp and ringed seals (Tryland et al. 1999).

Typing of the *Brucella* isolate by MLST (Whatmore et al. 2007) demonstrated that it belonged to the ST24 lineage of *Brucella pinnipedialis*. ST24 is the less common of 2 STs isolated predominantly from pinnipeds (Groussaud et al. 2007) and has previously been found associated with harbour seals, grey seals and a minke whale *Balaenoptera acutorostrata* which stranded in Scotland and from harbour seals and a beluga whale *Delphinapterus leucas* from North America (Groussaud et al. 2007, Whatmore et al. 2017).

Brucella-associated pathology was not found either grossly or histologically, although histology was not performed on the lymph node, and an association of *B. pinnipedialis* with the death of this animal was not established. This is in line with previous findings, which have revealed a paucity of pathologies following *Brucella* isolation from pinnipeds, including several apparently healthy harbour seals which had been shot by fishermen (Foster et al. 2002). In contrast, a broad range of pathologies have been reported for *Brucella* infection of various cetacean species which include lymphocytic meningoencephalitis, subcutaneous lesions, blubber abscessation, liver abscess, hepatic and splenic necrosis, macrophage infiltration in liver and spleen, lymph node inflammation, pneumonia, peritonitis, mastitis, osteomyelitis, spinal discospondylitis, diseased atlanto-occipital joint, endocarditis, epididymitis and abortion (Foster et al. 2002, Nymo et al. 2011, Guzman-Verri et al. 2012).

In vitro work has revealed differences between the classical terrestrial *Brucella* strains and *B. pinnipedialis*. The *B. pinnipedialis* reference strain NCTC 12 890 and *B. pinnipedialis* hooded seal strains were eliminated from murine and human macrophage cell lines and a human epithelial cell line within 72 to 96 h (Larsen et al. 2013b). Even more rapid elimination patterns were observed in hooded seal primary alveolar macrophages (Larsen et al. 2013a) and epithelial cells (Larsen et al. 2016). *B. pinnipedialis* NCTC 12 890 was also found to be attenuated in the BALB/c *Brucella* mouse model (Nymo et al. 2016). The reduced virulence in these models, when compared to the terrestrial virulent strain *Brucella suis* 1330 (Larsen et al. 2013b, Nymo et al. 2016), is in line with the limited virulence of the *B. pinnipedialis* strains in their natural hosts (Foster et al. 2002). Five seropositive pups were detected in the present study, all from Svalbard. The sampling took place in May, and peak birthing for bearded seals at Svalbard is in early May. The pups are thereafter weaned in approximately 24 d (Kovacs 2016). The seropositive pups were hence of very young age. At least one of the seropositive pups in the present study was the pup of a seropositive mother, and a strong relationship was identified between the titres (i.e. antibody levels) in the mothers and the pups in the 6 mother–pup pairs from Svalbard. These findings suggest a transfer of maternal antibodies between mother and pup. Seals have an endotheliochorial placenta (Stewart & Stewart 2009) where solely 5 to 10 % of the maternal antibodies are transferred to the fetus *in utero*. Passive immunity via the colostrum is there-

fore essential in species in which the type of placentation impedes contact between maternal and foetal circulation systems, hindering the transfer of antibodies (Tizard 2000). Indeed, when evaluating total IgG levels in harbour seal mothers and pups, the mothers showed a decreasing trend during lactation, while the total IgG levels in the pups were low at birth and higher at the end of lactation (Ross et al. 1993). In dogs, which also have an endotheliochorial placenta (Stewart & Stewart 2009), pups from *Brucella canis*-infected bitches have antibodies against *B. canis* (Carmichael & Kenney 1970). In animals having an epitheliochorial placenta, the young are born virtually agammaglobulinemic; however, after receiving colostrum, calves from dams with high and low *Brucella* antibody levels had corresponding high and low *Brucella* antibody levels (Sutherland & Searson 1990). Maternal transfer of antibodies against phocine distemper virus has been shown in harbour seals (Garnier et al. 2014), and maternal and pup antibody titres were shown to be strongly correlated in Scottish grey seals (Pomeroy et al. 2005). Even though the number of mother–pup pairs investigated in the present study is low, our findings and the literature support that the *Brucella* antibody levels of the pups are likely a reflection of the *Brucella* antibody levels of the mothers and the result of maternal transfer of antibodies rather than a vertical infection of the pups. However, further bacteriology work on organ samples from mother and pup pairs is needed to draw any conclusions on this matter. For hooded seals, however, no relation was found between *Brucella* serostatus and ovulation rate or neonatal body condition (Nymo et al. 2013b).

Bearded seals are largely solitary animals (Kovacs 2016). Ringed seals and hooded seals, from which *B. pinnipedialis* has been isolated and anti-*Brucella* antibodies detected (Forbes et al. 2000, Nymo et al. 2013b), are also generally described as being largely solitary (Kovacs 2002, Miyazaki 2002), though all 3 of these species do gather in the same areas where habitat is suitable for breeding, moulting and foraging. Contrary to cetaceans, where vertical transmission of *Brucella* was suggested (Ohishi et al. 2016), no evidence of vertical transmission of *Brucella* in true seals has been reported. Furthermore, the solitary behaviour of bearded seals suggests that opportunities for *Brucella* transmission between conspecifics are restricted. Altogether, this reinforces the possibility that *Brucella* infection may be acquired from the environment, possibly via diet, as suggested previously (Lambourn et al. 2013, Nymo et al. 2013b). In contrast, harp seals have also been shown to harbour infections with *B. pinnipedialis* (Tryland et al. 1999, Forbes et al. 2000), but this species demonstrates a much stronger tendency to congregate (Lavigne 2002), and transmission between conspecifics cannot be excluded.

Brucellosis is a significant zoonotic infection which causes a broad range of manifestations; it is especially associated with farmed animals and their products infected with *Brucella melitensis, B. abortus* and *B. suis* but also with *B. canis* contracted from dogs. While there have been 3 reports of human infections with marine mammal *Brucella*, none have involved *B. pinnipedialis*. Human infection has been reported in a laboratory infection scenario with ST23, a clade predominantly associated with porpoises, while naturally occurring infections have been reported only with ST27 (Whatmore et al. 2008), only isolated thus far from bottlenose dolphins *Tursiops truncatus* and California sea lions in the USA (Whatmore et al. 2017) and recently from a single bottlenose dolphin in the Mediterranean (Cvetnić et al. 2016).

While the lack of human infections with *B. pinnipedialis* is in contrast to the findings with *B. ceti* and the classical *Brucella* spp. mentioned in the previous paragraph, the zoonotic potential of *B. pinnipedialis* remains unknown at present. It is advisable, therefore, that those working with bearded seals and other pinniped species consider the infectious nature of the genus and follow appropriate safety procedures (Dierauf & Gulland 2001).

In conclusion, we report the first isolation of *B. pinnipedialis* from a stranded extralimital juvenile male bearded seal. In contrast to cetaceans, reports of *Brucella*-associated pathology in pinnipeds are lacking. Our study also provides novel serological evidence for *Brucella* spp. exposure in free-ranging bearded seal subpopulations. Future serological surveys and the isolation and characterization of *Brucella* isolates from stranded and free-ranging bearded seals, as well as other ice seals (ringed seal, ribbon seal, spotted seal), are needed to better understand the significance of *Brucella* infection in these northern pinnipeds.

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Research article

Validation of a novel method to create temporal records of hormone concentrations from the claws of ringed and bearded seals

Shawna A. Karpovich[1,*], Larissa A. Horstmann[2] and Lori K. Polasek[3]

[1]Alaska Department of Fish and Game, Marine Mammal Program, Fairbanks, AK 99701, USA
[2]College of Fisheries and Ocean Sciences, University of Alaska, Fairbanks, AK 99775, USA
[3]Alaska Department of Fish and Game, Marine Mammal Program, Juneau, AK 99811, USA

***Corresponding author**: Alaska Department of Fish and Game, Marine Mammal Program, Fairbanks, AK 99701, USA. Tel: 907 459 7322. Email: shawna.karpovich@alaska.gov

Ringed (*Pusa hispida*) and bearded seals (*Erignathus barbatus*) inhabit vast and often remote areas in the Arctic, making it difficult to obtain long-term physiological information concerning health and reproduction. These seals are experiencing climate-driven changes in their habitat that could result in physiological stress. Chronic physiological stress can lead to immunosuppression, decreased reproduction and decreased growth. Recently, keratin has become a popular matrix to measure steroid hormones, such as stress-related cortisol and reproduction-related progesterone. We developed and validated methods to extract cortisol and progesterone from the claws of adult female ringed ($n = 20$) and bearded ($n = 3$) seals using enzyme immunosorbent assays. As ringed and bearded seal claws grow, a pair of dark- and light-colored bands of keratin is deposited annually providing a guide for sampling. Two processing methods were evaluated, removal of claw material with a grinding bit or grinding followed by mechanical pulverization (102 paired samples from six claws, two each from three seals). Adding the mechanical pulverization step resulted in a 1.5-fold increase in hormone extraction. Progesterone from the proximal claw band was evaluated to biologically validate claw material as a measure of pregnancy in ringed seals ($n = 14$). Claws from pregnant seals had significantly higher claw progesterone concentrations than from non-pregnant seals. This suggests that the elevated progesterone associated with gestation was reflected in the claws, and that the most proximal claw band was indicative of pregnancy status at time of death. Thus, although the sample size was low and the collection dates unbalanced, this study demonstrates the potential to use claws to examine an extended time series (up to 12 yrs) of cortisol and progesterone concentrations in ringed and bearded seal claws.

Key words: Cortisol, keratin, phocid, pinniped, progesterone, steroid hormone

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Introduction

Arctic regions are undergoing declines in sea ice duration, extent and thickness (Shaftel *et al.*, 2015) and increases in water temperature (Stroh *et al.*, 2015). Ringed (*Pusa hispida*) and bearded (*Erignathus barbatus*) seals inhabit the Arctic, and in Alaska are found in the Beaufort, Chukchi and Bering seas (Allen and Angliss, 2010, Burns, 1970). For Arctic phocids, suitable sea-ice habitat is essential for resting, foraging and pup rearing (Gjertz and Lydersen, 1983, Kelly *et al.*, 2010). Decreases in sea ice extent may lead to changes in the timing, quality and quantity of prey, increases in water and air temperature, severe weather and exposure to disturbances, pathogens and toxins (e.g. Burek *et al.*, 2008). These changes could have considerable influence on the diet, health and reproduction of ringed and bearded seals, making them especially vulnerable to current and future habitat alterations. Therefore, an examination of how habitat changes influence the health, reproduction and survival of ice-dependent seals is warranted. Ringed seal reproductive rates are often estimated by the proportion of pups harvested by subsistence hunters (Crawford *et al.*, 2015, Ferguson *et al.*, 2005, Harwood *et al.*, 2000, Stirling, 2005). However, many factors (e.g. ice conditions, hunter preference, seasonal distribution) may influence the harvest proportions and bias these estimates. Reproduction can also be assessed by counting pups, but pupping locations are spread over large areas of ice (Harwood and Stirling, 1992) and can occur in snow covered dens (Born *et al.*, 2004) making pups difficult to detect. Alternatively, reproduction can be evaluated by examining reproductive tracts of harvested animals (Crawford *et al.*, 2015, Holst *et al.*, 1999), but this method is most accurate on samples collected in the autumn and winter when harvests are less common and assessing reproduction from tracts collected in other seasons can overestimate the number of successful births (Crawford *et al.*, 2015). Therefore, developing a method using other tissues to estimate pregnancy may reduce some of the difficulties associated with estimating pregnancy rates in these species.

If the habitat changes that ringed and bearded seals are experiencing are perceived as stressors, the hypothalamic-pituitary-adrenal axis will be activated leading to a complex suite of physiological and behavioral responses, including the release of glucocorticoids (Reeder and Kramer, 2005). Glucocorticoids, such as cortisol, then mobilize body energy stores to allow animals to cope with change or flee from danger (e.g. Romero and Butler, 2007). Thus, concentrations of cortisol are commonly used as an index of physiological stress in mammals (Gulland *et al.*, 1999, Möstl and Palme, 2002, Ortiz and Worthy, 2000, Thomson and Geraci, 1986). Short-term stress (triggering the fight-or-flight response) is an adaptive mechanism that increases the likelihood of survival; however, chronically elevated cortisol can result in negative physiological effects, such as immunosuppression, decreased reproduction (Dobson and Smith, 2000) and decreased growth (e.g. Möstl and Palme, 2002). Another hormone, progesterone, has similarly been used to assess reproduction in pinnipeds (Boyd, 1991, Greig *et al.*, 2007). Therefore, evaluating the levels of cortisol and progesterone would be especially valuable considering the effects of climate change on the Arctic, which could result in changes in chronic physiological stress or reproductive success for ice-dependent seals.

Long-established matrices to measure steroid hormones in pinnipeds include blood, feces, urine, blubber and saliva (Atkinson, 1997, Atkinson *et al.*, 2015). The period represented by these sample types is often short or difficult to estimate making it problematic to interpret the hormones measured. These samples are generally difficult and costly to collect in free-ranging wildlife. For most seals, they are almost impossible to obtain from the same individuals over time, which precludes long-term assessment of hormones. Progesterone concentrations can be difficult to interpret from single samples representing short periods, because the pinniped reproductive cycle includes a several month delayed implantation of the fertilized cells, during which circulating progesterone concentrations are similar between pregnant and non-pregnant animals (Greig *et al.*, 2007, Guinet *et al.*, 1998, McKenzie *et al.*, 2005). Cortisol concentrations from the above-mentioned tissues could also be difficult to interpret as capture or harvest events can cause acutely elevated levels of cortisol to be released into circulation that may obscure the pre-disturbance levels (Gulland *et al.*, 1999, Ortiz and Worthy, 2000, Thomson and Geraci, 1986). Challenges assessing physiological stress and reproduction over time highlight the need to develop methods for novel tissues, especially for species that are difficult to sample, like ringed and bearded seals.

Recently, the assessment of steroid hormones in hair as biomarkers for reproduction or chronic physiological stress has gained popularity (Koren *et al.*, 2019). Hair cortisol concentrations have been correlated with climate-related changes in habitat and contaminant load in polar bears (*Ursus maritimus*; Bechshøft et al., 2012a,b, 2013, 2015), nutritional and social stress in grizzly bears (*Ursus arctos*; Bryan *et al.*, 2013, Macbeth *et al.*, 2010) and hunting pressure in wolves (*Canis lupus*; Bryan *et al.*, 2015). However, mammalian hair has a relatively short period of growth (~1.5 mo in phocids; Ashwell-Erickson *et al.*, 1986). Thus, sampling keratinous tissue with an extended growing period would be more useful to examine long-term trends in hormone concentrations.

Seal claws grow continuously, and stable isotopes stored in ice seal claws contain diet information spanning up to the previous 12 years (Boucher *et al.*, 2020, Carroll *et al.*, 2013, Ferreira *et al.*, 2011). Whale baleen is also a continuously growing keratinous tissue that contains long-term records of several steroid hormones (Hunt *et al.*, 2014, 2017) and baleen progesterone concentrations have been correlated with known pregnant and non-pregnant periods (Hunt *et al.*, 2016). Similarly, steroid hormones have been extracted from the hooves of cattle (Comin *et al.*, 2014) and the claws of turtles (Baxter-Gilbert *et al.*, 2014), dogs (Fusi *et al.*, 2018,

Table 1: Individual IDs, collection date, pregnancy status, tooth and claw derived ages and proximal band progesterone concentration from adult female ringed seal claws. Pregnancy status was determined by examination of written collector notes or visual confirmation of the reproductive tract. Tooth ages were estimated using cementum growth layers and claw ages are the number of bands counted on the claw followed by a + indicating the estimated minimum age, as the number of bands lost to wear is unknown. 'Prox. Claw Prog.' is the progesterone measured in the most proximal claw band that was examined for correlation with the pregnancy status at the time of death

Individual ID	Collection date	Pregnancy status	Tooth age	Claw age	Prox. claw prog. (pg/mg)
ADFG:11SH015	2-Oct-11	Non-pregnant	12	11+	79.88
ADFG:11SH016	2-Oct-11	Non-pregnant	13	8+	78.82
ADFG:11SH099	13-Oct-11	Non-pregnant	18	9+	82.20
ADFG:10SH005	15-Oct-10	Non-pregnant	24	8+	103.68
ADFG:11GAM003	13-Nov-11	Non-pregnant	10	11+	44.41
UAM:Mamm:36830	12-Jan-67	Pregnant		11+	123.06
UAM:Mamm:36825	28-Jan-63	Pregnant		9+	111.73
UAM:Mamm:122131	10-Feb-67	Pregnant		11+	212.86
UAM:Mamm:36826	10-Feb-64	Pregnant		9+	118.35
ADFG:10SH053	10-Nov-10	Pregnant	25	12+	65.68
ADFG:10SH061	10-Nov-10	Pregnant	9	8+	93.52
UAM:Mamm:19062	13-Nov-66	Pregnant		6+	155.72
ADFG:14SH018	15-Nov-14	Pregnant	20	9+	155.15
UAM:Mamm:19059	30-Nov-65	Pregnant		8+	110.76

Veronesi *et al*., 2015) and chameleons (Matas *et al*., 2016), yet no such studies have examined marine mammal claws. Therefore, ice seal claws that grow continuously and have visibly discernable annual banding (Benjaminsen, 1973, McLaren, 1958) are an ideal matrix to examine long-term trends in hormone concentrations. This may be especially useful as ice seal habitats undergo climate change-related alterations.

Within this study, we developed and validated a technique for monitoring chronic physiological stress and reproductive status for ringed and bearded seals. The four main goals of our study were to (1) develop a protocol for hormone extraction from ringed and bearded seal claws, (2) validate enzyme immunosorbent assays (EIAs) for cortisol and progesterone using extracts from ringed and bearded seal claws, (3) compare methods for collecting claw powder by grinding with a diamond-tipped bit with and without an added mixer-mill pulverization step and (4) compare progesterone concentrations from the most recently deposited claw material (proximal band) to pregnancy status at time of harvest (pregnant *vs*. non-pregnant).

Methods

Sample collection

Claws were collected from 20 adult female ringed (3 laboratory validations, 3 processing methods, and 14 biological validations) and 3 adult female bearded seals (laboratory validations) harvested by Alaska Native subsistence communities in the Chukchi and Bering seas, Alaska, and were obtained from the University of Alaska Museum Mammalogy Collection (UAM:Mamm), the Alaska Department of Fish and Game (ADF&G) and the North Slope Borough (NSB). Laboratory validations required large samples, so extracts from two to four claws per seal were pooled from three ringed seals (NSB:RS602, NSB:RS51800 and NSB:RS700) and three bearded seals (NSB:BS602, NSB:BS702 and NSB:BS080801), respectively. Pools were created so that each individual seal was represented equally. To compare processing methods, the two longest claws each from three ringed seals ADFG:11GAM009, ADFG:10SH005 and NSB:2014–01 were used. Lastly, individual progesterone concentrations from the most proximal claw band from 14 adult female ringed seals were evaluated to compare pregnancy status at the time of death to claw progesterone concentrations (Table 1). Claws from the 1960s were stored at UAM in paper envelopes, claws collected from 2010 to 2014 were stored frozen as whole flippers, then removed from the flesh and stored in paper envelopes ≤1 yr prior to processing.

A fetus was collected with three of the six ringed seals from the museum collection (UAM:Mamm:19062, UAM:Mamm:19059 and UAM:Mamm:122131; Table 1), otherwise pregnancy status was determined by museum notes acquired from the original collectors. ADF&G collected claws and reproductive tracts from eight harvested ringed

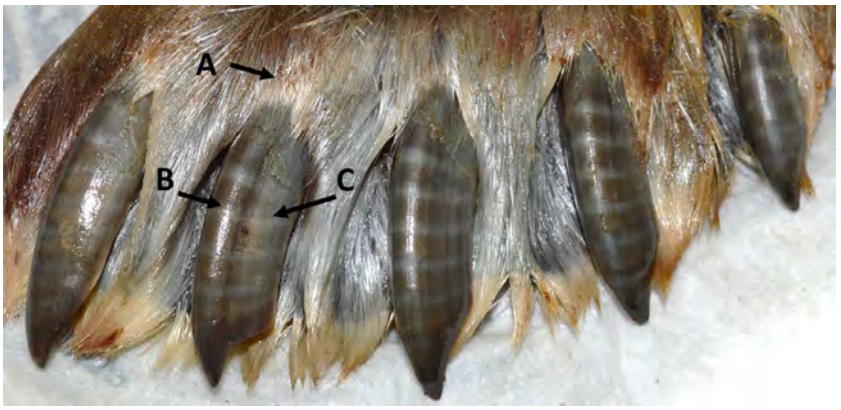

Figure 1: Front flipper from a subsistence harvested ringed seal showing the distinct claw bands. Pairs of bands are deposited annually (Benjaminsen, 1973, McLaren, 1958). The most proximal band (A), used for biological validations in this study, is hidden under the skin and fur at the insertion point of the claw. The central ridge (B) is the thickened dorsal and central portion of the claw (not sampled) and the lateral walls (C) are the area of the claw sheaths sampled in this study.

seals (Table 1) and pregnancy was determined by presence or absence of uterine implant sites as outlined in Crawford *et al.* (2015).

Seal age was estimated using teeth or claws (Benjaminsen, 1973, McLaren, 1958). For a subset of seals, a lower canine was sent to Matson's Laboratory, Milltown, Montana, USA, and ages were estimated by counting annual growth layers (cementum rings) in the tooth. Also, age was estimated using the banding pattern on the claws (Fig. 1) (Benjaminsen, 1973, McLaren, 1958). Because claw bands are lost from the distal end of the claw during wear, claw ages represent a minimum age estimate, denoted by a '+' after the estimate (Table 1).

Claw processing

Claws were submerged in a room temperature water bath until the keratinous claw sheath could be removed from the underlying bone and tissue (~1–14 days), then stored in paper envelopes at room temperature until processed. At the time of processing, claws were again soaked for 1–4 days to soften any remaining tissues adhered to the claw (see cuticle-like tissues at the top of the claws in Fig. 1), which were removed with a small weighing spatula. After soaking in the water baths, the claws were often coated with contaminants associated with other tissues in the flipper (e.g. blood and oils). To remove surface contaminants, claws were sonicated in deionized (DI) water for 30 min and immersed in 2:1 chloroform:methanol for 30–60 s. Finally, the outer surface was wiped with a cotton swab, and the inner surfaces scrubbed with a straw-cleaning brush, both wetted with 2:1 chloroform:methanol. Immersion and scrubbing/wiping was repeated four times, or more if the cotton swab continued to show discoloration from water bath contamination. Claws were dried in open containers at room temperature for ≥24 h prior to grinding.

Powdered samples of claw material were collected using a Dremel® tool at ~17 000 rpm with a diamond-coated engraving tip (part 7134; 2 mm bit diameter). For validations and comparison between processing methods, serial samples were collected from each claw. Sampling was restricted to the lateral walls of the claw sheaths, because the dorsal ridge of the claw contains keratin that is continuously deposited from the underlying tissue, which would confound the estimation of the timing of deposit (Ethier *et al.*, 2010). During sampling, the claw was ground to ~2 mm in depth and followed the contour of the visible bands. The claw powder was collected in a tin weighing dish, ~3–5 ml of DI water was added, mixed into a slurry, then poured into a 5-ml glass vial. Open vials were dried at ≤60°C for ≤6 days. To avoid cross contamination, gloves and weighing dishes were changed and the work area, claw and Dremel® were cleared with forced air between each sample.

Sample powdering methods

Bearded seal claws were processed first, as they are large (~6.5 × 1.4 cm) and collecting consistent powder using the grinding tip was unproblematic. Ringed seal claws are smaller (~5 × 0.8 cm) and large chunks of claw material often broke off while grinding, making the powder consistency more variable. Therefore, different processing methods were tested on pairs of claws from three individual ringed seals. One claw was processed by grinding with the Dremel® or grinding followed by pulverization of the powder using a Retsch® Mixer Mill MM400. Samples were collected from multiple bands along each claw (51 paired samples, $n = 102$). Post-grinding claw material was transferred into 2-ml Sarstedt® screwcap micro-centrifuge tubes with a silicon gasket, and for the grind+mill method, two 5-mm steel ball bearings were added to each tube and the sample was pulverized into a finer powder at 30 Hz for 15 min in the mill.

Hormone extraction

For hormone analyses, 5 ± 0.5 mg of dried claw powder was weighed to the nearest 0.1 mg and extracted in 1 ml of 100% ACS grade methanol by slowly rotating for 24 h. All samples were centrifuged for 13 min at 10°C and 10 500 g. Supernatant was transferred to a new 2-ml tube, and pellets were rinsed with 0.2 ml of methanol, centrifuged (same as above), and the rinse supernatant was added to the sample's extract. If less than 5 mg of claw powder was collected, the amount of methanol used during extraction and rinsing was reduced, so the ratio of powder to methanol was consistent among samples. The methanol extracts were frozen at ≤−20°C until analyzed (∼0–3 months).

Validations

Arbor Assays® kits (Ann Arbor, MI, USA) for progesterone (catalog # K025) and cortisol (catalog # K003) were validated using parallelism and accuracy tests. For validations, extracts from multiple claw bands and individuals were pooled. To verify that the claw extracts did not affect the kit hormone detection capabilities across the range of detection concentrations, accuracy tests were conducted. Kit standards were serially diluted, then combined with equal parts of the pooled sample. Accuracy was determined by plotting the expected and observed hormone concentrations of the standard-pool mixtures in SigmaPlot 13. To verify that the kit could accurately measure hormones from the claw extracts within the detection range, parallelism tests were conducted. Pooled claw extracts were serially diluted and assayed. The percent binding of extracts and standards was plotted against hormone concentration (pg/ml) expressed on a log scale.

Progesterone and cortisol hormone assays

Tubes of sample supernatant were removed from the freezer and centrifuged (13 min, 10 °C, 10500 g) to remove any residual powder from solution. The supernatant was placed into glass culture tubes and dried under forced air. For method comparisons and biological validations, 0.175 ml of ringed seal claw extract was dried and reconstituted with 0.12 ml of buffer, then analyzed for progesterone concentrations. Samples, standards, controls, non-specific binding and blank wells were assayed in duplicate. Results are presented as picograms of hormone per milligram of claw (pg/mg).

Biological validation

Based on the manner that claws grow, the most proximal claw material (base) contains the most recently deposited keratin. Therefore, for ringed seals ($n = 14$), claw progesterone concentrations from the most proximal material were compared to pregnancy status at the time of death (Table 1).

Statistics

All samples were run in duplicate and consistency between duplicates was examined using the mean intra-assay % coefficient of variation (*CV*). Plate-to-plate consistency was examined by calculating inter-assay % *CV* from the mean values of high and low controls included on each plate. For accuracy tests, the slope and 95% confidence interval *(CI)* of each line were examined for the inclusion of 1 (slope of a 1 to 1 relationship). Testing for parallelism was conducted with an analysis of covariance wherein two different models were fit and compared. One model was parameterized with parallel lines fitting paired sample and standard data while another model allowed for the slopes to vary between the sample and standards. A likelihood ratio test was subsequently performed to assess which of these two models were most supported by the data and is reported as '*F* statistic, *P* value'. To account for unequal variance, the comparison between the grind-only and grind+mill processing methods was conducted using a Wilcoxon Signed Ranks test, values are reported as 'median value (sample size, range)', and test results as '*Z* statistic, *P* value'. For biological validations, predicted claw progesterone concentrations were compared using a Mann–Whitney sum test and values are reported as 'median value (sample size, range)' and test results as '*U* statistic, *P* value'. All tests were considered significant at $P \leq 0.05$.

Results

Validations

Regression lines created by plotting the expected and observed concentrations from pooled claw extract/standard combinations (accuracy tests) were linear (Figs 2A, 2B, 3A, 3B). The 95% *CI* for all accuracy tests included 1 and had an $R^2 \geq 0.976$ (Table 2). This indicates that both high and low levels of progesterone and cortisol could be accurately differentiated using extracts from ringed and bearded seal claw powder. Pairs of regression lines created by plotting the percent binding and the hormone concentration of serially diluted sample and standard pools (parallelism tests, Figs 2C, 2D, 3C, 3D), had similar slopes and showed substantial overlap of the 95% *CI*s between sample/standard pairs with $R^2 \geq 0.983$ (Table 2). The likelihood ratio test rejected the model where slopes could vary, thus confirming that the serially diluted sample lines were parallel to the corresponding serially diluted standard lines ($F = 0.108$, $P = 0.744$). This indicates that no significant interference or magnification of binding was observed while using extracts from claw powder in the EIAs.

Intra-assay % *CV*s for individual samples run in duplicate averaged 6.5% for cortisol ($n = 126$) and 4.5% for progesterone ($n = 126$). Inter-assay % *CV*s derived from high and low controls included on each plate were 11.2% ($n = 5$) and 7.4% ($n = 12$), for cortisol and progesterone, respectively.

Sample powdering methods

Adding the mill pulverizing step significantly increased the concentration of progesterone extracted from the claw

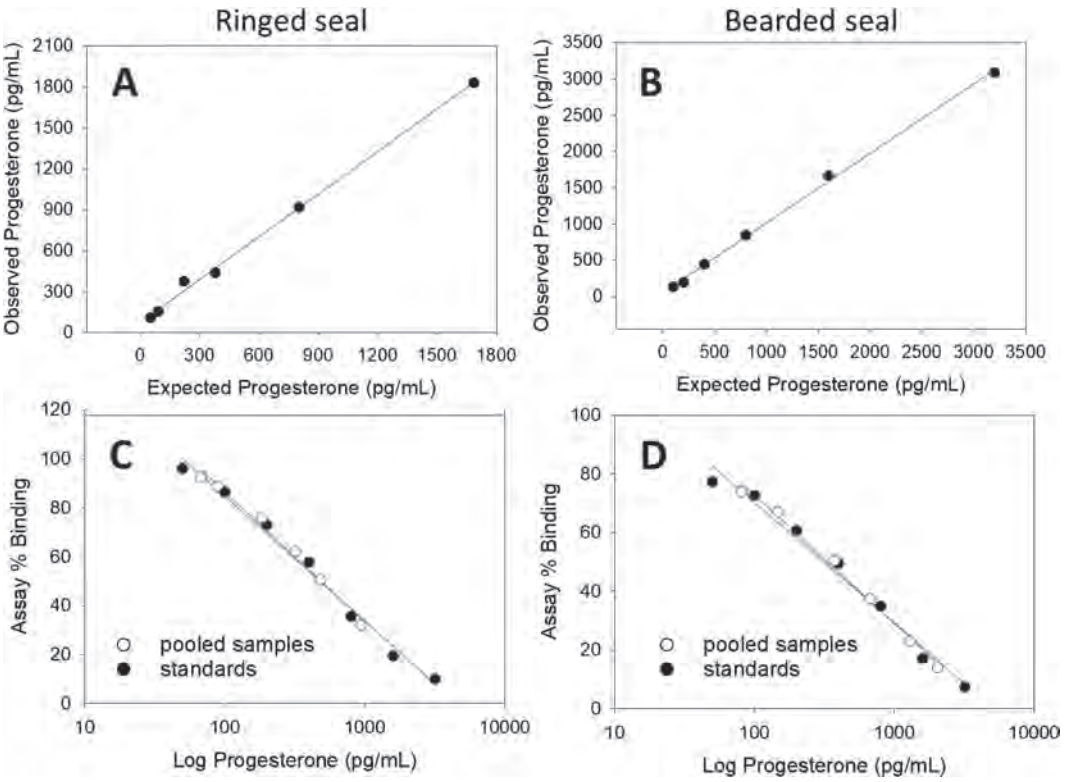

Figure 2: Validations of progesterone EIAs using pooled methanol extracts from powdered ringed (A, C) and bearded seal (B, D) claws. Accuracy tests (A, B) were conducted by combining equal parts of pooled sample with each standard and the *CI* of each slope included 1 (slope of a 1 to 1 correlation). Parallelism tests (C, D) compared the slope of serially diluted pooled samples with serially diluted standards; the *CI*s of the slopes had strong agreement. Slopes, 95% *CI* of slopes and R^2 values are reported in Table 2.

Table 2: Slope, upper and lower 95% *CI*, R^2 and the corresponding figure panel for each line created during validations of progesterone and cortisol EIAs using pooled methanol extracts from powdered ringed and bearded seal claw material.

Species	Hormone	Test	Slope	Upper 95% *CI*	Lower 95% *CI*	R^2	Figure
Ringed	Progesterone	Accuracy	1.04	1.12	0.96	0.994	2A
Ringed	Progesterone	Parallelism (standards)	−22.12	−19.41	−24.83	0.989	2C
Ringed	Progesterone	Parallelism (samples)	−22.96	−19.32	−26.60	0.987	2C
Bearded	Progesterone	Accuracy	0.96	1.01	0.91	0.998	2B
Bearded	Progesterone	Parallelism (standards)	−17.87	−14.93	−20.81	0.983	2D
Bearded	Progesterone	Parallelism (samples)	−19.03	−16.79	−21.27	0.992	2D
Ringed	Cortisol	Accuracy	1.04	1.12	0.97	0.999	3A
Ringed	Cortisol	Parallelism (standards)	−21.57	−18.61	−24.53	0.990	3C
Ringed	Cortisol	Parallelism (samples)	−21.62	−18.38	−24.86	0.993	3C
Bearded	Cortisol	Accuracy	1.05	1.28	0.82	0.976	3B
Bearded	Cortisol	Parallelism (standards)	−21.53	−19.69	−23.36	0.996	3D
Bearded	Cortisol	Parallelism (samples)	−23.17	−21.44	−24.91	0.998	3D

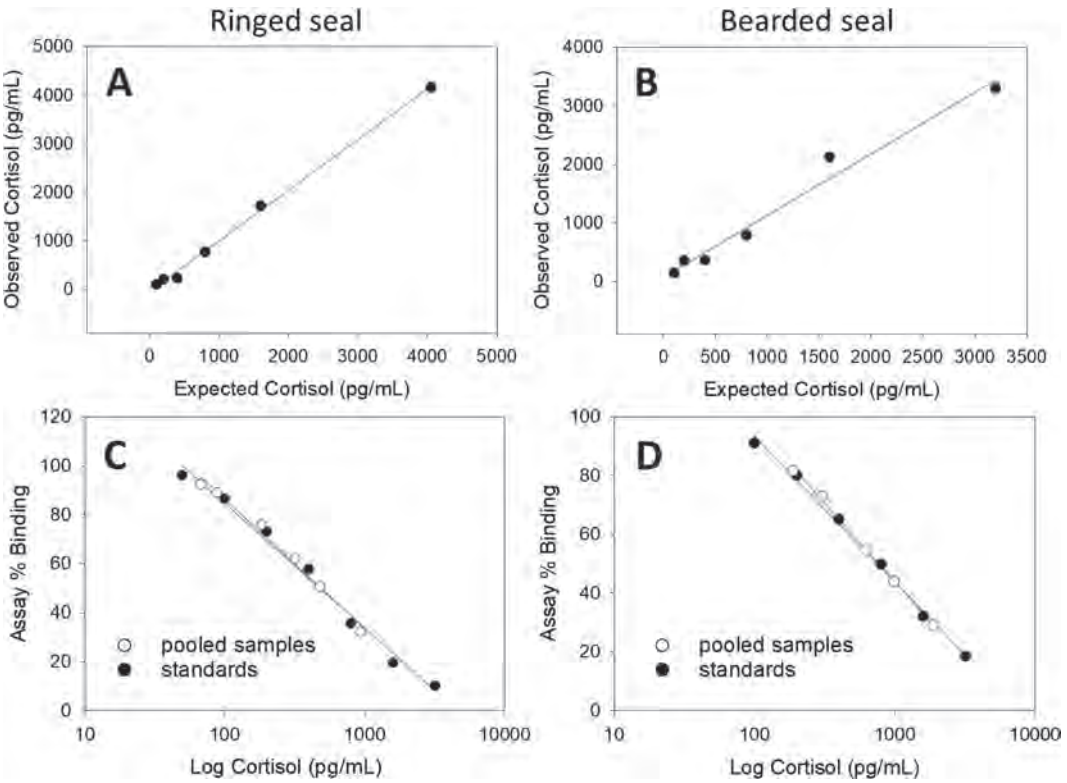

Figure 3: Validations of cortisol EIAs using pooled methanol extracts from powdered ringed (A, C) and bearded (B, D) seal claws. Accuracy tests (A, B) were conducted by combining equal parts of pooled sample with each standard and the *CI* of each slope included 1 (slope of a 1 to 1 correlation). Parallelism tests (C, D) compared the slope of serially diluted pooled samples with serially diluted standards; the *CI*s of the slopes had strong agreement. Slopes, 95% *CI* of slopes and R^2 values are reported in Table 2.

material (Fig. 4). Concentrations of claw progesterone extracted from the grind-only powder was 50.6 pg/mg ($n = 51$, 17.8–72.9 pg/mg), which was significantly lower than concentrations from the grind+mill powder of 118.7 pg/mg ($n = 51$, 66.1–162.8 pg/mg) ($Z = 6.2$, $P < 0.001$). Subsequently, validations for ringed seal samples (Figs 2 and 3) and all individual ringed seal claw values (Table 1 and Fig. 4) were processed using the grind+mill method.

Biological validation

Progesterone concentrations in the most recently deposited claw material from adult female ringed seals that were identified as pregnant at the time of death was 118.3 pg/mg ($n = 9$, 65.7–212.9 pg/mg), which was significantly higher than claw progesterone concentrations from seals that were identified as non-pregnant 79.9 pg/mg ($n = 5$, 44.4–103.7 pg/mg) ($U = 5$, $P = 0.02$), (Table 1 and Fig. 5). However, when claw progesterone values from January and February harvested pregnant seals were removed and comparisons were only made among seals harvested in October and November, the difference was no longer significant ($U = 5$, $P = 0.151$).

Discussion

Recent studies document that keratinized tissue can store long-term information associated with physiological stress and reproduction. Cortisol or corticosterone in the claws of other species have been correlated with proximity to roads (Baxter-Gilbert *et al.*, 2014), premature birth (Veronesi *et al.*, 2015) and social dominance associated with body size (Matas *et al.*, 2016). Progesterone concentrations from other keratinized tissues, such as hooves (Comin *et al.*, 2014) and baleen (Hunt *et al.*, 2016), have been correlated with known pregnancies. The validations presented here show that both cortisol and progesterone can be extracted from ringed and bearded seal claws, and to our knowledge, this is the first study to measure hormone concentrations in pinniped claws. This may be especially important for these ice-dependent seals that are vulnerable to climate change-related changes such as declines in duration, quality, or quantity of sea ice.

The visibly discernable annual claw banding in ice seal claws (Benjaminsen, 1973, McLaren, 1958) makes the collection of several years of information from one sampling event attractive. However, several overlapping longitudinal

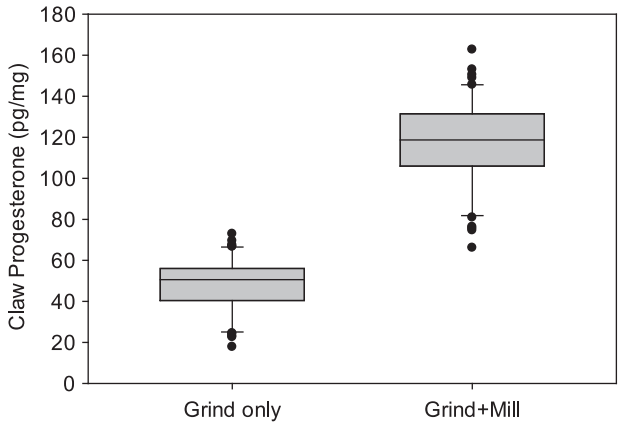

Figure 4: Effect of claw processing on the progesterone concentrations from claw bands of three adult ringed seals. Paired claws from each individual seal were processed using two methods. The bands of one claw were processed using the 'Grind only' method ($n = 51$) and bands from a second claw were processed using the 'Grind+Mill' method ($n = 51$). Progesterone concentrations were significantly higher when the mill pulverization step was added. Boxplots encompass the 25% and 75% quartiles, and the median is depicted by a line in the middle of the gray box. Whiskers extend to the 10th and 90th percentiles, and values beyond that (outliers) are shown as individual points.

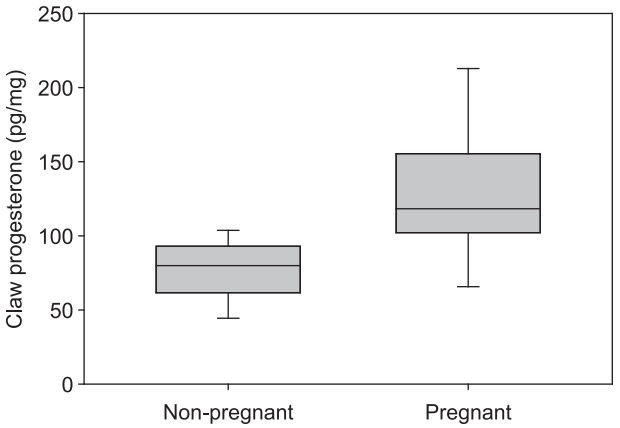

Figure 5: Progesterone concentrations in the proximal band of ringed seal claws extracted using the 'Grind+Mill' method. Seals were identified as either non-pregnant ($n = 5$) or pregnant ($n = 9$) at time of death based on observation of a fetus or an embryonic implant site. Claw progesterone concentrations were significantly higher comparing seals that were pregnant at time of death to seals that were not. Boxplots encompass the 25% and 75% quartiles, the median value is depicted by a solid line in the middle of the gray boxes, and whiskers extend to the 10th and 90th percentiles. Outliers were not identified due to the small sample sizes.

layers of keratin were observed in bearded seal claws (Benjaminsen, 1973). If this is the case in the present study, the arrangement of these layers would mean that the 2 mm depth sampled would contain two or three distinct layers. However, the previous report does not describe which portion of claw was cross sectioned and due to the width of the claw material shown (~4 mm; Benjaminsen, 1973), we suspect the thickened central ridge of the claws was used. Examinations of other mammalian claws have concluded that keratin is continuously deposited under the central ridge, while the lateral walls are deposited exclusively at the insertion point of the claw (germinal matrix; Ethier *et al.*, 2010). In other words, time series information stored in the central ridge of claws is confounded by layering of keratin over time, while the lateral walls contain an undiluted time series of information. In this study, we sampled exclusively from the lateral walls, and only sampled the most proximal band where, even if additional material were to be deposited under the lateral walls of the claws, the animal was harvested before it would have been possible.

The comparison of progesterone from paired claws collected from individual ringed seals requires that the hormone concentrations are similar among neighboring claws. Carroll *et al.* (2013) found that stable isotope signatures did not vary among claws sampled from individual ringed seals; therefore, we assumed that hormone deposits would also be similar. Yet, when the mill pulverization step was added to a powder collected from a second claw from the same seal, the concentration of progesterone extracted was 1.5 times higher. This suggests that methods used during claw processing can have a large influence on the hormone concentrations reported. Several methods have been utilized to process claws from other species for steroid hormone extraction, including mincing of claw tips (Fusi *et al.*, 2018), collecting shavings of claw tips (Veronesi *et al.*, 2015), freezing and crushing claw tips (Baxter-Gilbert *et al.*, 2014) and producing powder by grinding or grinding then pulverizing in a mill (this study). Pulverization in a mill is preferable to other claw processing methods, because it is mechanical and timed, which decreases the likelihood of human error adding variability to the sample consistency. Mill grinding was also preferred for preparation of hair samples for hormone extraction (e.g. Meyer *et al.*, 2014). Additionally, the mill pulverization step resulted in higher concentrations of hormones extracted, allowing for smaller samples sizes or tissues with lower hormone concentrations to be analyzed. Regardless of the method used, with consistent processing steps, the hormone concentrations measured within each study should be comparable. However, direct comparisons of hormone concentrations among studies that use dissimilar processing methods may be invalid.

In phocids, serum progesterone can be used to detect pregnancy (Gardiner *et al.*, 1996, Reijnders, 1990). In the present study, ringed seals that were pregnant when the claw was collected had higher progesterone concentrations in the proximal claw material than those that were non-pregnant. This suggests that the progesterone deposited into the claw was correlated with circulating progesterone and that the proximal band of claws indicated reproductive status during the period of keratin deposition. A similar comparison

of progesterone concentrations in bearded seal claws could not be conducted because all harvested females with information about reproductive status were either pregnant or post-partum and claws from non-pregnant individuals were unavailable for comparison. Further research is required to determine if claw progesterone can also be used as an indicator of pregnancy in bearded seals. Yet, the ringed seal results suggest that retrospective examinations of reproduction from the previous 6 to 12 years could be conducted by analyzing serial bands along the length of the claws.

During phocid gestation, serum progesterone levels rise gradually (Boyd, 1991, Reijnders, 1990). This implies that pregnancy would be easier to detect closer to pupping (May–June for ringed seals; Kelly *et al*., 2010). In this study, the harvest dates were not balanced, and for some pregnant seals the proximal bands contained more material deposited while the seal was pregnant including some material deposited during late pregnancy when progesterone was highest. This may explain why the difference between pregnant and non-pregnant claw progesterone levels became non-significant after the removal of seals that had been gestating for 3 additional months. Accordingly, future studies with larger sample sizes and balanced harvest dates would be beneficial. However, unbalanced harvest dates would not affect all other bands along the claws as those bands were deposited during previous years and thus contain keratin deposited during the entire gestation period.

This study only presents ringed seal claw progesterone concentrations from the most proximal claw band. However, the validations and methods presented here may also be used to assess cortisol and progesterone from other bands along the length of ringed and bearded seal claws. As some claws contained up to 12 years' worth of bands, this study introduces the potential to describe changes in progesterone and cortisol for a substantial portion of a seal's lifetime. Assuming the lateral walls of seal claws contain an undiluted time series of information, the hormones in claws represent concentrations in circulation and are inert once deposited; analyses of serial bands of claw growth give researchers the ability to examine annual pregnancy status and changes in chronic physiological stress, among other things. This would be especially valuable for retrospective studies examining responses to unexpected changes when researchers do not have notice to collect pre-event data (e.g. unusual mortality events, extreme weather, chemical spills, or any other abrupt changes). Furthermore, the long-term physiological data available from claws could contribute to species management and conservation planning and be used for a broad range of ecological studies.

Recommendations for future studies

(1) Standardized processing methods should include a mixer-mill pulverization step so that claw hormone concentrations can be comparable among studies. (2) Studies examining the timing and pattern of keratin deposition into ringed and bearded seal claws would be invaluable, especially to confirm that the lateral walls of the claw sheaths contain an undiluted time series of information. (3) In this study, claws were soaked in water for extended periods to allow the connective tissue that holds the claw sheath to the underlying bone to degrade. This required that the claw remain wet, otherwise the underlying tissues would dry, and the claw sheath would not disassociate. However, cortisol concentrations in primate hair decreased significantly with repeated exposure to soap and water (Hamel *et al*., 2011, Li *et al*., 2012). This raises the possibility that some claw hormone could have been lost in the water baths. We could not directly measure this because the baths contained oils and other substances from non-keratin tissues. Perhaps future studies using claws could prevent drying using alternate measures such as sealed containers or exposure to humidity. (4) The use of hormone concentrations stored in claws to recreate a timeline of hormone concentrations requires that claw hormones represent concentrations in circulation and remain inert once deposited. However, cortisol concentrations in baleen plates of bowhead whales (*Balaena mysticetus*; Hunt *et al*., 2014) were highest at the base and declined moving toward the tip. This pattern suggests hormone loss over time or inclusion of hormone from sources other than circulation at the base of the baleen. Yet, the same pattern was not found in the concentrations of progesterone, another steroid hormone, in the baleen of North Atlantic right whales (*Eubalaena glacialis*; Hunt *et al*., 2016). This demonstrates the need for a better understanding of the source and stability of steroid hormones in keratinous material.

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Examination of relationships between stable isotopes and cortisol concentrations along the length of phocid whiskers

Shawna A. Karpovich,[1] Marine Mammals Program, Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701, U.S.A.; **John P. Skinner,** Marine Mammals Program, Alaska Department of Fish and Game, 525 W. 67th Avenue, Anchorage, Alaska 99518, U.S.A.; **Luciene A. Kapronczai,** Department of Veterinary Biomedical Sciences, University of Saskatchewan, 44 Campus Drive, Saskatoon, Saskatchewan S7N 5B3, Canada; **Justin A. Smith,** Institute of Arctic Biology, Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, Alaska 99775, U.S.A.; **David M. Janz,** Department of Veterinary Biomedical Sciences, University of Saskatchewan, 44 Campus Drive, Saskatoon, Saskatchewan S7N 5B3, Canada.

Abstract

Alaskan seals are found in remote and sometimes inaccessible locations, making it difficult to collect time-series information. This study explores a novel method to examine temporal changes in diet and physiological status of ringed (*Pusa hispida*), spotted (*Phoca largha*), and harbor (*Phoca vitulina*) seals using cortisol concentrations and $\delta^{15}N$ and $\delta^{13}C$ stable isotopes (SIs) measured in serial sections of whiskers. As whiskers grow, whisker tissue is deposited sequentially making these measurements temporally aligned. Whisker cortisol presented in a distinct pattern with elevated concentrations at the root section followed by a curvilinear decline moving toward the tip of most whiskers. Comparing SIs at the root to the rest of the whiskers, $\delta^{13}C$ values were slightly lower in ringed and harbor seal whiskers and $\delta^{15}N$ values were slightly higher in harbor seal whiskers. The data were modeled controlling for the observed trends in cortisol concentrations and further associations between cortisol concentrations and SIs were detected in spotted and harbor seal whiskers. Additional research examining the source and stability of whisker cortisol is warranted. However, the methods presented here demonstrate that whiskers could prove valuable to gather long-term and naturally aligned dietary and physiological information.

Key words: whisker, vibrissa, cortisol, stable isotope, $\delta^{13}C$, $\delta^{15}N$, *Pusa hispida*, *Phoca largha*, *Phoca vitulina*.

[1]Corresponding author (e-mail: shawna.karpovich@alaska.gov).

Alaskan pinnipeds are experiencing changes in climate and sea ice extent, and these changes are predicted to intensify over time (*e.g.*, Douglas 2010). Unfortunately, the current status of ringed and spotted seal populations are poorly understood due to the difficulty associated with obtaining accurate counts of seals dispersed across the vast and sometimes inaccessible sea ice. Each pinniped species will be uniquely affected by the ongoing and future changes to their habitats resulting from differences in prey and habitat requirements, therefore it is essential to develop methods to assess changes in diet and physiological status in difficult to study species.

As marine environments are altered, prey species will likely be affected leading to changes in phocid diets. Direct methods of diet investigations *via* stomach and scat content include biases such as underrepresentation of partially consumed or soft-bodied prey (Harvey and Antonelis 1994, Arim and Naya 2003). More recently indirect methods, such as stable isotope (SI) analyses, have been used to assess diet in many species (Hobson 1999, Dehn *et al.* 2007, Herreman *et al.* 2009). Variations in isotope ratios of N and C can provide information on trophic level and foraging area. Within a food web, the ratios of naturally occurring stable nitrogen isotopes (expressed as $\delta^{15}N$) are predictably enriched in a stepwise fashion with trophic level (Newsome *et al.* 2010). Stable carbon isotopes (expressed as $\delta^{13}C$) are enriched to a lesser extent by trophic level; instead the largest differences are associated with habitat use or foraging locations as carbon is more depleted in offshore pelagic prey than nearshore benthic prey (*e.g.*, Newsome *et al.* 2010). SI values in most animal tissues represent an average of diet consumed over a tissue-specific period, *e.g.*, days to months due to different rates of tissue regeneration or turnover (Tieszen *et al.* 1983, Hobson *et al.* 1996). Therefore, methods commonly used to describe diets provide information over distinct periods, and repeated sampling is required to characterize temporal changes in diet. In remote areas or during winters in Alaska, repeated sampling of pinnipeds is time consuming, expensive, and at times impossible. Therefore, whiskers that contain metabolically inert information deposited during the previous ≥1 yr for phocids and several years for otariids (Hall-Aspland *et al.* 2005, Stricker *et al.* 2015, Beltran *et al.* 2016, Lübcker *et al.* 2016, McHuron *et al.* 2016) are becoming an increasingly popular tool to create dietary reconstructions (Hobson *et al.* 1996, Newland *et al.* 2011, de la Vega *et al.* 2016). These studies emphasize the utility of whiskers for tracking diet, yet to date there are no methods that pair physiological parameters with the stable isotope information gained from whiskers.

The current and predicted changes in habitat and prey will have negative, positive, or neutral effects on seal species, depending on their life history and ecology; therefore it is essential to develop tools to assess physiological responses to changing conditions, particularly diet. Cortisol, the primary glucocorticoid in pinnipeds (DeRoos and Bern 1961), is released when animals perceive a stressor leading to a suite of behavioral and physiological changes (*e.g.*, Sapolsky 1990, Wingfield 2003) including mobilizing fatty acids and increasing gluconeogenesis (Gil *et al.* 1985, Atkinson *et al.* 2015). Cortisol also plays a significant role during phocid fasting, lactation (Engelhard *et al.* 2002, Ortiz *et al.*

2003), and molt (Riviere *et al.* 1977, Ashwell-Erickson *et al.* 1986, Kershaw and Hall 2016). Further, cortisol is associated with energy balance (Strack *et al.* 1995), and changes in body condition or diet have been associated with circulating stress-related hormones in birds (Kitaysky *et al.* 1999, Cockrem *et al.* 2006), terrestrial mammals (Barboza *et al.* 2004, George *et al.* 2014), and seals (Bartsh *et al.* 1992). Therefore, elevated cortisol concentrations can indicate energetic demand or deviation from physiological homeostasis (Bonier *et al.* 2009). Consequently, a method that tracks cortisol concentrations in association with SI values will be beneficial for understanding how animals are responding to a changing environment.

Steroid hormones, including cortisol, accumulate in keratinized mammalian tissues such as hair (Macbeth *et al.* 2010, 2012; Meise *et al.* 2016) and baleen (Hunt *et al.* 2014), suggesting that it may be possible to use these keratinized tissues to evaluate stress during the period the keratin was deposited. For example, hair cortisol concentrations were elevated following administration of adrenocorticotropic hormone (ACTH) in lynx (*Lynx canadensis*) (Terwissen *et al.* 2013), and in response to chronic stress in rhesus monkeys (*Macaca mulatta*) (Davenport *et al.* 2008) and domestic dogs (*Canis lupus familiaris*) (Siniscalchi *et al.* 2013). Hair cortisol concentrations were also used to assess environmental stressors and contaminant levels in polar bears (*Ursus maritimus*) (Bechshøft *et al.* 2012*a*, 2012*b*, 2013, 2015) and nutritional stress in brown bears (*Ursus arctos*) (Bryan *et al.* 2013). These findings signify that cortisol, measured in hair, can be used as an index of circulating concentrations, even though the mechanisms of cortisol incorporation into keratin are not understood.

An advantage of examining cortisol concentrations in metabolically inert tissues, such as hair or whiskers, is the ability to avoid the acutely elevated serum cortisol concentrations associated with capture or harvest that can obscure baseline circulating levels (Harcourt *et al.* 2010, Champagne *et al.* 2012, Keogh *et al.* 2013). However, in phocids, hair growth occurs over about 2.5 mo (Ashwell-Erickson *et al.* 1986), which is a relatively short period compared to whiskers that may continue to grow for a year or more (Zhao and Schell 2004, Beltran *et al.* 2015, Lübcker *et al.* 2016, McHuron *et al.* 2016). Due to the similarity between whiskers and hair and the utility of other keratin tissues, such as baleen, to measure steroid hormones (Hunt *et al.* 2014); it is likely that cortisol is incorporated into whiskers during growth, and analyses of sections along the length of the whisker will allow researchers to recreate a timetable of changes in cortisol concentrations over time.

This study measured cortisol concentrations and stable carbon and nitrogen values in serially sampled sections of whiskers from three species of phocids that inhabit Alaskan waters: ringed seals (*Pusa hispida*), spotted seals (*Phoca largha*), and harbor seals (*Phoca vitulina*). The objectives of this study were: (1) determine whether measurable levels of cortisol were present in phocid whiskers, (2) compare whisker cortisol concentrations among and within three phocid species, (3) determine whether cortisol concentrations varied along the length of whiskers, and (4) characterize associations between $\delta^{13}C$ and $\delta^{15}N$ values and cortisol

concentrations from serial sections of whiskers. It is not understood by which process cortisol is incorporated into keratin, a nonlipid matrix. As a result, the relationships shown in this study are largely a result of exploratory analyses and we do not endeavor to explain the factors driving differences in cortisol concentrations or stable isotope values measured among or within phocid whiskers. Instead, results are presented to show that this novel method, using serial sections of whiskers, has the potential to describe concurrent changes in diet (SIs) and physiological homeostasis (cortisol) over time.

Methods

Whisker Collection and Handling

During 2009 and 2010 the longest whiskers were collected from adult and subadult (age estimate > 3 yr) ringed (n = 20,), spotted (n = 20), and harbor seals (n = 28) (Table 1). The ringed and spotted seal whiskers were obtained from animals harvested for subsistence purposes. The spotted seal whiskers were collected at Shishmaref in the Chukchi Sea, the ringed seal whiskers were collected at Shishmaref, Diomede in the Bering Strait, and Gambell and Hooper Bay in the Bering Sea. Whole cheek pads were collected and stored at −20ºC for ∼2–3 yr until whiskers were extracted and stored in paper envelopes at room temperature for <1 yr prior to analysis. Harbor seals were live-captured in Tracy and Endicott Arms, Southeast Alaska, sedated (Diazepam 0.25 mg/kg), and manually restrained. Whiskers were extracted with pliers so that the root portion remained intact and stored in whirl-paks or paper envelopes at room temperature for 2–4 yr until analyzed.

Whisker Preparation and Sectioning

Whiskers were decontaminated using methods similar to those used for hair preparation in Macbeth *et al.* (2010). Some whiskers had small amounts of follicle tissue that remained adhered to the proximal (root) end, which was removed using fine forceps under a dissecting microscope. Whole whiskers were washed in 100% methanol then sonicated in deionized water for 30 min and dried in an oven at 60ºC for ≥ 12 h. Whiskers were then serially sectioned (Fig. S1) and individual sections were analyzed separately for concentrations of cortisol or SI values (Table 1). To track locations along the whisker (Lw), zero was assigned to the root end and each 1 mm increment along the whisker shaft was assigned a sequential number. For SI analysis, 8–17 sections (1–4 mm) were excised intermittently along the whisker shaft. The spacing between SI sections was shorter at the root than at the tip since whisker growth slows over time and a longer period is represented per unit of length at the root for phocids (Zhao and Schell 2004, Beltran *et al.* 2015, Lübcker *et al.* 2016, McHuron *et al.* 2016). Generally, four sections were removed from the first 11 mm at 0–1 mm, 4–5 mm, 7–8 mm, and 10–11 mm. Distal to 11 mm, 1–4 mm sections were removed at about every 10 mm until the tip was reached (Fig. S1). Depending on the

Table 1. Sample sizes, mean ($\overline{x}$), SD, and ranges of whisker lengths, collection day, cortisol concentrations, and $\delta^{15}N$ and $\delta^{13}C$ values from whisker sections for each species.

	Ringed	Spotted	Harbor
Whiskers			
sample size (whole whiskers)	20	20	28
whisker length $\overline{x}$ ± SD (mm)	95 ± 12	108 ± 18	109 ± 13
whisker length range (mm)	69–124	60–130	72–130
collection day of year $\overline{x}$ ± SD	30 Nov ± 49 d	8 Oct ± 7 d	15 May ± 26 d
collection day of year range	3 Oct–27 Apr	2 Oct–25 Oct	29 Apr–14 Jul
collection years	2009–2010	2009–2010	2009–2010
Cortisol			
combined sections (lost *via* error)	1 (7)	0 (0)	32 (5)
sections analyzed	67	112	115
sections below detectable limit	5	4	18
concentration $\overline{x}$ ± SD (pg/mg)	6.0 ± 5.8	3.1 ± 2.0	2.5 ± 1.9
concentration range (pg/mg)	0.6–28.4	0.3–10.9	0.3–12.6
Stable isotopes			
sections analyzed	245	257	445
$\delta^{15}N$ $\overline{x}$ ± SD (‰)	17.2 ± 1.3	17.3 ± 1.0	15.6 ± 0.7
$\delta^{15}N$ range (‰)	13.6–20.8	13.7–19.3	13.7–17.8
$\delta^{13}C$ $\overline{x}$ ± SD (‰)	−17.6 ± 1.3	−16.6 ± 0.7	−14.4 ± 0.5
$\delta^{13}C$ range (‰)	−21.3–−15.2	−20.8–−14.9	−17.0–−12.9

degree of narrowing at the whisker tip, distal SI section lengths were usually increased to 2–4 mm. After sections for SI analysis were removed, the remaining pieces were combined to form 3–7 larger sections. Samples that were analyzed for cortisol concentrations, from single or combined sections, were ≥15 mm and ranged from 0.7 and 15.5 mg (Fig. S1).

Cortisol Extraction

Whisker sections (n = 294, Table 1) designated for cortisol analysis were homogenized to a fine powder using a mixer mill (Retsch model

MM301, Retsch Inc., Newtown, PA) or a Wig-L-Bug Almalgamator (Crescent Dental MFG Co.). Cortisol was extracted by adding 0.5 mL of HPLC-grade methanol to each powdered sample (0.7–15.5 mg) and placing samples on a slow rotator for 24 h at room temperature. Samples were then centrifuged for 15 min at 2,150 g at 20ºC and the supernatant was collected. To ensure all extracted cortisol was recovered, the powdered samples were rinsed twice more with 0.5 mL methanol, vortexed for 1 min, centrifuged, and the supernatant from each methanol rinse was combined in the same tube and dried under a gentle stream of nitrogen gas at 38ºC. The extract was reconstituted with 0.2 mL phosphate buffer for 24 h at 4ºC. Sample cortisol concentrations were analyzed in triplicate using an enzyme-linked immunosorbent assay (ELISA) kit (Oxford EA-65 Cortisol EIA kit, Oxford Biomedical, Lansing, MI). To ensure sufficient sample mass for cortisol extraction two (n = 29) or three (n = 4) adjacent sections were combined prior to analysis, 12 samples produced no results due to lab error, and 27 were below the detection limit (BDL) of the assay (Table 1). If the result was BDL, 50% of the detectable limit was used to estimate cortisol concentrations for that section (Cohen and Ryan 1989).

Performance characteristics of the ELISA were determined using five harbor seal whiskers. Intraassay and interassay percent coefficients of variation (% CV) were 7.36% (n = 5) and 9.41% (n = 10), respectively. Parallelism between serially diluted harbor seal whisker extracts and the ELISA kit standard curve was observed (r^2 = 0.998, P < 0.001). Cortisol extraction efficiency was 99.8% ± 4.8% (± SEM, n = 3). Optimal sample mass was determined to be 5 mg, approximately 15 mm of whisker length, but some distal sections were longer to accommodate for the narrowing of the whisker toward the tip and some samples had lower masses from material loss when transferring between containers.

Stable Isotopes

Whisker sections (n = 947, Table 1) designated for SI analysis were prepared using modified methods from Nash *et al.* (2009). Each section was placed individually into a vial, sonicated in a 2:1 chloroform and methanol solution for 30 min, dried at 60ºC for 30 min, sonicated in deionized water for 30 min, dried again, weighed, and sealed into a tin capsule for SI analysis. Stable carbon and nitrogen isotope analyses of whisker sections were conducted at the Alaska Stable Isotope Facility at the University of Alaska Fairbanks using an elemental analyzer (Costech Analytical Technologies, Inc., ESC 4010) interfaced to an isotope ratio mass spectrometer (ThermoFisher, DeltaPlusXP). Typical precision for sample analysis was SD ≤ 0.05‰ for $\delta^{13}C$ values and SD ≤ 0.07‰ for $\delta^{15}N$ values. Results are presented in the conventional delta (δ) notation as parts per thousand (‰) deviation from the international standards VPDB (carbon) and N_{AIR} (nitrogen), using the equations $\delta^{15}N = [(\text{sample } {}^{15}N/{}^{14}N)/(\text{standard } {}^{15}N/{}^{14}N) - 1] \times 1{,}000$ or $\delta^{13}C = [(\text{sample } {}^{13}C/{}^{12}C)/(\text{standard } {}^{13}C/{}^{12}C) - 1] \times 1{,}000$.

Table 2. Generalized additive mixed models (GAMs) describing differences in cortisol concentrations, $\delta^{13}C$, or $\delta^{15}N$ along the length of whiskers (*Lw*) and between sexes (*Sx*). Data for each species were modeled separately and predictor variables were considered statically significant. Adjusted proportions of variance explained (r^2_{adj}), model log-likelihoods [log($\mathscr{L}$)], second order Akaike information criterion (AICc), delta AIC (Δ_{ic}), and model weights (w_i) are shown. See Tables S1–S3 for a list of the top five models.

Measure	Seal sp.	Model terms	r^2_{adj}	log($\mathscr{L}$)	AICc	Δ_{ic}	w_i
Cortisol	Ringed	*Lw*	0.46	−69.4	163.7	0.00	0.55
Cortisol	Spotted	*Lw*	0.72	−27.1	103.9	0.00	0.55
Cortisol	Harbor	*Sx* + *Lw* + *Lw* × *Sx*	0.59	−63.3	193.3	0.00	0.93
$\delta^{13}C$	Ringed	*Lw*	0.53	−303.5	657.0	0.00	0.48
$\delta^{13}C$	Spotted	null	0.15	−252.4	539.1	1.41	0.19
$\delta^{13}C$	Harbor	*Lw*	0.45	−168.7	407.5	0.00	0.61
$\delta^{15}N$	Ringed	null	0.29	−357.0	755.0	1.68	0.16
$\delta^{15}N$	Spotted	null	0.39	−283.8	611.3	0.00	0.41
$\delta^{15}N$	Harbor	*Sx* + *Lw*	0.49	−249.9	573.9	0.00	0.57

Factors influencing whisker cortisol and SIs—To estimate trends in whisker cortisol, $\delta^{13}C$, and $\delta^{15}N$ values (response variables) with respect to seal sex (*Sx*) and the location along the whiskers where sample sections were obtained (*Lw*), generalized additive mixed models (GAMs) were used. Models were run independently for each species. For each response variable, a global model was constructed that included sex as a factor variable, location along the whisker as a penalized cubic regression spline (CRS) smoother term, and a sex and location along the whisker (*Lw* × *Sx*) interaction term as a CRS smoother term. Smoother terms were limited to five degrees of freedom ($k = 6$). Statistically significant predictors were chosen using AIC model selection (Burnham and Anderson 2002) (Table 2, see Appendix S1 for all model results).

Associations between cortisol and SIs—SI sections were both smaller than and unevenly distributed within cortisol sections (Fig. S1). As a result, the timescale represented by SIs was mismatched with respect to that of cortisol concentrations. To address this, trends in $\delta^{13}C$ and $\delta^{15}N$ values were modeled with respect to location along the whisker of each whisker using least-squares second-degree local polynomial regressions (Cleveland and Devlin 1988). Optimal fit was achieved by adjusting the degree of smoothing (*i.e.*, "span" parameter, 0.33 for all splines) to ensure suitable visual fit to SIs. To allow a comparison between cortisol concentrations and SIs on the same time scale, estimates for $\delta^{13}C$ and $\delta^{15}N$ values, temporally aligned with cortisol concentrations, were calculated as the mean of 100 evenly spaced predicted values along the polynomial trend curves within lengths of whisker representing each section that was homogenized and analyzed for cortisol concentrations.

After the trends in cortisol and $\delta^{13}C$ and $\delta^{15}N$ values were described, we then used GAMs to estimate the strength of associations between cortisol and averaged $\delta^{13}C$ and $\delta^{15}N$ values while controlling trends in

cortisol concentrations associated with sex and location along the whisker (described above in *Factors influencing whisker cortisol and SIs)*. A global model was constructed with cortisol as the response variable, and sex, location along the whisker, $\delta^{13}C$, and $\delta^{15}N$ values included as predictor variables both as main effects and in two-way interactions. Sex was included as a factor variable while all other effects were included as smoother terms (CRS, $k = 6$).

All GAM analyses were conducted using R software version 3.3.1 (R Core Team 2015) and model fitting was performed using R package "gamm4" (Wood and Scheipl 2016). All GAMs used an identity link function, assumed a Gaussian (*i.e.*, normal) distribution for residuals, and were fit by maximum likelihood. Models included a whisker-specific random intercept and *Lw* slope term to account for sample correlations. Model residuals were visually inspected to detect nonlinear trends and ensure that they were normally distributed against all predictors. During preliminary inspections, it was found that cortisol concentrations (response variable) needed to be transformed using a natural logarithm to meet modeling assumptions. For more detailed modeling information, see Appendix S1.

Results

Whisker Cortisol Concentrations

Cortisol concentrations were highest near the root and declined in subsequent sections moving toward the tip (Fig. 1A, 2B, 3A, and 3B). However, all three species had some notable exceptions to the observed patterns. For example, whiskers from five ringed (Fig. 1A), two spotted (Fig. 1B), and two harbor seals (Fig. 2B) had the highest cortisol concentrations in sections distal to the root, and two of these whiskers had the highest concentrations in the distal half of the whiskers (Fig. 1B, 2B). The highest cortisol concentrations and the largest degree of variation were found in ringed seal whiskers (Table 1). Average cortisol concentrations in spotted and harbor seal whisker sections were lower than ringed seal whiskers by 2.9 pg/mg and 3.1 pg/mg, respectively (Table 1).

Whisker SIs

There was a high degree of inter- and intrawhisker variability in whisker SI values. Harbor seal whisker $\delta^{15}N$ values were lower than ringed and spotted seal whiskers by 1.7‰ and 1.6‰, respectively (Table 1). Harbor seal whiskers had the highest average $\delta^{13}C$ value, spotted and ringed seal whiskers averages were 2.2‰ and 3.2‰ lower, respectively (Table 1).

Trends in Whisker Cortisol Concentrations

Location along the whisker was a significant predictor of cortisol concentrations in the whiskers of both ringed ($F = 44.6$, $P < 0.001$; estimated degree of freedom, edf = 1.0) and spotted seals ($F = 23.9$, $P < 0.001$, edf = 2.9), however, sex was not (Table 2). In harbor seal whiskers, sex ($\beta_{\text{males}} = -0.04$, SE = 0.15), location along the whisker

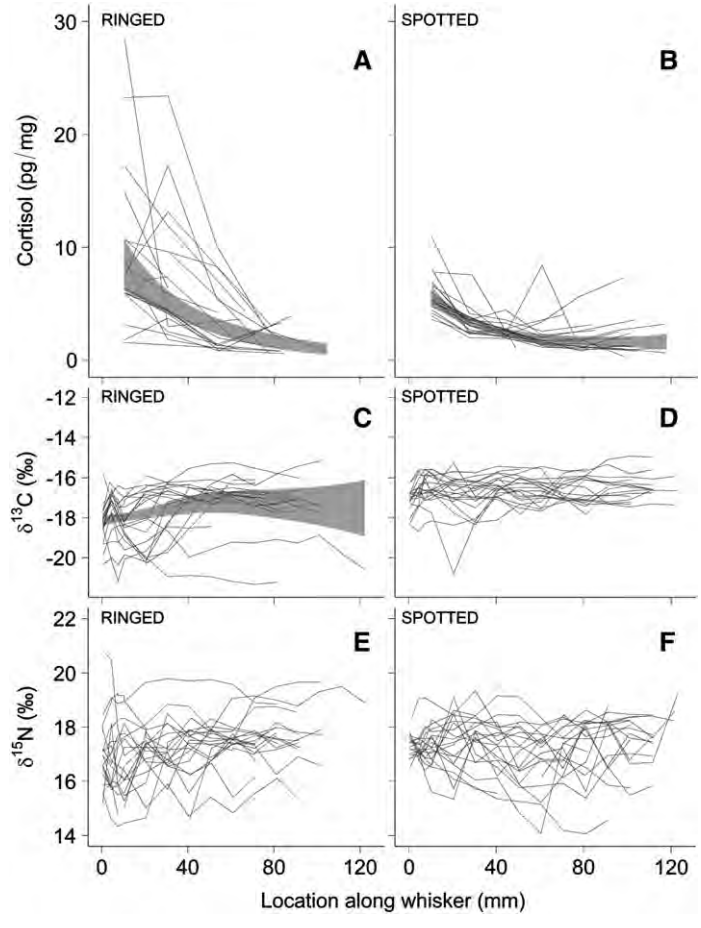

Figure 1. Measured values of cortisol concentrations, δ^{13}C, and δ^{15}N along the length of whiskers of ringed and spotted seals (black lines) and the 95% confidence intervals for model predicted responses (gray regions). Predicted response trends were based on best fit generalized additive mixed models. Cortisol concentrations declined curvilinearly across the length of whiskers of both ringed (A) and spotted (B) seals while δ^{13}C values exhibited a slight increase from root to about 50 mm (C). There was no significant trend in δ^{13}C along whiskers of spotted seals (D). Additionally, no significant trends were found for δ^{15}N along whiskers of ringed (E) or spotted seals (F).

($F = 20.8$, $P < 0.001$, edf = 2.1), and an interaction between the two (*Lw* × *Sx)* ($F = 4.94$, $P = 0.029$, edf = 1.0), were statistically significant predictor variables for cortisol concentrations (Table 2). The shape and amplitude of the trends in cortisol concentrations along the length of the whiskers differed markedly between species. Cortisol concentrations declined sharply from root to tip in ringed seal whiskers (Fig. 1A), from root to about 80 mm in spotted seal whiskers (Fig. 1B), and gradually from root to tip in harbor seal whiskers (Fig. 2A, B). Compared to

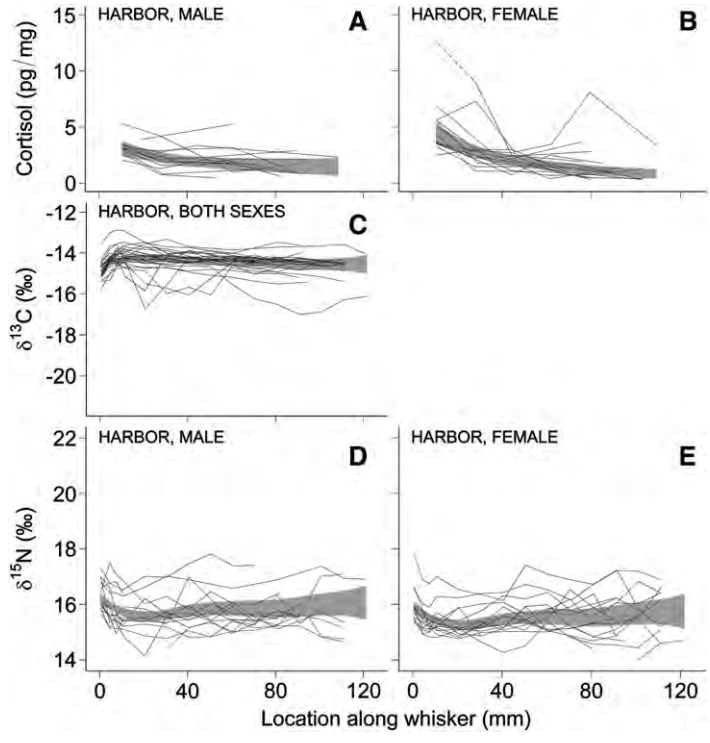

Figure 2. Measured values of cortisol concentrations, $\delta^{13}C$, and $\delta^{15}N$ along the length of whiskers of male and female harbor seals (black lines) and 95% confidence intervals for the predicted response (gray region). Predicted response trends were based on the best fit generalized additive mixed models. Along the length of harbor seal whiskers, cortisol concentrations declined in both sexes but the degree of decline was less steep in males (A) compared to females (B). Values of $\delta^{13}C$ exhibited a short and steep rise followed by a long gradual decline over the length of whiskers. Overall whisker $\delta^{15}N$ values were higher for males (D) than for females (E) but both sexes exhibited a similar trend of a short decline and then gradual rise along the lengths of whiskers.

female harbor seals, whisker cortisol was slightly lower near the root for males (Fig. 2A, B). GAM trends suggested that cortisol concentrations in harbor seal whiskers were lower near the root and declined less steeply over whisker length compared to ringed and spotted seal whiskers.

Trends in Whisker $\delta^{13}C$ Values

Location along the whisker was a significant predictor of the $\delta^{13}C$ value trends in the whiskers of both ringed ($F = 4.23$, $P = 0.005$, edf = 2.7) and harbor seals ($F = 5.84$, $P < 0.001$, edf = 5.07) but not spotted seals (Table 2). Additionally, $\delta^{13}C$ values did not vary by sex in any species (Table 2). In ringed seal whiskers, $\delta^{13}C$ values increased gradually from the root to about 50 mm but then exhibited no significant trend toward the tip (Fig. 1C). In harbor seal whiskers, $\delta^{13}C$ values increased

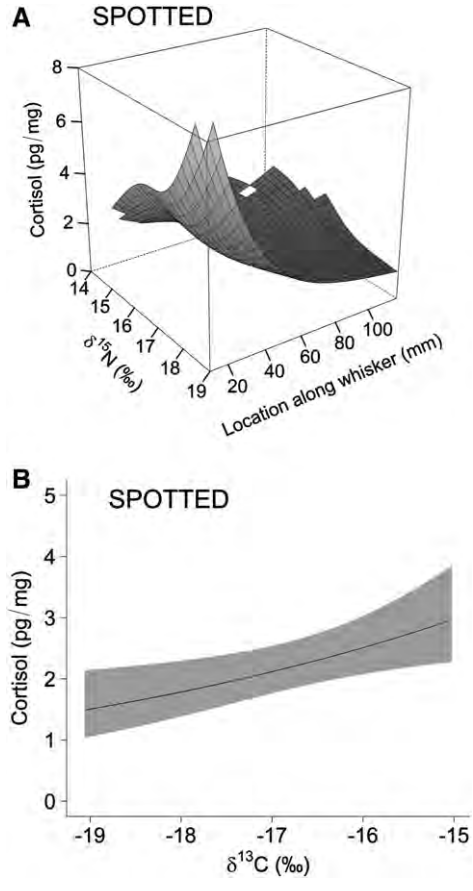

Figure 3. Response plots showing changes in spotted seal whisker cortisol concentrations in association with SI values. Trends in cortisol over the length of spotted seal whiskers varied with values of $\delta^{15}N$ (A) while cortisol was positively associated with $\delta^{13}C$ values independent of seal sex or location along the whiskers (B) with 95% confidence intervals for the predicted response (gray region).

sharply from the root to about 10 mm and then declined gradually toward the tip (Fig. 2C). No significant trends were found for $\delta^{13}C$ values in spotted seal whiskers because values, with some exceptions, appeared to remain relatively stable within and across whiskers (Fig. 1D).

Trends in Whisker $\delta^{15}N$ Values

Neither location along the whisker nor sex adequately explained variation in spotted or ringed seal whisker $\delta^{15}N$ values (Fig. 1E, F). In harbor seal whiskers, both location along the whisker ($F = 5.83$, $P < 0.001$, edf = 5.1) and sex ($\beta_{males} = 0.31$, SE = 0.08) were significant predictors of $\delta^{15}N$ values (Table 2). Male harbor seal whiskers had slightly higher

Table 3. Generalized additive mixed models (GAMs) describing associations between cortisol concentrations and interpolated $\delta^{13}C$ and $\delta^{15}N$ values after controlling for variation along the length of whiskers (*Lw*) and between sexes (*Sx*). Results are presented separately for each species. The model with only *Lw* was the best fit for ringed seal whisker data, which indicated no significant association between whisker cortisol concentrations and SI values. See Table 2 for complete description and definitions for statistics abbreviated below and Table S4 for a list of the top five models.

Seal sp.	Model terms	r^2_{adj}	log()	AICc	Δ_{ic}	w_i
Ringed	*Lw*	0.62	−53.5	149.9	0	0.32
Spotted	$Lw + \delta^{13}C + \delta^{15}N + \delta^{15}N \times Lw$	0.68	−32.5	126.0	0	0.36
Harbor	$Sx + Lw + Lw \times Sx + \delta^{15}N + \delta^{15}N \times Lw$	0.58	−62.7	197.0	0	0.62

$\delta^{15}N$ values overall, and for both sexes, $\delta^{15}N$ values declined from the root to 20 mm and then gradually increased toward the tip (Fig. 2D, E).

Associations Between Cortisol and SIs

After controlling for the previously found significant trend between whisker cortisol concentrations and location along the whisker, no additional associations were found between cortisol concentrations and SIs in ringed seal whiskers (Table 3).

For spotted seal whiskers, additional variation in cortisol concentrations was explained by $\delta^{13}C$ values ($F = 6.93$, $P = 0.01$, edf = 1.0), $\delta^{15}N$ values ($F = 1.87$, $P = 0.19$, edf = 3.0), and an interaction between $\delta^{15}N$ values and location along the whisker ($\delta^{15}NxLw$) ($F = 9.94$, $P = 0.002$, edf = 1.0) (Tables 3, S4). This indicates that once the trend in cortisol concentrations associated with location along the whisker (Fig. 1B) was accounted for, cortisol concentrations were highest in sections that also had higher $\delta^{15}N$ values near the whisker root, and cortisol concentrations near the root diminished in magnitude with respect to middle $\delta^{15}N$ values and disappear at the lowest $\delta^{15}N$ values (Fig. 3A). About 5 pg/mg of variation in cortisol concentrations could be attributed to $\delta^{15}N$ values near spotted seal whisker roots. At the tips of spotted seal whiskers, the association between cortisol concentrations and $\delta^{15}N$ values reverses and cortisol concentrations were lowest, instead of highest, in sections that had higher $\delta^{15}N$ values, accounting for a difference of ~1 pg/mg of variation in cortisol concentrations. Cortisol concentrations in spotted seal whiskers were positively associated with $\delta^{13}C$ values. This indicates that after the trend in cortisol associated with location along the whisker (Fig. 1B) was accounted for, about 1 pg/mg of increase in cortisol was associated with a 4‰ increase in $\delta^{13}C$ values (Fig. 3B).

Harbor seal whisker cortisol concentrations were associated with $\delta^{15}N$ values ($F = 2.43$, $P = 0.06$, edf = 2.41) and an interaction between $\delta^{15}N$ values and location along the whisker ($\delta^{15}N \times Lw$) ($F = 4.80$, $P = 0.01$, edf = 2.0). This means that after the trends in cortisol concentrations

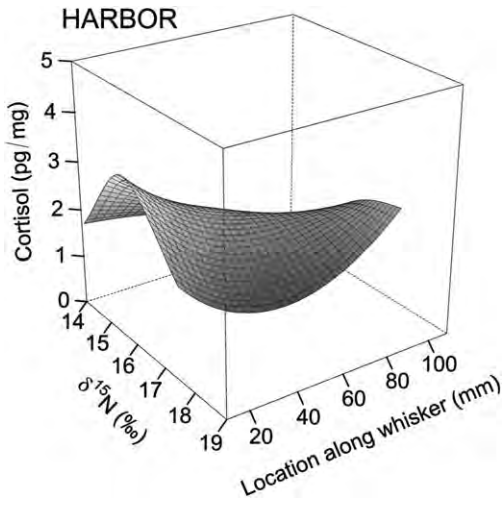

Figure 4. Three-dimensional predicted response of harbor seal whisker cortisol concentration trends in association with $\delta^{15}N$ values along the length of the whiskers.

associated with location along the whisker and sex (Fig. 2A, B) were accounted for, cortisol concentrations in harbor seal whisker root sections were highest in the mid-range of $\delta^{15}N$ values, and at the tip, cortisol concentrations were highest at the highest $\delta^{15}N$ values (Fig. 4).

Discussion

To our knowledge, this is the first study to show that steroid hormones can be measured in mammalian whiskers. Furthermore, we showed that all three phocid species had a persistent pattern of elevated cortisol near the whisker roots; and after controlling for this trend in cortisol concentrations across the whisker lengths and by sex, associations exist between cortisol concentrations and $\delta^{15}N$ and $\delta^{13}C$ values. These findings demonstrate that whiskers could provide important information about associations between diet and physiological status over time because SI values and cortisol concentrations are deposited sequentially with whisker growth and are therefore temporally aligned.

Cortisol

The general premise is that as hair or whiskers grow, cortisol is deposited into the keratin, concentrations measured in keratinous tissues are an index of circulating concentrations, and cortisol remains biochemically unchanged once deposited (Kirschbaum *et al.* 2009). We found that for all three species, the majority of cortisol concentrations measured in serial sections of whiskers exhibited a general pattern with the highest concentrations near the root followed by a curvilinear decrease moving towards the tip. The reason for this observed pattern in cortisol

concentrations is unknown, and possible causes include: (1) temporal changes in circulating cortisol concentrations, (2) differing rates of cortisol inclusion associated with nonlinear whisker growth rates (*i.e.*, concentration or dilution), (3) water immersion causing leaching of cortisol out of the whisker tissue, or (4) inclusion of nonkeratin tissues near the root. Therefore, future research should focus on the mechanisms of cortisol deposition and retention in growing whiskers.

To interpret cortisol concentrations in whiskers, it is necessary to understand when the whisker material included in each section was deposited. Phocid whiskers grow in an asymptotic pattern with very rapid growth that slows to little or no growth within a few months (Beltran *et al.* 2015, Lübcker *et al.* 2016, McHuron *et al.* 2016). Spotted seal whiskers in the McHuron *et al.* (2016) study initiated growth in April and reached asymptotic length within 4 mo. Assuming the whiskers in this study grew and molted in a similar fashion, only a small proportion of the proximal section would be added during the period after asymptotic length was acquired which could be up to 8 mo depending on collection date. It is possible that the elevated cortisol concentrations near the root of ringed, spotted, and harbor seal whiskers reflects a period of high cortisol concentrations in circulation that occurred during the time that the section was deposited. However, the timing associated with the elevated cortisol is difficult to estimate due to the slow growth rate at the whisker root section, the wide range of whisker collection dates, and the fact that the proximal section represents an average of cortisol over 20 mm of whisker material.

It is also possible that cortisol is incorporated into the whisker material at different rates. During the initial rapid whisker growth, cortisol concentrations may be diluted by the large amount of material being deposited, and then more concentrated when whisker growth slows near the root. The whiskers in this study were collected at or near asymptotic lengths, and thus all of the root sections represent the slowest portion of the growth curves; consequently we could not determine if cortisol is deposited differentially during different whisker growth rates.

Another possibility is that persistent immersion in sea water could cause the cortisol to leach out of the whisker material over time. In serial sections of hair from monkeys and humans, cortisol concentrations decreased significantly with repeated exposure to soap and water (Hamel *et al.* 2011, Li *et al.* 2012). This would suggest that phocid whiskers could lose cortisol with repeated exposure to sea water. However, the permeability of the cuticle of hair or whiskers may be substantially different when comparing hair or whiskers handled in the lab to those on live animals. For example, archived harbor seal whiskers, when dyed with commercially available hair dye, readily absorbed the dye and turned a dark black color that was maintained during several months of exposure to moving seawater; yet when the same dye was applied to the whiskers of live seals, the whiskers turned slightly gray and no apparent coloration could be detected after 24 h (Alaska Department of Fish and Game, unpublished data). It seems that the dye readily permeated the cuticle of archived whiskers but could not penetrate the

whisker cuticle on live animals. This suggests that permeability of the whisker cuticle may have changed after the whisker was removed from the animal. Therefore, leaching may occur more readily in a laboratory setting when the hair or whisker cuticle may be different, perhaps more desiccated, than when on a live animal.

Lastly, blood or skin was manually removed from the exterior of whiskers in this study to avoid affecting the results. However, nonkeratin material may be included in the whisker medulla because reddish spots have been observed in the interior of some phocid whiskers root sections (Alaska Department of Fish and Game, unpublished data). This tissue would not be removed by cleaning the outer surface prior to analyses, and the inclusion of blood-related material would cause cortisol concentrations to be elevated due to the stress-related release of cortisol into circulation during capture or harvest (*e.g.*, Sapolsky 1990, Wingfield 2003). Further, inclusion of blood or plasma in the whisker root would result in lower $\delta^{13}C$ but unchanged $\delta^{15}N$ compared to the rest of the whisker (Beltran *et al.* 2016). Lower $\delta^{13}C$ and higher $\delta^{15}N$ at the root of southern elephant seal (*Mirounga leonina*) whiskers was attributed to the inclusion of molecules other than keratin at the whisker root (Lübcker et al. 2016). In this study, spotted seal whiskers had no SI trends; ringed seal whiskers had lower $\delta^{13}C$ at the root compared to the rest of the whisker length and no trend in $\delta^{15}N$; and harbor seal whiskers had lower $\delta^{13}C$ but higher $\delta^{15}N$ at the root compared to the rest of the whisker. Thus, it is unclear from the results of this study if the elevated cortisol concentrations and the SI values at the root of the whiskers sampled could reflect the inclusion of blood-related or other compounds at the root.

Associations Between Cortisol and SIs

The approach used in this study revealed associations between whisker cortisol concentrations and $\delta^{13}C$ and $\delta^{15}N$ values after controlling for variation in cortisol across sexes and along the length of whiskers, but, we do not endeavor to explain these trends. However, associations between these measurements could provide evidence about how life history and behavior can influence individual physiology, and thus success and survival. For example, higher $\delta^{13}C$ is associated with consumption of nearshore/benthic prey and lower $\delta^{13}C$ with offshore/pelagic prey (*e.g.*, Hobson and Welch 1992). Extending that understanding, one might hypothesize that changes in foraging locations, prey availability, or both (as described by changes in $\delta^{13}C$) contributed to different levels of physiological stress for seals (as indicated by changes in cortisol concentrations).

The associations between cortisol concentrations and $\delta^{15}N$ values in spotted and harbor seal whiskers include an interaction with location along the whisker indicating that the association changes throughout the period of whisker growth. For spotted seals, during the period that the most proximal whisker section was deposited, cortisol concentrations were much higher (4 pg/mg) at 19‰ compared to 14‰ $\delta^{15}N$; suggesting that foraging at higher trophic levels contributed to

physiological changes resembling stress (*i.e.*, higher cortisol concentrations). Alternately, the elevated $\delta^{15}N$ values and cortisol concentrations could represent a period of fasting or nutritional stress when protein stores were being mobilized. While seals initially utilize blubber stores to meet energetic needs, there is a point that they switch to protein mobilization to maintain a layer of blubber for insulation (Rosen and Renouf 1997, Mellish *et al.* 2007), and use of body protein stores would increase $\delta^{15}N$ values (*e.g.*, Hobson *et al.* 1993). In spotted seal whisker sections distal to the root, the association between cortisol concentration and $\delta^{15}N$ values flattens, and near the tip the association reverses and higher cortisol is associated with feeding at a lower tropic level. If the lower tropic level prey is of lower quality or more difficult to access, this would agree with previous studies on mammals and birds showing stress-related hormones are inversely correlated food availability (Kitaysky *et al.* 1999) and body mass and condition (Bartsh *et al.* 1992, Barboza *et al.* 2004, Cockrem *et al.* 2006, George *et al.* 2014).

For harbor seals, during the period that the most proximal whisker section was deposited, cortisol concentrations were slightly higher (~1 pg/mg) when $\delta^{15}N$ was 15‰ and 16‰ compared to both higher and lower $\delta^{15}N$ values. This association is lower in magnitude than the spotted seal whiskers. Furthermore, moving toward the tip of harbor seal whiskers, higher cortisol concentrations were associated with higher $\delta^{15}N$ values; this could mean that during the period that the whisker tip was grown, higher stress was associated with feeding on higher trophic level prey or foraging in a manner or area that allows consumption of higher trophic level prey.

The associations described here demonstrate the potential utility of extracting naturally aligned dietary and physiological information from whiskers. The methods presented in this study could facilitate future research tracking how marine or terrestrial mammals are responding to habitat alterations associated with changes in climate or anthropogenic influences.

Conclusions and Recommendations

To understand how cortisol and SIs in whiskers are related, controlled studies should be conducted. Skin or blood-related tissues in whiskers could contribute to elevated cortisol concentrations at the proximal sections, nonlinear whisker growth could cause concentration or dilution of cortisol, and leaching could cause depressed cortisol concentrations in distal sections. It is, therefore, important to determine whether cortisol concentrations relate to seal physiology in a similar way throughout the whisker and, if not, what factors affect the relationship between the concentration of cortisol at the time of deposition and the measurements of cortisol and SIs obtained from whiskers in the laboratory.

For future studies, we recommend several approaches for studying trends in whisker cortisol. First validations of whisker cortisol should be conducted using captive seals *via* exposure to different stress scenarios or ACTH injections, paired with marking of the whiskers to track whisker growth. Whisker collections should be conducted across all months,

and paired measurements of cortisol concentrations in whisker roots and circulation should be conducted. Additionally, to determine if whisker growth rate affects cortisol deposition, cortisol concentrations in phocid whiskers collected prior to being fully grown (less than at asymptotic lengths) and whiskers from other species with constant growth rates should be examined. Studies should be conducted to assess if skin or blood-related tissues are incorporated at the root of whiskers and if cortisol is lost through leaching. Finally, in this study we assumed the laboratory validation of harbor seal whiskers was sufficient for ringed and spotted seal whiskers. However, prior to using whisker cortisol concentrations, laboratory validations should be conducted on whiskers from each species.

The cortisol concentrations presented here for phocid whiskers raise many questions. However, this study has determined that cortisol is present in measurable concentrations in phocid whiskers, varies along the length of the whiskers, and can be measured with commercially available ELISAs. Furthermore, after the unexplained curve of cortisol concentrations was controlled for, we found associations between $\delta^{13}C$ and $\delta^{15}N$ values and cortisol concentrations in spotted and harbor seal whiskers. Analysis of whisker cortisol concentrations could be a powerful tool to gather long-term physiological information from marine and terrestrial animals, but further research is needed to gain a better understanding of the source and stability of the cortisol in whiskers.

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Supporting Information

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Appendix S1. This appendix contains additional details about the model selection process, tables showing the top five candidate models for each comparison, and a map of the sections removed from each whisker for analyses.

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Prevalence of algal toxins in Alaskan marine mammals foraging in a changing arctic and subarctic environment

Kathi A. Lefebvre [a,*], Lori Quakenbush [b], Elizabeth Frame [a,1], Kathy Burek Huntington [c], Gay Sheffield [d], Raphaela Stimmelmayr [e], Anna Bryan [b], Preston Kendrick [a], Heather Ziel [f], Tracey Goldstein [g], Jonathan A. Snyder [h], Tom Gelatt [f], Frances Gulland [i], Bobette Dickerson [f], Verena Gill [h,2]

[a] Northwest Fisheries Science Center, NOAA Fisheries, Seattle, WA, USA
[b] Alaska Department of Fish and Game, Arctic Marine Mammal Program, 1300 College Road, Fairbanks, AK, USA
[c] Alaska Veterinary Pathology Services (AVPS), 23834 The Clearing Drive, Eagle River, AK, USA
[d] University of Alaska Fairbanks, Alaska Sea Grant, Marine Advisory Program, PO Box 400, Nome, AK, USA
[e] North Slope Borough Department of Wildlife Management, PO Box 69, Barrow, AK, USA
[f] Marine Mammal Laboratory, Alaska Fisheries Science Center, NOAA, Seattle, WA, USA
[g] One Health Institute, School of Veterinary Medicine, University of California, Davis, CA, USA
[h] U.S. Fish and Wildlife Service, Marine Mammals Management, 1011 East Tudor Rd., Anchorage, AK, USA
[i] The Marine Mammal Center, 2000 Bunker Road, Fort Cronkhite, Sausalito, CA, USA

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ABSTRACT

Current climate trends resulting in rapid declines in sea ice and increasing water temperatures are likely to expand the northern geographic range and duration of favorable conditions for harmful algal blooms (HABs), making algal toxins a growing concern in Alaskan marine food webs. Two of the most common HAB toxins along the west coast of North America are the neurotoxins domoic acid (DA) and saxitoxin (STX). Over the last 20 years, DA toxicosis has caused significant illness and mortality in marine mammals along the west coast of the USA, but has not been reported to impact marine mammals foraging in Alaskan waters. Saxitoxin, the most potent of the paralytic shellfish poisoning toxins, has been well-documented in shellfish in the Aleutians and Gulf of Alaska for decades and associated with human illnesses and deaths due to consumption of toxic clams. There is little information regarding exposure of Alaskan marine mammals. Here, the spatial patterns and prevalence of DA and STX exposure in Alaskan marine mammals are documented in order to assess health risks to northern populations including those species that are important to the nutritional, cultural, and economic well-being of Alaskan coastal communities. In this study, 905 marine mammals from 13 species were sampled including; humpback whales, bowhead whales, beluga whales, harbor porpoises, northern fur seals, Steller sea lions, harbor seals, ringed seals, bearded seals, spotted seals, ribbon seals, Pacific walruses, and northern sea otters. Domoic acid was detected in all 13 species examined and had the greatest prevalence in bowhead whales (68%) and harbor seals (67%). Saxitoxin was detected in 10 of the 13 species, with the highest prevalence in humpback whales (50%) and bowhead whales (32%). Pacific walruses contained the highest concentrations of both STX and DA, with DA concentrations similar to those detected in California sea lions exhibiting clinical signs of DA toxicosis (seizures) off the coast of Central California, USA. Forty-six individual marine mammals contained detectable concentrations of both toxins emphasizing the potential for combined exposure risks. Additionally, fetuses from a beluga whale, a harbor porpoise and a Steller sea lion contained detectable concentrations of DA documenting maternal toxin transfer in these species. These results provide evidence that HAB toxins are present throughout Alaska waters at levels high enough to be detected in marine mammals and have the potential to impact marine mammal health in the Arctic marine environment.

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* Corresponding author. Tel.: +206 302 2454.
E-mail address: Kathi.Lefebvre@noaa.gov (K.A. Lefebvre).
[1] Current address: Aquatic Toxicology Unit, King County Environmental Laboratory, Seattle, WA, USA.
[2] Current address: Bureau of Ocean Energy Management, 3801 Centerpoint Dr., Anchorage, AK, USA.

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1. Introduction

Harmful algal blooms (HABs) most commonly occur in temperate and tropical regions; however, current climate trends such as ocean warming and loss of seasonal sea ice, are likely to expand the geographic distribution and the duration of conditions that support blooms (Moore et al., 2008; Van Dolah, 2000), making HAB exposure potentially more common among marine mammals in Alaskan waters (Burek et al., 2008). Species of phytoplankton known to be toxic are not new to Alaskan waters. Diatoms of the genus *Pseudo-nitzschia*, have been documented as far north as the eastern Beaufort Sea (Bursa, 1963) and produce domoic acid (DA), the toxin responsible for amnesic shellfish poisoning (ASP). Domoic acid was first detected in low levels in razor clams in Kachemak Bay in July 1992 (RaLonde and Wright, 2011). Dinoflagellates of the genus *Alexandrium* produce toxins that cause paralytic shellfish poisoning (PSP) and have been well documented in Alaskan waters (Gessner and Middaugh, 1995; Gessner et al., 1997; Gessner and Schloss, 1996; Lewitus et al., 2012; Trainer et al., 2014). Saxitoxin (STX) is the most potent of the PSP causing toxins. Both DA and STX affect the central nervous system of vertebrates. Saxitoxin acts as a sodium channel blocker and prevents action potential activity in nerves causing paralysis primarily of the respiratory system (Cusick and Sayler, 2013). Domoic acid is an excitotoxin that over-stimulates glutamate receptors in the vertebrate central nervous system causing stimulation of nerves (Berman et al., 2002; Berman and Murray, 1997; Todd, 1993). While PSP has been documented in humans in Alaska since 1799, the only documented cases of ASP in humans occurred in Southeastern Canada in 1989 (Perl et al., 1990). Unlike temperate regions, no incidences of DA toxicosis and very few incidences of STX toxicosis have been documented in Alaskan marine mammals.

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

The potential for health effects on Alaskan marine mammals may be high considering more than 40% of marine mammal unusual mortality events (UMEs) in the contiguous USA during the last 20 years have been attributed to algal toxin exposure (Flewelling et al., 2005; Gulland and Hall, 2007; Landsberg et al., 2014; Scholin et al., 2000; Torres De La Riva et al., 2009). The number of HAB-related strandings appears to be increasing in the contiguous USA as these events were relatively rare even in temperate regions only two decades ago (Landsberg et al., 2014). The negative impacts of algal toxins on marine mammal health have been well documented along the west coast of the USA. For example, the neurotoxic effects of DA were first reported in stranded California sea lions (*Zalophus californianus*) in 1998 through exposure from toxic planktivorous prey such as northern anchovies and Pacific sardines (Gulland, 2000; Lefebvre et al., 1999; Scholin et al., 2000). Clinical signs of acute DA poisoning in marine mammals include ataxia, head weaving, seizures or coma and/or death (Gulland et al., 2002). The frequency of DA-associated California sea lion strandings has increased since 1998 and strandings now occur annually, affecting hundreds of sea lions per year (Bargu et al., 2010). Additionally, a chronic neurological syndrome associated with repetitive sub-lethal exposure to the toxin is now recognized by behavioral changes, seizures, and atrophy of the hippocampal formation (Cook et al., 2015; Goldstein et al., 2008). Domoic acid has also been documented to cross the placenta of California sea lions and be present in milk, thus, neonates may be exposed *in utero* and after birth until weaning (Brodie et al., 2006; Rust et al., 2014). In addition to contributing to reproductive failure, *in utero* and lactational exposure to DA can result in developmental abnormalities leading to neurological and behavioral deficits in surviving offspring. Given that California sea lions and humans share a common prey base, sea lions serve as important food safety sentinels regarding the presence of HABs near California. The findings associated with DA exposure in California sea lions demonstrate the potential health effects for other marine mammal species, as well as the potential for marine mammals in other regions to be sentinels for public health threats. The effects of STX on marine mammals are not as well documented as they are for DA. The first reported STX-related mortality event involved humpback whales in the late 1980s when 14 humpback whales died near Cape Cod Bay after ingesting mackerel containing STX (Geraci et al., 1989). Saxitoxin was suspected (although not substantiated) to be a factor in 60 sea otter deaths in Alaska (Degange and Vacca, 1989) and in 117 Mediterranean monk seal (*Monachus monachus*) deaths in Western Sahara, Africa (Costas and Lopez-Rodas, 1998).

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

2. Methods

2.1. Marine mammal sample collection:

A variety of samples (feces, stomach contents, intestinal contents, serum, milk, urine, amniotic fluid, bile, aqueous humor, and pleural, peritoneal and pericardial fluid) were collected from Alaskan marine mammals that were stranded, harvested for subsistence purposes, or captured for research. Samples were also collected during the Northern Alaska Pinniped Unusual Mortality Event (UME) (http://www.nmfs.noaa.gov/pr/health/mmume/events.html). Not all sample types were collected from each animal. The majority of the samples consisted of feces, urine, serum and stomach and intestinal contents. Samples were frozen as soon as possible after collection to prevent degradation, although some stranded animals had various levels of degradation. Samples were stored frozen until shipped to the Northwest Fisheries Science Center's Wildlife Algal-Toxin Research and Response Network (WARRN-West) laboratory (Seattle, WA, USA) for algal toxin testing. All live and stranded animal handling was consistent with approved humane practices under the following

permits: Marine Mammal Protection Act (MMPA) permit number MA041309-5, and National Marine Fisheries (NMFS) research permit numbers 358-1787, 15324 and 10091. A summary of the total number of animals, collection period, and locations is shown in Table 1. Additional detailed information on sample collection is provided in the sections below.

*2.1.1. Humpback whale fecal, stomach and intestinal contents, aqueous humor, pleural fluid and urine samples (*n *= 8 animals)*

During 2007–2011, samples were collected from stranded humpback whales from Southeast Alaska ($n = 5$), Kodiak ($n = 2$) and The Alaska Peninsula ($n = 1$). Samples were stored frozen in Whirl-Pak® bags at −40 or −80 °C until analyzed for algal toxins.

*2.1.2. Bowhead whale fecal samples (*n *= 25 animals)*

During 2006–2011, fecal samples from bowhead whales harvested for subsistence purposes were collected during the spring and fall in Barrow, Alaska. Sections of colon were cut and fecal matter was removed using plastic spoons. Samples were stored frozen in Whirl-Pak® bags at −20 °C until analyzed for algal toxins.

*2.1.3. Beluga whale fecal, stomach contents, amniotic fluid, pericardial fluid and urine samples (*n *= 15 animals)*

During 2007–2012, samples were collected from stranded Cook Inlet beluga whales. Three females were pregnant and samples were collected from both the mother and the fetus in all three cases. Stomachs from two belugas harvested for subsistence purposes at Hooper Bay were also collected. Samples were stored frozen in Whirl-Pak® bags at −40 or −80 °C until analyzed for algal toxins.

*2.1.4. Harbor porpoise fecal, stomach and intestinal contents, aqueous humor and urine (*n *= 5 animals)*

During 2010–2013, samples were collected from five stranded harbor porpoises from Cook Inlet, with both mother and fetus analyzed in one case. Samples included feces ($n = 2$), aqueous humor ($n = 1$), stomach ($n = 1$) and intestinal contents ($n = 1$) and urine ($n = 2$) with some animals having multiple sample types analyzed. Samples were stored in Whirl-Pak® bags at −40 or −80 °C until analyzed for algal toxins.

*2.1.5. Northern fur seal fecal and serum samples (*n *= 179 animals)*

Between 7 and 15 October 2010, serum samples were collected from 131 live-captured adult female northern fur seals with pups on Saint George Island (Pribilof Islands) and fecal samples were collected from 48 northern fur seals harvested on Saint Paul Island (Pribilof Islands) for subsistence purposes. Samples were frozen in cryovials or Whirl-Pak® bags and stored at −20 °C until analyzed for algal toxins.

*2.1.6. Steller sea lion fecal, stomach and intestinal contents, amniotic, pleural, peritoneal and pericardial fluid, bile and urine samples (*n *= 42 animals)*

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

*2.1.7. Harbor seal bile, feces, aqueous humor, placenta and urine samples (*n *= 9 animals)*

During 2008–2012, samples were collected from nine stranded harbor seals; three in Southeast Alaska (Bartlett Cove in Glacier Bay, Lynn Canal, and Sitka), four in Southcentral Alaska (Kachemak Bay, Resurrection Bay, Kenai, and Cook Inlet), one in Southwest Alaska (Izembek Lagoon), and one in Bristol Bay (Egegik). Samples were collected in amber vials (bile), Whirl-Pak® bags (feces and placenta) or cryovials (aqueous humor and urine) and stored at −60 °C until analyzed.

*2.1.8. Ice seals (ringed (*n *= 113), bearded (*n *= 55), spotted (*n *= 158), and ribbon seals (*n *= 21)) stomach and intestinal contents, fecal and urine samples*

During 2006–2013, samples from ice seals harvested during spring and fall for subsistence purposes were collected from the coastal communities of Hooper Bay, Savoonga, Gambell, Little Diomede, Shishmaref, Kotzebue, Point Hope, Wainwright and Barrow in the Bering Strait region and Chukchi Sea. Whole stomachs or a piece of intestine were collected in Ziploc® bags. Urine was collected in a centrifuge tube. All samples were frozen and shipped to the Alaska Department of Fish and Game (ADF&G) laboratory in Fairbanks and stored at −20 °C. Stomachs and intestines were thawed and 5 ml of content was removed from each, placed in centrifuge tubes with screw caps, and refrozen. During May of 2009–2010, live captures were conducted by the National Marine Mammal Lab (Alaska Fisheries Science Center's Polar Ecosystems Program) and samples were obtained from ice floes and collected using a metal shovel to scoop the urine soaked ice which was placed in Whirl-Pak® bags and frozen at −80 °C until analyzed for algal toxins.

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

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

2.1.9. Pacific walrus stomach and intestinal content samples (n = 82 animals)

During May 2012–2013, stomach and intestinal contents were collected from walruses harvested for subsistence purposes from the coastal communities of Gambell and Savoonga on Saint Lawrence Island. Hunters collected the samples in situ and brought them to shore where they were frozen on site at −18 °C and shipped to the ADF&G laboratory in Fairbanks and subsequently stored at −20 °C until algal toxin analysis. Stomachs and intestines were thawed and 5 ml of content was removed and placed in centrifuge tubes with screw tops and refrozen.

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

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

2.2. Sample extraction for toxin analysis

All samples were thawed at room temperature. Depending on the amount of sample available, 1–4 g was weighed out or 1–4 ml was aliquoted into a 15 ml polypropylene screw-cap tube (Falcon-BD). The initial extraction step was carried out by adding 50% aqueous methanol (for DA extraction) or 80% ethanol (for STX extraction) to the sample in a 1:4 wt/wt ratio (1 part sample, 3 parts solvent) and thoroughly vortexing the sample. For fecal material, stomach contents and intestinal contents, samples were homogenized for at least 60 s using an Omni ES homogenizer. The homogenized sample was then centrifuged at 10,000*g* (Sorvall RC 5C Plus centrifuge) for 20 min at 4 °C. The supernatant was then filtered through a 0.22 μm membrane microcentrifuge tube filter (Millipore Ultrafree-MC centrifugal concentration device, Durapore membrane, 0.22 μm pore size) and spun in a desk-top microcentrifuge (Eppendorf model 5415C) for at least 10 min at a setting of 14. For urine, serum and other body fluids, samples were sonicated (Branson Sonifier 450) at 50% pulse for 45 s at a setting of 5. Samples were then centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was then filtered through a 25 mm diameter, 0.45 μm pore size syringe filter (Pall Gelman Acrodisc PSF GxF with GHP membrane). All sample extracts were stored at 4 °C until analysis by ELISA.

2.3. Quantification of algal toxins in marine mammal extracts:

Algal toxins were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits; Biosense® DA ELISA for DA and Abraxis saxitoxin ELISA for STX, following the instruction protocol supplied by the manufacturer (Biosense® Laboratories, Bergen, Norway and Abraxis LLC, Warminster, PA) with slight modifications based on sample matrix. These kits were originally developed for testing shellfish rather than marine mammal samples. Consequently, testing in order to determine matrix effects for feces, stomach and intestinal contents, urine, bile, aqueous humor, serum, and milk were performed in a previous study (Lefebvre et al., 2010). For DA ELISAs, the minimum dilutions of the 1:4 50% MeOH extracts required to eliminate all matrix effects were 1:100 for feces and bile, 1:50 for milk and stomach and intestinal contents and 1:10 for urine, aqueous humor and serum, using our extraction methods and ELISA kits. For STX ELISAs, a 1:50 dilution of the 1:4 80% ethanol extracts was sufficient to eliminate matrix effects in all sample types. Additionally, the Abraxis ELISA kit is designed to measure only STX (with some limited cross-reactivity to several other PSP toxins, as listed in the Abraxis product documents), consequently all PSP levels are listed as STX equivalents and as such, may underestimate the presence of other congeners. With these minimum dilutions, the detection limits for DA in sample material were, 4 ng/g or ml for feces and bile, 2 ng/g or ml for stomach and intestinal contents and milk, and 0.4 ng/ml urine, aqueous humor and serum. The detection limit for STX in all sample matrices was 3 ng/ml.

3. Results

Algal toxins were detected in at least one animal from all 13 species of marine mammals sampled ($n = 905$ total animals; Tables 2 and 3). In addition, 46 individuals contained detectable concentrations of both DA and STX, including 3 of 8 humpbacks,

Table 2
Summary of the number of domoic acid-positive individuals from 13 species of Alaskan marine mammals, including the sample matrix with the highest concentration.

Species	Number of animals	Number positive	% Positive	Max conc. (ng/g or ml)	Sample matrix
Cetaceans					
Humpback whale	8	3	38	51	F
Bowhead whale	25	17	68	359	F
Beluga whale	15	2	13	7	SC
Harbor porpoise	5	2	40	15	F
Otariids					
Northern fur seal	179	8	5	14	S
Steller sea lion	44	12	27	7	SC
Phocids					
Harbor seal	9	6	67	8	F
Ringed seal	113	19	17	127	F
Bearded seal	55	14	25	48	IC
Spotted seal	158	5	3	40	SC
Ribbon seal	21	5	24	7	F
Odobenids					
Pacific walrus	82	34	41	6457	SC
Mustelids					
Northern sea otter	172	43	25	162	U
Total number	886	188	21		

F = feces, SC = stomach contents, S = serum, IC = intestinal contents, U = urine.

Table 3
Summary of the number of saxitoxin-positive individuals from 13 species of Alaskan marine mammals, including the sample matrix with the highest concentration.

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

F = feces, SC = stomach contents, IC = intestinal contents, U = urine, na = not applicable.

6 of 25 bowheads, 5 of 110 ringed seals, 3 of 44 bearded seals, and 20 of 82 walruses tested for both toxins (Table 4). Saxitoxin and DA were present in marine mammals sampled throughout the study area, from the southeastern Gulf of Alaska to the eastern Beaufort Sea (Fig. 1). Domoic acid was detected in more animals (Table 2) and species (all 13) than STX (10 species; Table 3).

3.1. Domoic acid

Bowhead whales had the greatest prevalence of DA (68%), followed by harbor seals (67%), walruses (41%), harbor porpoises (40%), humpback whales (38%), Steller sea lions (27%), bearded seals (25%), northern sea otters (25%), ribbon seals (24%), ringed seals (17%), beluga whales (13%), northern fur seals (5%) and spotted seals (3%; Table 2). The highest concentrations were found in feces, stomach contents, intestinal contents and urine (Table 2). The maximum DA concentration detected was from the intestinal contents of a 15-year-old female walrus (6457 ng/g) from the northern Bering Sea. Domoic acid was also detected in three fetuses (one beluga, one harbor porpoise and one Steller sea lion). Fig. 2 shows all DA positive fecal, gastrointestinal and urine samples for all species examined. Additionally, DA concentrations in feces and urine from ten California sea lions (CSLs) sampled from the central California coast that were exhibiting clinical signs of DA toxicosis (seizures) are shown in red in Fig. 2 for comparison. These CSLs were selected from Appendix A, Table 1 of a report on toxin detection methods for marine mammals where both urine and feces were analyzed for comparison in multiple animals (Frame and Lefebvre, 2012). The identification numbers were used to access the Marine Mammal Center Database to determine which animals were observed to have seizures.

3.2. Saxitoxin

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

4. Discussion

The number of species and the extensive geographic range in which DA and STX were detected demonstrates that HABs are present throughout the Alaskan marine environment and thus the potential for health effects due to exposure is present for all 13 Alaskan marine mammal species tested in this study. For adult marine mammals, DA and STX are being ingested through prey, however, in the case of DA, fetuses and suckling young can also be exposed through amniotic fluid and milk (Rust et al., 2014). Maternal transfer of DA has been well documented by laboratory studies and natural environmental exposures with California sea

Table 4
Summary of animals ($N = 46$) that tested positive for both domoic acid (DA) and saxitoxin (STX) and mean (±sd) toxin values (ng/g or ml).

Species	N	DA	STX	Matrix	Collection locations
Humpback whale	3	21 ± 26	30 ± 28	F	Southeast Alaska
Bowhead whale	6	83 ± 137	48 ± 11	F	Barrow
Ringed seal	5	6 ± 2	41 ± 73	F/SC	Chukchi Sea, Bering Sea
Bearded seal	3	11 ± 14	8 ± 6	F/SC/U	Chukchi Sea, Bering Sea
Pacific walrus	20	524 ± 1432	64 ± 80	IC	St. Lawrence Island
Northern sea otter	9	2 ± 2	7 ± 3	U	Kachemak Bay, Juneau, Glacier Bay
Total number	46				

Sample matrix includes: F = feces, SC = stomach contents, IC = intestinal contents, U = urine.

Fig. 1. Locations where algal toxins were detected in stranded (s) and harvested (h) marine mammals. Red images represent species positive for domoic acid (DA) and purple images represent species positive for saxitoxin (STX). Marine mammal species are listed as follows: (A) humpback whales, (B) bowhead whales, (C) beluga whales, (D) harbor porpoises, (E) northern fur seals, (F) Steller sea lions, (G) harbor seals, (H) ringed seals, (I) bearded seals, (J) spotted seals, (K) ribbon seals, (L) Pacific walruses and (M) northern sea otters.

lions (Maucher and Ramsdell, 2005, 2007; Ramsdell and Zabka, 2008). In the present study, one beluga whale fetus, one harbor porpoise fetus, and one Steller sea lion fetus contained detectable concentrations of DA in stomach contents and feces, further documenting the risk of maternal transfer of toxins from pregnant females with environmental exposures to these biotoxins. Additionally, several sea otter pups and harbor seal neonates contained detectable concentrations of DA, however whether they were actively nursing is unknown. The diets of the 13 species tested in this study are varied due to their diverse marine mammal life histories and range from zooplankton, to benthic invertebrates, to finfish.

4.1. Cetaceans

4.1.1. Humpback whales

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

4.1.2. Bowhead whales

The entire population of western Arctic bowhead whales ranges through Arctic Alaskan waters from the central Bering Sea to the Canadian Beaufort Sea during their annual migration cycle. Bowhead whales are an important subsistence species for western and northern Alaska providing more than ten villages with substantial meat and blubber each year. This stock of bowhead whales is listed as endangered under the Endangered Species Act (ESA), however the population is increasing (3% annually) and believed to have recovered substantially (George et al., 2004; Gerber et al., 2007; Givens et al., 2013; Zeh and Punt, 2005), suggesting that the current reduction in sea ice has had no detectable negative effects on population growth. Bowheads feed on small zooplankton consisting mainly of calanoid copepods and euphausiids, both of which consume phytoplankton (Moore et al., 2010) and are likely the source of the high occurrence rates of DA (68%) and STX (32%) in fecal samples.

4.1.3. Beluga whales

In Alaska, there are four stocks of beluga whales, which range from the Bering Sea to the Canadian Beaufort Sea. These stocks are abundant and support subsistence harvests. In addition, there is a fifth stock in Cook Inlet, a tidal estuary located in the northern Gulf of Alaska. All but two of the animals sampled were part of the Cook Inlet stock. This stock is the most genetically isolated (O'Corry-Crowe et al., 2002), was listed in 2008 as "endangered" under the ESA, and is not currently showing signs of recovery; no harvest is currently allowed. Generally, beluga whales prey on a wide variety of fish, crustaceans, and cephalopods. In Cook Inlet, primary prey species consist of at least three species of Pacific salmon (Chinook,

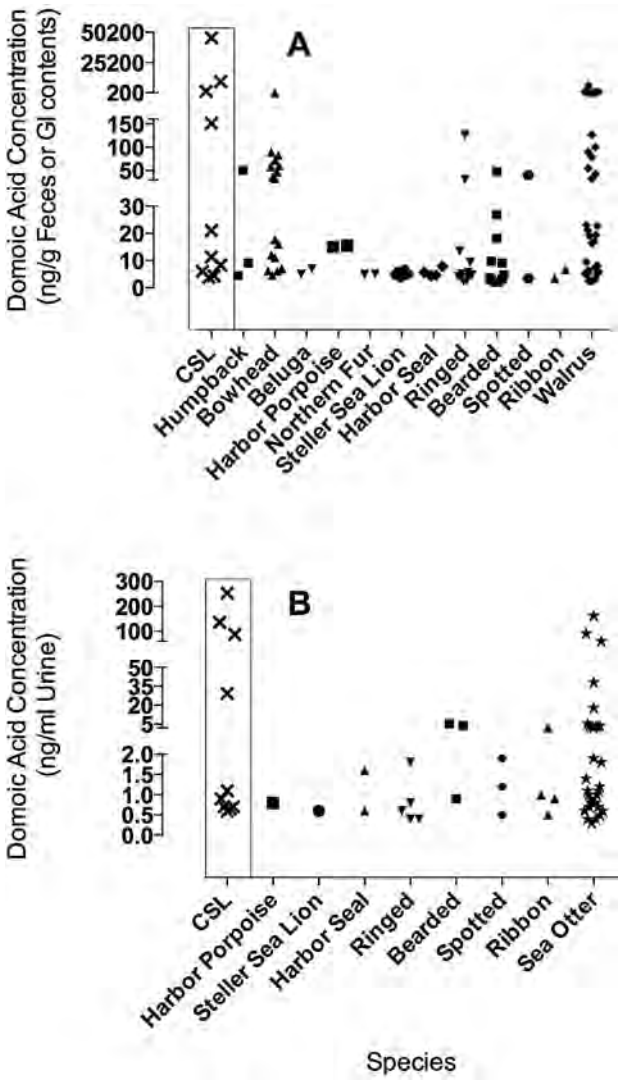

Fig. 2. Domoic acid (DA) concentrations quantified in (A) feces and gastrointestinal (GI) contents and (B) urine for all Alaskan species sampled. Domoic acid concentrations detected in 10 California sea lions (CSLs) exhibiting signs of DA toxicosis (seizures) are included for reference and shown in the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

Oncorhynchus tshawytscha; chum, *Oncorhynchus keta*; and coho, *Oncorhynchus kisutch*), which have been found in beluga stomachs, however it is likely that sockeye (*Oncorhynchus nerka*) and pink salmon (*Oncorhynchus gorbuscha*) are also eaten when they are available (Quakenbush et al., 2015). In addition, eulachon (*Thaleichthys pacificus*), Pacific cod (*Gadus macrocephalus*), walleye pollock (*Theragra chalcogramma*), saffron cod (*Eleginus gracilis*), starry flounder (*Platichthys stellatus*) and yellowfin sole (*Limanda aspera*) have also been found in the stomachs of Cook Inlet belugas. Seven types of invertebrates were found in Cook Inlet beluga stomachs, with the frequency of occurrence in non-empty stomachs being highest for shrimp (39%), followed by polychaetes and amphipods (Quakenbush et al., 2015). Invertebrates appear to be much less important to Cook Inlet beluga diet compared to the other stocks. Therefore, analysis of HABs in other beluga stocks would be of interest (Moore et al., 2000).

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

4.1.4. Harbor porpoises

Harbor porpoises are widespread in the Northern Hemisphere, found in most cool temperate and subpolar waters (Jefferson et al., 1993), including coastal and inland waters, and are seldom found in waters with an annual average temperature above 17 °C (Read, 1999). They generally forage on small, pelagic schooling fish in waters less than 200 m deep (Shelden et al., 2014). Harbor porpoises are not harvested for food in Alaska. In Alaskan waters, harbor porpoise stock structure is unclear, but three stocks are currently recognized for management purposes: Southeast Alaska, Gulf of Alaska and Bering Sea (Shelden et al., 2014), all of which belong to the subspecies *Phocasna phocaena yomerina*. Harbor porpoises eat a wide variety of fish, cephalopods and benthic invertebrates with the main prey items varying by region and season (Culik, 2004; Jefferson et al., 1993; Reyes, 1991). In Cook Inlet, harbor porpoises feed on schooling planktivorous fish such as smelt (Family Osmeridae) and Pacific herring (*Clupea pallasii pallasii*) (Shelden et al., 2014), which are known to accumulate DA. Harbor porpoise samples were collected primarily from Cook Inlet with one animal each from Prince William Sound and Kachemak Bay. The highest concentration of DA was 15.3 ng/g, which occurred in the feces of one animal and the intestinal contents of another animal. One of these was pregnant and the fetus also contained DA at 8 ng/g in its feces documenting maternal transfer of algal toxins. Saxitoxin was not detected in any of the harbor porpoise samples. The higher prevalence of DA is likely a result of a planktivorous fish diet.

4.2. Otariids

4.2.1. Northern fur seals

Northern fur seals breed and give birth on the Pribilof Islands of Alaska in the southern Bering Sea during the summer (June–August) and pups remain dependent until mid-November when the rookeries are abandoned for the winter and weaning occurs abruptly. Immature males are harvested for food in summer. In Alaska, the majority of northern fur seals winter in the North Pacific and return to Alaskan waters the following spring. Movements from seals tagged with satellite-linked transmitters indicate that all sex and age classes can migrate thousands of kilometers as far west as Kamchatka and east to the west coast of the USA (Baker, 2007; Lea et al., 2009; Pelland et al., 2014; Ream et al., 2005; Sterling et al., 2014). Newly weaned pups may remain south of the Aleutian chain for two or more years before returning to the Pribilofs to breed. Northern fur seals in Alaska predominantly forage on schooling fish and gonatid squid with walleye pollock representing approximately 40–75% of the prey observed in scat collections (Ream et al., 2005; Sinclair et al., 1994). Little is known about the recent winter diet since pelagic sealing and the collection of seals for scientific purposes has ceased. Collections made during 1958–1974 in the eastern Bering Sea, however, indicated that in addition to pollock, northern fur seals consumed capelin, Pacific herring, and squid (Perez and Big, 1986). The serum

samples for this study were collected from adult female fur seals with young pups. Provisioning pups with milk every few days limits the distance that females can forage to relatively local waters near the Pribilof Islands and may explain the relatively low occurrence of DA and STX (both at 5% of the animals tested; Tables 2 and 3).

4.2.2. Steller sea lions

Steller sea lions range throughout the Pacific Rim from southern California, across the Gulf of Alaska, to Northern Honshu Island in Japan, and north into the Bering Strait (Lander et al., 2009). In Alaska, they are harvested for food and managed as two stocks, the eastern distinct population segment (DPS) and western DPS. From 1980 to 2000 there was a greater than 80% population decline in the western DPS, which included Russian and Alaskan waters of the Gulf of Alaska, North Pacific Ocean, and Bering Sea, leaving fewer than 55,000 individuals (Lander et al., 2009). Steller sea lions were listed as threatened under the ESA in 1990, and the western portion of the population was reclassified as endangered in 1997. The cause of the decline is unknown. The population has stabilized in the Gulf of Alaska and the eastern DPS, but continues to decline in the western and central Aleutian Islands. Adult Steller sea lions eat a wide variety of fish, with either walleye pollock or Atka mackerel (*Pleurogrammus monopterygius*) predominant in most areas. Other prey consists of schooling fish, including Pacific herring and salmon (*Oncorhynchus* spp.), with smaller numbers of Pacific sand lance (*Ammodytes hexapterus*), capelin, eulachon, Pacific cod, Pacific hake (*Merluccius productus*), flatfish, demersal fish, and cephalopods (Merrick et al., 1997). Several of these prey species are planktivorous including herring, juvenile chum salmon, walleye pollock and sand lance.

4.3. Harbor seals

In Alaska, harbor seals are primarily found in coastal waters throughout the Gulf of Alaska, Aleutian Islands, and Southeastern Bering Sea where they are harvested for food (Small et al., 2003). They are found in diverse habitats including glacial and non-glacial areas and are generally non-migratory (Bigg, 1981). Harbor seals mainly forage on fish including Pacific herring, rainbow smelt (*Osmerus mordax*), salmon (Salmonidae), walleye pollock, Pacific cod, greenling (*Hexagrammidae*), sculpins (*Cottidae*), Pacific sand lance, and flatfish (*Pleuronectidae*) (Pitcher, 1980a, 1980b). Invertebrates such as octopus, squid, and shrimp are also consumed (Pitcher, 1980a, 1980b). The importance of these prey items varies by location. In the Bering Sea and Gulf of Alaska, pollock and octopus are the most common prey items, whereas shrimp and capelin are most common in the southeastern Gulf of Alaska (Pitcher, 1980b). No harbor seals tested positive for STX, however six of nine animals tested positive for DA, although the maximum concentration was low; 8 ng/g feces (Table 2). Harbor seals had a much higher percentage of individuals that tested positive (67%) than spotted seals (3%) even though they consume similar fish species. This could be due to the more southern range of harbor seals compared to the spotted seal's more northerly range in the Bering Sea. Most harbor seals sampled were from the Gulf of Alaska, which has warmer waters and as such are more likely to be exposed to HABs, although no data are available on HAB or shellfish toxicity for verification. Additionally, the samples sizes tested were vastly different ($n = 9$ for harbor seals and $n = 158$ for spotted seals) making direct comparisons difficult.

4.4. Ice seals (ringed, bearded, spotted and ribbon)

Ringed, bearded and spotted seals are sea ice-associated seals that range throughout the Bering, Chukchi and Beaufort seas in Alaska (Burns, 1970). Of these species, only ringed seals are currently ESA listed. Bearded seals were listed under ESA, but a court overturned the decision, which is being appealed by the National Marine Fisheries Service (NMFS). Ribbon seals are also ice-associated and occur throughout the Bering and Chukchi seas, but are not often found in the Beaufort Sea (Burns, 1981). Although movements in winter months are restricted by sea ice, these seals move widely in spring, summer, and fall (Burns, 1970, 1981; Crawford et al., 2012; Harwood et al., 2012a, 2012b; Lowry et al., 2000). Ringed and bearded seals tend to inhabit areas that are seasonally ice covered and are found in heavy pack ice. Spotted and ribbon seals are less ice dependent at certain times of the year and can be found near the ice edge, in the broken pack ice, of the Bering Sea in winter and spring. The distribution of spotted seals shifts northward and toward the coasts as sea ice recedes in May and June and many spotted seals enter bays and rivers and haul out on sand bars and barrier islands (Burns et al., 1981). The distribution of ribbon seals also shifts northward as sea ice recedes in May and June. When sea ice melts, however, the majority of the ribbon seal population likely becomes pelagic in the North Pacific and the central Bering Sea, although some seals follow receding ice into the Chukchi Sea (Burns et al., 1981). All four species are harvested for food, mostly in spring and fall.

The diets of ringed, bearded, spotted and ribbon seals vary widely. Ringed seals feed mostly in the water column on pelagic and semi-demersal fish (including arctic cod, *Boreogadus saida*; saffron cod, walleye pollock, and sculpins) and invertebrates (including mysids, amphipods, shrimps and echiurids) (Crawford et al., 2015; Dunbar, 1941; Fedoseev, 1965; Johnson et al., 1966; Lowry et al., 1980; McLaren, 1958). Bearded seals feed on a wide variety of benthic invertebrates (including bivalves, gastropods, cephalopods, isopods, amphipods, shrimp, crab, echiurids and polychaetes) and fish (including arctic and saffron cod; sculpins; snailfish (Liparidae); pricklebacks (Stichaeidae); Pacific sandlance, and flatfish) (Antonelis et al., 1994; Burns, 1981; Chapskii, 1938; Crawford et al., 2015; Dunbar, 1941; Quakenbush et al., 2011; Smith, 1981). Spotted seals eat mostly pelagic fish including arctic and saffron cod; Pacific herring; and smelt (Bukhtiyarov et al., 1984; Frost and Lowry, 1981; Quakenbush et al., 2009). Ribbon seal diet is less well documented because most have empty stomachs when they are harvested in the late spring. But their diet is most similar to spotted seals and includes fish (arctic and saffron cod and pollock), shrimp (*Crangonid* and *Pandalid* species) and octopus (Dehn et al., 2007; Frost and Lowry, 1980; Quakenbush and Citta, 2008).

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

4.5. Pacific walrus

Pacific walruses are migratory, following the southern margins of the pack ice from the Bering Sea to the Chukchi Sea in the spring, where foraging is optimal in the relatively shallow shelf waters (Estes and Gilbert, 1978; Fay, 1982; Gilbert, 1989). Walruses are

harvested for subsistence and in addition to walrus tissues, clams found in the stomach during butchering are also eaten by harvesters. The Pacific walrus population is currently a candidate species under the ESA due to concern regarding the species' response to changes in summer sea ice habitat (Robards and Garlich-Miller, 2013; USFWS, 2011). Walruses feed primarily on benthic invertebrates including marine worms (e.g., polychaetes, sipunculids and echiurids priapulids), mollusks (e.g., bivalves and gastropods), and crustaceans (e.g., amphipods, shrimp and crabs) (Born et al., 2003; Bowen and Siniffand, 1999; Dehn et al., 2007; Fay, 1982; Sheffield et al., 2001; Sheffield and Grebmeier, 2009) although fish and other vertebrates (including seals) are also occasionally reported (Fay, 1982; Seymour et al., 2014; Sheffield et al., 2001; Sheffield and Grebmeier, 2009). Walruses are not physiologically adapted for deep diving and concentrate foraging efforts in shallower waters, typically using the sea ice as a resting platform between feeding trips (Fay, 1982). Since 2007, walruses summering in the Chukchi Sea have been hauling out in large numbers at two terrestrial haulout sites on the eastern Chukchi Sea (Icy Cape and Point Lay) beginning in late summer when sea ice over the Continental Shelf disappears (Robards and Garlich-Miller, 2013).

Stomach contents from walruses sampled near St. Lawrence Island had the highest measured concentrations of both DA and STX of any species examined in this study (Tables 2 and 3). That 41% and 28% of walruses sampled contained elevated concentrations of DA and STX, respectively, is surprising due to the sampling location. These walruses were sampled in the northern Bering Sea during May, as they were moving northward with the receding sea ice (Fay, 1982). Water temperatures with sea ice present are not considered favorable to support HABs, although the DA and STX could have come from invertebrates eaten farther south. The elevated toxin concentrations in walruses suggest that DA and STX producing phytoplankton are well established in seasonally ice covered waters to accumulate in clams within the foraging range of walruses. That the highest concentrations of both DA and STX in this study came from walruses and that the walrus with the highest concentration of STX also had relatively high DA is cause for continued monitoring and investigation. The DA concentrations detected in walruses are similar to those detected in California sea lions suffering from DA toxicosis, although hunters did not report abnormal behavior in any of the sampled walruses (Fig. 2; Lefebvre et al., 1999; Scholin et al., 2000).

Walruses and bearded seals are typically benthic feeders with overlapping ranges, but the percent positive and maximum concentration for both DA and STX were higher for walruses than bearded seals. This could be because bearded seals are more generalist foragers than walruses.

4.6. Sea otters

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

The primary prey of sea otters in the Gulf of Alaska (Southeast Alaska, Prince William Sound, Kachemak Bay and Kodiak Island) are clams, such as butter clams (*Saxidomus giganteus*) (Calkins, 1978; Doroff and Bodkin, 1994; Doroff and Degange, 1994; Hoyt et al., 2014; Kvitek et al., 1993). In contrast, the diet in the Aleutian Islands is dominated by sea urchins and a variety of finfish, including those in the families of Hexagrammidae, Gadidae, Cottidae, Cyclopteridae and Scorpaenidae (Estes et al., 1982; Kenyon, 1969). The majority of the sea otter carcasses recovered and sampled for this study were from the northern Gulf of Alaska (i.e. Kachemak Bay) where clams are an important prey item (Doroff et al., 2012; Newsome et al., 2015).

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

Additionally, between May 12 and 28, 2011 sea otters ($n = 21$) were live-captured around Kuiu Island in Southeast Alaska (Fig. 1). Urine was collected and they were implanted with VHF transmitters and released (Hoyt et al., 2014). Twenty of these otters contained detectable concentrations of STX (3.0–28.4 ng/ml urine). During capture and handling, none of these animals exhibited any clinical signs (i.e., paralysis, difficulty breathing) associated with STX toxicity. After being released they were relocated and feeding was observed between August 2011 and May 2013. Tagged sea otters consumed a total of 32 unique prey types (Hoyt et al., 2014). In terms of biomass, the three most common prey items were clams (primarily *Saxidomus giganteus*) followed by green urchins (*Strongylocentrotus droebachiensis*) and Dungeness crab (*Metacarcinus magister*). Continuing to track DA and STX concentrations in sea otters will be important in understanding threats to their populations especially in the ESA-listed stock. As otters are a nearshore, highly visible species that do haul out on land in some human inhabited Alaskan locations, it may be prudent to set up protocols for documenting and reporting signs of HAB-related toxicosis.

4.7. Toxic exposure levels and data limitations

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

A major limitation in the assessment of health risks is that toxin concentrations in marine mammal samples are not directly related to the magnitude of an animal's exposure. For example, not all California sea lions with acute behavioral signs of toxicity (e.g., seizures) have high concentrations of DA in feces and urine because elimination rates are rapid (Lefebvre et al., 1999; Fig. 2). Passage rates for captive sea lions fed Pacific herring averaged less than 5 h (Helm, 1984) and laboratory studies have reported 99% of algal toxin is eliminated through urine within 24 h of dosing (Suzuki and Hierlihy, 1993). Therefore, the concentrations presented here provide proof of exposure risk and evidence for

potential neurotoxic impacts to several marine mammal species in Alaska.

5. Conclusions

These results demonstrate that the algal toxins DA and STX are present in Alaskan Subarctic and Arctic ecosystems and have the potential to affect most marine mammal species in USA waters farther north than expected. Given the current trend of decreasing sea ice and warming ocean waters that will extend the open water season favorable to HABs, the prevalence and concentrations of DA and STX documented in this study are expected to increase creating a greater risk to marine mammals. Clinical signs of neurotoxicity were not confirmed in the present study, however many of the animals were dead when sampled. Additionally, toxin effects could contribute to an increase in ship strikes for large cetaceans and increased vulnerability to subsistence harvested seals, walruses and whales, both of which would be difficult to detect due to concurrent increases in ship traffic and changes in ice and weather patterns that affect hunting. Sea lions along the central California coast provide a cautionary example of increasing HAB impacts on marine mammal health. This threat to marine mammals was first recognized in 1998, and now has a major impact on sea lions annually. Recent studies have suggested that HABs are also affecting large cetaceans in southern latitudes such as the Minke whale (*Balaenoptera acutorostrata*) (Fire et al., 2010). This study documents the presence of HAB toxins in marine mammals from southeast Alaska to the Arctic Ocean revealing a potentially growing exposure risk to northern marine mammal populations. Unless unknown factors inhibit HABs in northern waters, warming water temperatures and increased light availability due to loss of sea ice are likely to support more blooms increasing toxin concentrations and the health risks they present for northern marine mammal species as they have for southern species.

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Brucella Antibodies in Alaskan True Seals and Eared Seals—Two Different Stories

Ingebjørg H. Nymo[1], Rolf Rødven[2], Kimberlee Beckmen[3], Anett K. Larsen[1], Morten Tryland[1], Lori Quakenbush[3] and Jacques Godfroid[1]*

[1] Arctic and Marine Biology, UiT – The Arctic University of Norway, Tromsø, Norway, [2] Bioscience, Fishery and Economy, UiT – The Arctic University of Norway, Tromsø, Norway, [3] Division of Wildlife Conservation, Alaska Department of Fish and Game, Fairbanks, AK, United States

Brucella pinnipedialis was first isolated from true seals in 1994 and from eared seals in 2008. Although few pathological findings have been associated with infection in true seals, reproductive pathology including abortions, and the isolation of the zoonotic strain type 27 have been documented in eared seals. In this study, a *Brucella* enzyme-linked immunosorbent assay (ELISA) and the Rose Bengal test (RBT) were initially compared for 206 serum samples and a discrepancy between the tests was found. Following removal of lipids from the serum samples, ELISA results were unaltered while the agreement between the tests was improved, indicating that serum lipids affected the initial RBT outcome. For the remaining screening, we used ELISA to investigate the presence of *Brucella* antibodies in sera of 231 eared and 1,412 true seals from Alaskan waters sampled between 1975 and 2011. In eared seals, *Brucella* antibodies were found in two Steller sea lions (*Eumetopias jubatus*) (2%) and none of the 107 Northern fur seals (*Callorhinus ursinus*). The low seroprevalence in eared seals indicate a low level of exposure or lack of susceptibility to infection. Alternatively, mortality due to the *Brucella* infection may remove seropositive animals from the population. *Brucella* antibodies were detected in all true seal species investigated; harbor seals (*Phoca vitulina*) (25%), spotted seals (*Phoca largha*) (19%), ribbon seals (*Histriophoca fasciata*) (16%), and ringed seals (*Pusa hispida hispida*) (14%). There was a low seroprevalence among pups, a higher seroprevalence among juveniles, and a subsequent decreasing probability of seropositivity with age in harbor seals. Similar patterns were present for the other true seal species; however, solid conclusions could not be made due to sample size. This pattern is in accordance with previous reports on *B. pinnipedialis* infections in true seals and may suggest environmental exposure to *B. pinnipedialis* at the juvenile stage, with a following clearance of infection. Furthermore, analyses by region showed minor differences in the probability of being seropositive for harbor seals from different regions regardless of the local seal population trend, signifying that the *Brucella* infection may not cause significant mortality in these populations. In conclusion, the *Brucella* infection pattern is very different for eared and true seals.

Keywords: harbor seal, ribbon seal, ringed seal, serology, spotted seal, Steller sea lion, Northern fur seal, disease

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Scott Napper, University of Saskatchewan, Canada

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Julio Alvarez, University of Minnesota, United States

****Correspondence:***
Ingebjørg H. Nymo
ingebjorg.nymo@vetinst.no

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INTRODUCTION

Alaskan waters accommodate a number of pinniped species, both true seals (family *Phocidae*) including Eastern North Pacific harbor seals (*Phoca vitulina richardsii*), spotted seals (*Phoca largha*), ribbon seals (*Histriophoca fasciata*), Arctic ringed seals (*Pusa hispida hispida*), and bearded seals (*Erignathus barbatus*), as well as eared seals (family *Otariidae*) including Steller sea lions (*Eumetopias jubatus*) and Northern fur seals (*Callorhinus ursinus*) (1). Currently, bearded seals of the Bering Sea distinct population segment, and Steller sea lions in the western distinct population segment have been listed as threatened ("*likely to become an endangered species within the foreseeable future*") under the Endangered Species Act (ESA). Although the Northern fur seal Pribilof Island stock has not been listed under the ESA, they have been deemed depleted ("*below its optimum sustainable population*") under the Marine Mammal Protection Act (MMPA) (2). Listings for seals were based on their predicted negative responses to climate change, while for sea lions and fur seals it is due to population declines for unknown reasons. Furthermore, the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species considers several of these species as "Data deficient" ("*inadequate information to make a direct, or indirect, assessment of its risk of extinction based on its distribution and/or population status*") (3). Clearly there are concerns about how well these species and populations are able to adapt to future climate change scenarios where disease prevalence is predicted to increase as new species with novel pathogens appear to exploit warmer waters and longer open water seasons, and the host–pathogen balance may be altered (4). Thus, health and disease status of these animal populations are of prime importance for the purpose of management and conservation.

Brucella spp. was first reported in true seals in 1994 (5) and *Brucella pinnipedialis* has since been isolated from numerous true seal species (6). Persistence in macrophages—causing chronic infections—is the hallmark of brucellosis (7). However, *B. pinnipedialis* isolated from harbor (*Phoca vitulina vitulina*) and hooded seals (*Cystophora cristata*), both true seal species, did not multiply *in vitro* in human, murine or hooded seal macrophages, or in human and hooded seal epithelial cells (8–10) suggesting it may be a less virulent *Brucella* subspecies with a lower zoonotic potential. Furthermore, hooded and harbor seal brucellae are attenuated in the BALB/c mouse model (11, 12), in contrast to virulent pathogenic terrestrial brucellae, such as *Brucella suis*, which show a great ability to multiply and persist in this model (12). *Brucella* infections are further characterized by bacterial replication in the reproductive system of primary hosts, associated with pathology in the reproductive organs, causing abortion and sterility (7). Interestingly, although *B. pinnipedialis* has often been detected, pathology associated with it in true seals is virtually absent (6).

Previous studies on hooded seals from the east side of Greenland (13) and harbor seals from Alaska and the East coast of the USA (14, 15) have shown an age-dependent serological pattern, with a low probability of being seropositive for pups, a higher probability for yearlings, followed by a decreasing probability with age. This indicates that exposure occurs during the first year of life rather than *in utero* with a subsequent clearing of the infection (13). However, whether a similar age-dependent serological pattern is present in other Alaskan seal species and populations had not been documented.

In contrast to the situation in true seals, brucellae are rarely isolated or detected by PCR in eared seals, making it difficult to evaluate the presence or absence of *Brucella*-associated pathology in these species. However, it is worth noticing that the few cases reported in eared seals have been associated with reproductive pathology (16–18) and that transplacental transmission has been suggested (16). Furthermore, certain eared seal species are able to host infections with the zoonotic strain type (ST) 27 (16) and terrestrial brucellae (19), and could hence pose a zoonotic risk.

Serologic tests for detecting antibodies against a specific etiologic agent are the first screening tools for wildlife. The Rose Bengal test (RBT) is a simple and reliable test recommended by the World Organization for Animal Health (OIE) for the detection of *Brucella* antibodies. However, when using it in marine mammals, fat globules being wrongly identified as agglutinates may interfere with the interpretation of the results (20, 21). Serum lipids may be partly removed by chloroform cleanup (20) and has previously been shown to greatly improve the agreement between RBT and an enzyme-linked immunosorbent assay (ELISA) when detecting *Brucella* antibodies (21). The first objective of the present study was to compare the results of RBT and ELISA in a subset of serum samples before and after chloroform cleanup. Thereafter, the second objective was to use the best technique to determine the seroprevalence of *Brucella* antibodies adjusting by other potential covariates.

For the remaining screening, we used ELISA to investigate the seroprevalence of *Brucella* antibodies in a large number of harbor seals, spotted seals, ribbon seals, ringed seals, Steller sea lions, and Northern fur seals from Alaskan waters, sampled between 1975 and 2011. The aim of the present study was to analyze how the likelihood of seropositivity varied between species, sex, age, sampling year, and regions in order to draw further conclusions on whether a *Brucella* infection may be negatively impacting the health and population dynamics of these species. Knowledge about to what degree these species harbor the infection is also of importance as many of these species are subsistence harvested and hence may pose a zoonotic threat.

MATERIALS AND METHODS

Sampling

Samples were collected (1975–2011) from Alaskan pinnipeds by biologists during live/capture release studies, scientific collections or Alaska native subsistence harvested animals and stored at −40 to −80°C at the Alaska Department of Fish and Game (ADF&G) in Fairbanks, Alaska until subsampled for this study ($n = 1{,}643$). Sample sizes and distribution by sex and age category (pups; <1 year, juveniles; 1–3 years, adults; >3 years) are depicted in **Table 1**. Age category was known for 1,420 seals (86%); 1,039 harbor seals (93%), 73 spotted seals (86%), 50 ribbon seals (91%), 75 ringed seals (50%), 46 Steller sea lions from the Western distinct population segment (61%), 31 Steller sea

TABLE 1 | Alaskan seals analyzed for *Brucella* antibodies.

Species	Pups (f/m/u)	Juveniles (f/m/u)	Adults (f/m/u)	Unknown (f/m/u)	Total (f/m/u)
Harbor seal	244 (120/123/1)	311 (154/156/1)	484 (239/242/3)	83 (32/43/8)	1,122 (545/564/13)
Spotted seal	12 (5/7/0)	34 (16/18/0)	27 (16/11/0)	12 (1/6/5)	85 (38/42/5)
Ribbon seal	0 (0/0/0)	21 (10/11/0)	29 (14/15/0)	5 (0/4/1)	55 (24/30/1)
Ringed seal	7 (3/4/0)	16 (6/10/0)	52 (20/31/1)	75 (25/40/10)	150 (54/85/11)
Steller sea lion (WDPS)	23 (9/14/0)	8 (6/2/0)	15 (13/2/0)	30 (9/12/9)	76 (37/30/9)
Steller sea lion (EDPS)	15 (8/7/0)	0 (0/0/0)	16 (16/0/0)	17 (0/0/17)	48 (24/7/17)
Northern fur seal	93 (60/32/1)	0 (0/0/0)	13 (13/0/0)	1 (0/0/1)	107 (73/32/2)
Total	394 (205/187/2)	390 (192/197/1)	636 (331/301/4)	223 (67/105/51)	1,643 (795/790/58)

Age categories (pups; <1 year, juveniles; 1–3 years, adults; >3 years) and sex (f, females; m, males; u, unknown) for harbor seals (1975–2001), spotted seals (1978–2008), ribbon seals (1978–2003), ringed seals (1978–2011), Steller sea lions from the Western (1977–1996) and Eastern (1993–1995) distinct population segment, and Northern fur seals (1996–2000) investigated for Brucella antibodies in the present study.

lions from the Eastern distinct population segment (65%), and 106 Northern fur seals (99%). Age by year was determined by assessing morphometric measurements (22), tooth annuli [e.g., (23)] or claw annuli [e.g., (24)] as validated for each species. Age by year was determined for 916 seals (56%): 599 harbor seals (53%, 0–30 years), 58 spotted seals (68%, 0–25 years), 49 ribbon seals (89%, 1–25 years), 75 ringed seals (50%, 0–16 years), 28 Steller sea lions from the Western distinct population segment (37%, 0 – 10 years), 15 Steller sea lions from the Eastern distinct population segment (31%, all pups), and 93 Northern fur seals (87%, all pups). The animals included in the study were from Alaskan waters (**Figure 1**) and seven ringed seals were from Argo Bay, Canada.

Antibody Detection

Serum samples were analyzed for *Brucella* antibodies using a Protein A/G ELISA, as previously described (25). A subsample of sera was also tested using RBT (IDEXX Laboratories, Pourquier, Hoofddorp, the Netherlands) (20 ELISA-negatives/species, $n = 140$, and up to 20 ELISA-positives/species, $n = 66$). These sera ($n = 206$) were cleaned with chloroform to remove lipids (20, 21) and re-analyzed by ELISA ($ELISA^{chl}$) and RBT (RBT^{chl}).

Statistical Analysis

Pairwise agreement among the serological tests before and after chloroform cleanup was assessed (Cohen's kappa, κ) (26, 27). RBT and RBT^{chl} results were categorized as negative, positive, or impossible to interpret. ELISA and $ELISA^{chl}$ results were categorized as negative or positive. The remaining statistical analyses were performed using ELISA results.

Differences in seroprevalence between sex and age groups were estimated using generalized linear models with a binomial error distribution and a logit link. To account for the possibility that age effects may differ between males and females, the interaction *age* × *sex* was included in the models. Influence of age was analyzed in two ways: first, age was treated as a categorical variable with three age categories: pups, juveniles, and adults. Second, for animals older than pups, we used age as a continuous variable. Each species was modeled separately instead of using "species" as a covariate to reduce the number of model parameters.

We limited the examination of how seropositivity varied spatially to harbor seals because this was the only species with a sufficient sample size from all sampling regions (**Figure 1**), with exception of the Aleutian Islands, which was excluded from the analysis. Region was modeled as a categorical variable. Age category and sex, as well as their interactions, were included as covariates in the full model. Not all regions, age categories, sex, and species, were sampled all years. Hence, we chose not to include sampling year as a covariate as preliminary analysis indicated that this could bias estimates, in particular for species with small sample sizes. However, a univariate analysis of trends in seroprevalence over time did not reveal any overall trend (logit regression, slope = 0.00, 95% CI = −0.02, 0.02).

The most parsimonious models where selected using Akaikes information criterion corrected for small sample sizes (AICc), using an all possible regression approach [library *MuMIn* in R (28)] on the sample size for all possible combinations (i.e., a fixed minimum sample size for all models ranked). Estimates are considered significant if their 95% confidence intervals do not include zero. All statistical analyses were performed in the program R, version 3.3.0 [R (29)].

RESULTS

Results from testing sera without chloroform cleanup (i.e., containing lipids), revealed moderate agreement between ELISA and RBT (κ: 0.66, SE: 0.04). Chloroform cleanup greatly improved agreement between the ELISA/$ELISA^{chl}$ (identical results) and RBT^{chl} (κ: 0.90, SE: 0.03), while the agreement between RBT and RBT^{chl} was only moderate (κ: 0.68, SE: 0.04). Detailed information about how the chloroform cleanup affected the results are available in **Table 2**. The remaining results are ELISA results.

Brucella antibodies were detected in 276/1,122 harbor seals (24.6%), 16/85 spotted seals (18.8%), 9/55 ribbon seals (16.4%), and 21/150 ringed seals (14.0%). *Brucella* antibodies were detected in 2/124 Steller sea lions, both of unknown age, one from the Western distinct population segment (1.3%) and one from the Eastern distinct population segment (2.1%). All 107 Northern fur seals were seronegative.

For harbor and ringed seals, juveniles had a higher probability of being seropositive than pups, and adults for harbor seals (45.3%, 95% CI = 40.0, 50.9 versus 7%, 95% CI = 4.2, 10.6 and 19.4%, 95% CI = 16.1, 23.1, and 56.3%, 95% CI = 32.3, 78.2 versus

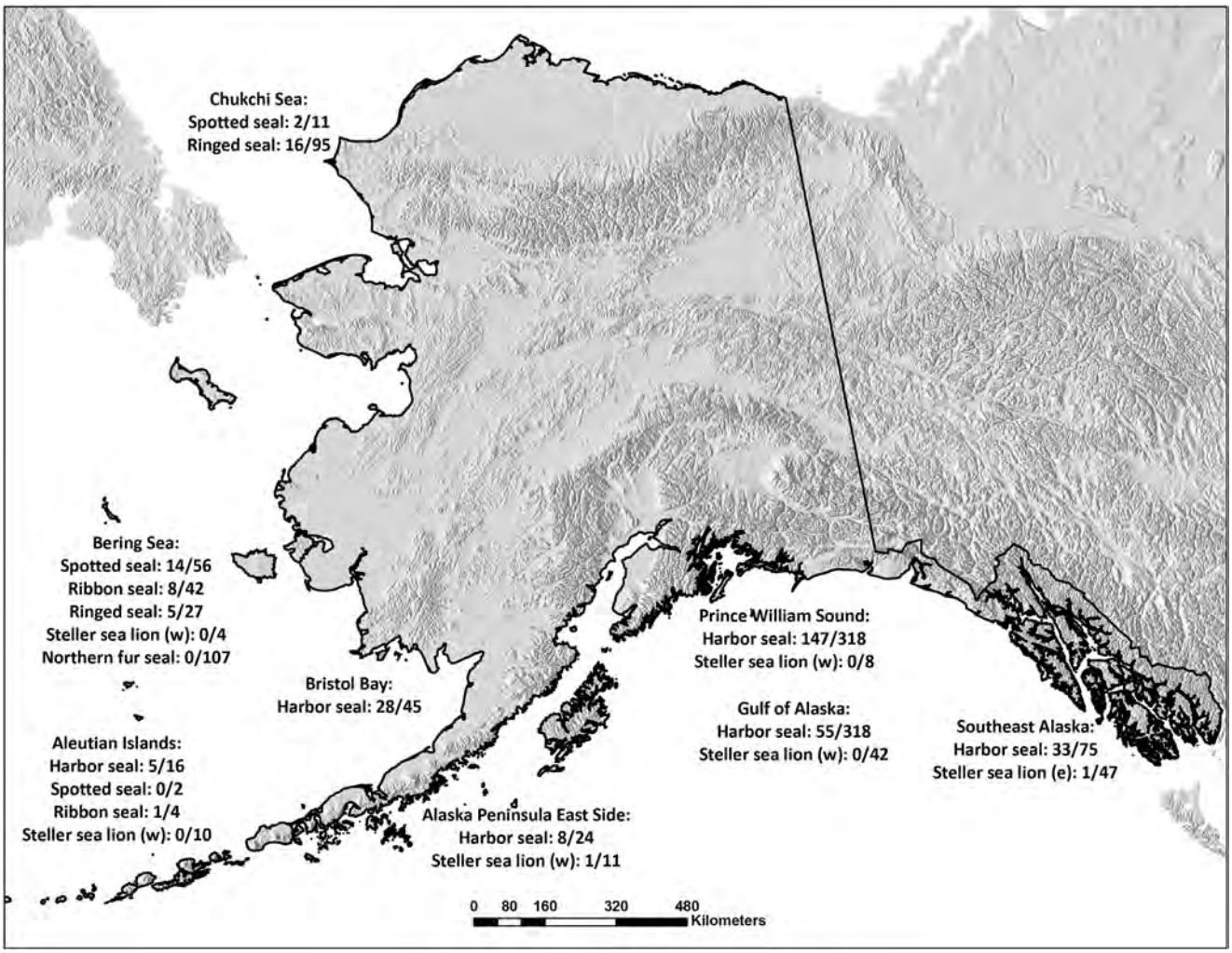

FIGURE 1 | Number of *Brucella*-seropositive seals per species and sampling area. Serum samples obtained from Alaskan harbor seals, spotted seals, ribbon seals, ringed seals, Steller sea lions from the Western (w) and Eastern (e) distinct population segments, and Northern fur seals in the Chukchi and Bering Seas, in Bristol Bay, around the Aleutian Islands, on the Eastern side of the Alaska Peninsula, in Prince William Sound, in the Gulf of Alaska, on the coast of Southeast Alaska and in Argo Bay, Canada. The samples were investigated for the presence of *Brucella* antibodies and the numbers of positives per species and sampling spot are given.

TABLE 2 | Comparison of results before and after chloroform cleanup.

Tests		RBT			ELISA/ELISA^{chl}	
	Results	**Positive**	**Negative**	**Unknown**	**Positive**	**Negative**
RBT^{chl}	Positive	41	1	19	61	0
	Negative	4	128	7	3	136
	Unknown	0	2	4	2	4
ELISA/	Positive	42	2	22	66	0
ELISA^{chl}	Negative	3	129	8	0	140

Serum samples (n = 206) were analyzed for Brucella antibodies using a Protein A/G indirect enzyme-linked immunosorbent assay (ELISA) and the Rose Bengal test (RBT). The samples were thereafter subjected to a chloroform cleanup to remove lipids and re-analyzed by ELISA (ELISAchl) and RBT (RBTchl). RBT and RBTchl results were categorized as negative, positive, or impossible to interpret (unknown). ELISA and ELISAchl results were categorized as negative or positive. The results are pairwise compared in the table.

14.3%, 95% CI = 0.9, 49.4 and 3.8%, 95% CI = 0.6, 11.4), though for the ringed seals the contrast to pups was not significant (**Figure 2**; **Table 3**; Table S1 in Supplementary Material). There was no such significant age category pattern evident for spotted or ribbon seals in the best approximating models (**Figure 2**; **Table 3**). While lower ranked models for both species included age category (δAICc < 2, Table S1 in Supplementary Material), these estimates did not significantly differ (95% CI for contrasts of pups and adults versus juveniles for spotted seal: [−4.71, 0.19] and [−2.75, 0.27], and for adults versus juveniles for ribbon seal: [−1.17, 1.95], all logit-transformed).

When splitting up age categories into age by year, there was an overall significant decreasing probability of being seropositive with age from the age of 1 year for all true seals except ribbon seals (**Figure 3**; **Table 4**; Table S2 in Supplementary Material).

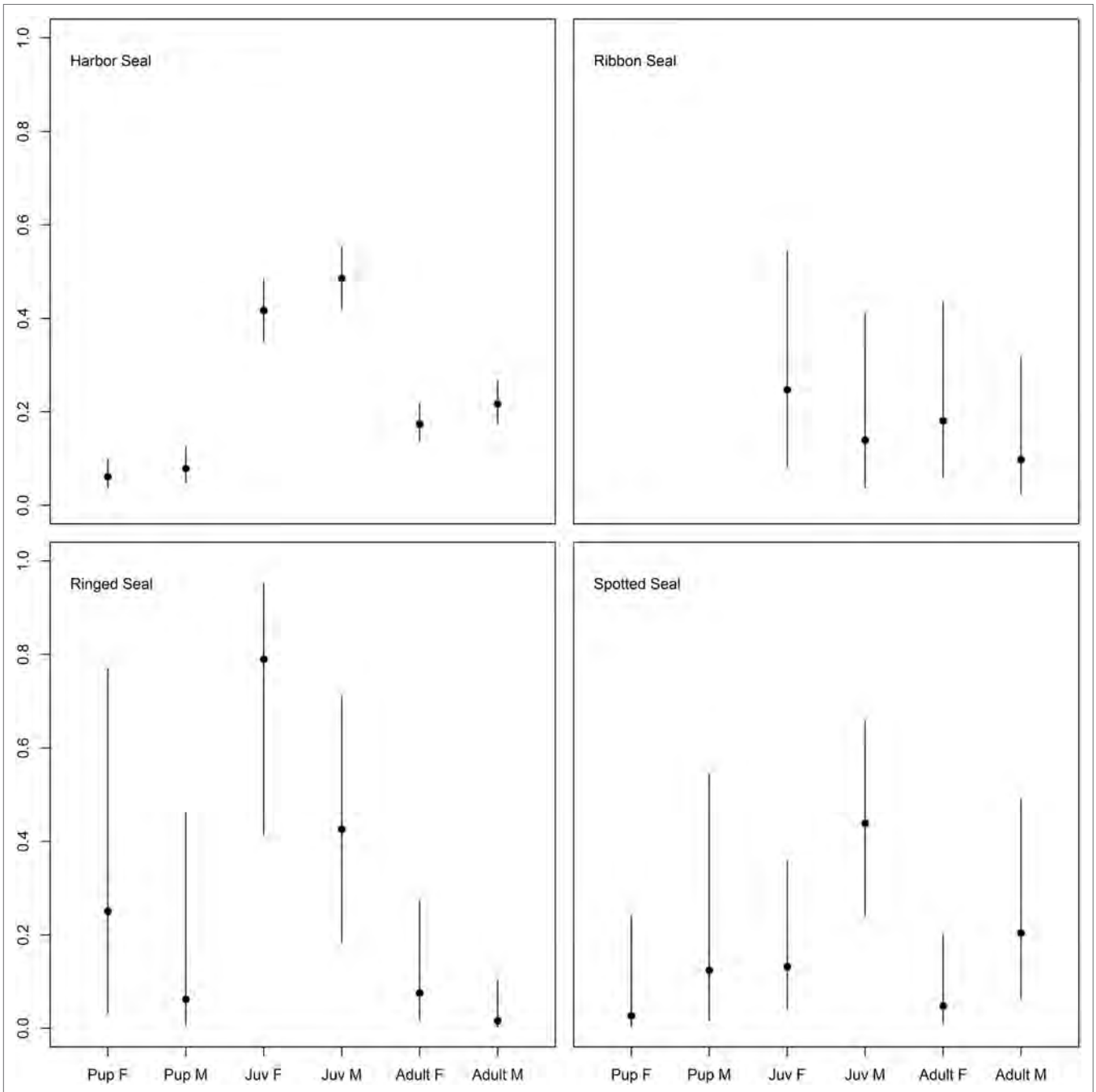

FIGURE 2 | Differences in probability of being seropositive between age categories in true seals. Differences in probability of being *Brucella* spp. seropositive among age categories (pups < 1 year, juveniles 1–3 years, adults > 3 years) and sexes (males; M and females; F) for the four true seal species. Points indicate predicted mean, while error bars indicate 95% confidence intervals.

While seropositive animals were absent among individuals older than 5 years in spotted seals and 6 years in ringed seals, they were present among harbor seals until the age of 16, though at a very low prevalence for older ages (**Figure 3**).

There was an inconsistent pattern for difference in probability of seropositivity between sexes (Tables S1 and S2 in Supplementary Material). Spotted seal males had a significant and harbor seal males a near significant higher probability of being seropositive, while the pattern was the opposite for ringed seals (**Figures 2** and **3**; **Tables 3** and **4**). Like ringed seals, ribbon seal showed higher probability for seropositivity among females, though not significant [95% CI = [−0.66, 2.54] and [−0.78, 2.44], for lower ranked age category (δAICc = 1.4) and age models (δAICc = 1.2), respectively].

Differences were found in seropositivity among regions (δAIC = 9.2 to the best model not including region, controlled

TABLE 3 | Parameter estimates (logit-transformed) for best approximating models, with age is included as a categorical predictor.

Species	Predictor	Estimate	SE	*Z*-value	95% CI
Harbor seal	Intercept	−1.56	0.14	−10.93	(−1.85, −1.28)
n = 1,034	Age category—juveniles	1.23	0.16	7.55	(0.91, 1.55)
	Age category—pups	−1.18	0.28	−4.25	(−1.75, −0.66)
	Sex—males	0.28	0.15	1.79	(−0.02, 0.58)
Ribbon seal	Intercept	−1.63	0.36	−4.48	(−2.41, −0.97)
n = 55					
Ringed seal	Intercept	−2.51	0.77	−3.27	(−4.38, −1.23)
n = 74	Age category—juveniles	3.83	0.99	3.85	(2.10, 6.14)
	Age category—pups	1.41	1.34	1.06	(−1.79, 3.99)
	Sex—males	−1.62	0.90	−1.80	(−3.67, 0.02)
Spotted seal	Intercept	−2.14	0.53	−4.05	(−3.35, −1.22)
n = 80	Sex—males	1.22	0.63	1.95	(−0.06, 2.58)

For age category, adult is set as reference level, while for sex, female is. SE is standard error, CI is confidence interval, and n is sample size used for parameter estimation (see Materials and Methods for details).

for age category and sex), mainly due to a significant lower probability of being seropositive for harbor seals in the Gulf of Alaska (**Figures 1** and **4**).

DISCUSSION

Past evaluations of *Brucella* infection status in marine mammals have likely been inaccurate if the results were based solely on agglutination tests. RBT is a much used, simple, cheap, and reliable test recommended by the OIE (30); however, the results may be influenced by the presence of hemolysis and globules of fat (21), often present in marine mammal sera. For marine mammals, it is necessary to run a combination of tests to determine if discrepancies are found between RBT and other serological tests. A chloroform cleanup may be required, followed by repetition of the tests, as shown in the present study where the agreement between ELISA and RBT was moderate, while after chloroform cleanup, the coherence between ELISA and RBT^{thl} was greatly improved. The improved agreement between the tests and the reduced number of non-interpretable RBT-results following chloroform cleanup indicate that the original serum quality likely contained lipids that reduced the accuracy of the initial RBT-outcome.

Even with serological techniques that are less sensitive to poor serum quality and lipemic serum (e.g., ELISA), positive serological results presume exposure (31), as serological cross-reactions and false positives bias (i.e., inflate) the results. Several potential agents cross-reacting with *Brucella* are identified (32), however, little is known about their presence in wildlife. Therefore, the gold standard in brucellosis diagnostics is bacterial isolation or detection of *Brucella* spp. specific DNA-fragments. Ideally serological tests should be used to screen sera and seropositives should be further tested by bacterial isolation and PCR to address *Brucella* status among Alaskan pinnipeds in the future.

An age-dependent serological pattern, with a low probability of being seropositive for pups, a higher probability for yearlings, followed by a decreasing probability with age, previously identified in hooded (13) and harbor seals (14, 15) was identified in harbor seals in the present study, and similar patterns were present also for the other true seal species, however, solid conclusions could not be made due to sample size. Harbor and ringed seal juveniles had a higher probability of being seropositive than pups and adults. Likewise, when analyzing juveniles and adults by exact ages, there was a falling probability of seropositivity with age for harbor, spotted and ringed seals. Seropositive animals were lacking among individuals older than 5 years in spotted seals and 6 years in ringed seals, but were present among harbor seals until the age of 16, though at a very low prevalence. Almost all seropositive ribbon seals were between 1 and 6 years, though one was 12 years. The lack of significant results for ribbon seals, although the same trend was present, is likely due to the small number of animals sampled, and the lack of pups among the sampled animals. These results suggest that the investigated true seal species may be clearing the infection with increasing age.

Seals have an endotheliochorial placenta where 5–10% of the maternal antibodies are transferred to the fetus *in utero* and the rest are transferred through the colostrum. The immunity transmitted is determined by the level of systemic immunity in the mother (33). The low numbers of seropositive harbor, spotted, and ringed seal pups in this study indicates that these (1) have not received maternal antibodies against *Brucella* and (2) are not exposed to brucellae and hence have not mounted an antibody response yet. The low seroprevalence among pups is consistent with our finding that the majority of the females had no detectable levels of *Brucella* antibodies by the time they reached sexual maturity.

Brucella spp. in terrestrial animals causes reproductive pathology and may be transmitted during breeding and lactation or by crossing the placenta from mother to offspring (34). However, reproductive pathology is not associated with *B. pinnipedialis* infections in true seals and vertical transmission of *B. pinnipedialis* has never been described in a true seal species (6). The limited number of serologically and bacteriologically positive true seals, of reproductive age, in previous studies (13, 15, 35, 36), and the herein presented age-dependent serological patterns, further indicates that maternal transmission is unlikely as females have become seronegative for *Brucella* by the time they reach sexual maturity. Furthermore, the mean probability of being seropositive increased from pups to juveniles in previous studies (13–15) as well as in this study, suggesting that exposure to *B. pinnipedialis* is primarily during the post-weaning period and during the first few years of life, and is not transmitted *in utero* or to neonates.

Although underlying reason for this age-related serological pattern is unknown, it may be related to changes in diet. Stable isotopes and mercury biomarkers have indicated that in general, adult harbor and ringed seals feed at a higher trophic level than pups (37). Stable isotope analysis has shown that ribbon and spotted seals also feed at increasing trophic levels with age (38). Hence, there may be a general shift in diet composition toward higher trophic levels with increasing age, which coincides with the age at which seropositive juveniles start to appear, indicating a possible reservoir of *B. pinnipedialis* in one or more lower trophic level prey species.

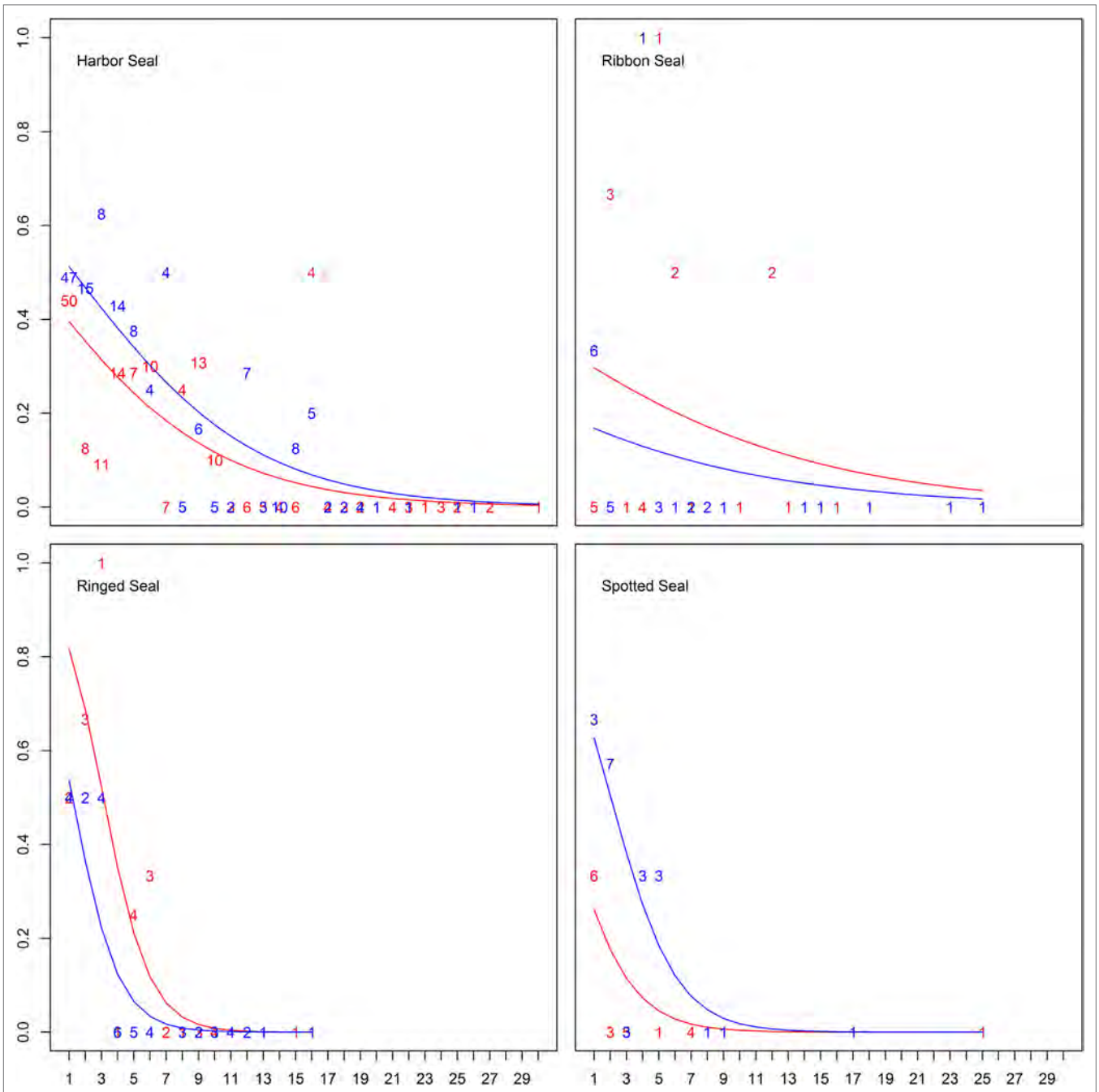

FIGURE 3 | Differences in probability of being seropositive between ages in years for true seals. Differences in probability of being *Brucella* spp. seropositive among four true seal species at different age and sex (males in blue, females in red). Lines show the predicted probabilities, while numbers indicate empirical frequencies for different ages.

Analysis by region showed a decrease in the probability of being seropositive for harbor seals in the Gulf of Alaska compared to the other regions. At Nanvak Bay, the largest haul-out in northern Bristol Bay, harbor seals declined in abundance between 1975 and 1990, but have increased since (2). Samples from harbor seals were from the time period 1975 to 2001, however, as different sites were sampled different years, we chose not to include year as a covariate in the statistical analysis. Still, univariate analysis of trends in seroprevalence over time did not reveal any overall trend suggesting that the *Brucella* infection in the harbor seal population in Bristol Bay may not contribute to higher mortality rates.

Considering the lack of impact on the harbor seal population trends, the age-dependent serological and bacteriological patterns (13–15, 35, 36), the lack of *Brucella*-associated pathology in true seals (6) and the lack of multiplication in established *in vitro*

TABLE 4 | Parameter estimates (logit-transformed) best approximating models, when age is included as a continuous predictor (for animal equal to, or older than 1 year).

Species	Predictor	Estimate	SE	*Z*-value	95% CI
Harbor seal	Intercept	−0.25	0.23	−1.10	(−0.70, 0.19)
n = 351	Age	−0.18	0.03	−5.84	(−0.24, −0.12)
	Sex—males	0.47	0.26	1.82	(−0.04, 0.99)
Ribbon seal	Intercept	−1.63	0.39	−4.23	(−2.47, −0.93)
n = 49					
Ringed seal	Intercept	2.18	1.06	2.06	(0.28, 4.57)
n = 67	Age	−0.70	0.22	−3.17	(−1.21, −0.33)
	Sex—males	−1.35	0.86	−1.56	(−3.18, 0.29)
Spotted seal	Intercept	−0.54	0.86	−0.63	(−2.33, 1.13)
n = 46	Age	−0.50	0.27	−1.86	(−1.12, −0.08)
	Sex—males	1.56	0.82	1.93	(0.04, 3.34)

For sex category, female is set as reference level. SE is standard error, while CI is confidence interval, and n is sample size used for parameter estimation (see Materials and Methods for details).

(8–10) and *in vivo* models (11, 12), it is possible that true seals may not be the primary hosts of *B. pinnipedialis*, but rather a spillover host. *B. pinnipedialis* has been isolated from lungworms in seals (35) and a recent experimental infection showed that a *B. pinnipedialis* hooded seal strain survived in Atlantic cod (*Gadus morhua*) (39). Moreover, a novel *Brucella* strain has been isolated from a fish, a bluespotted ribbontail ray (*Taeniura lymma*) (40). In addition, *Brucella microti* has been isolated from soil (41) and novel brucellae strains have been isolated from frogs (42–44), indicating an extended ecological niche of brucellae. Further investigation of marine sources for exposure to *B. pinnipedialis* should be performed in order to further reveal the epizootiology of *Brucella* infection in true seals.

The infection pattern in eared seals seems to be very different from that found in true seals. We detected *Brucella* antibodies in only two Steller sea lions, and none of the Northern fur seals. These findings are consistent with the low number of *Brucella*

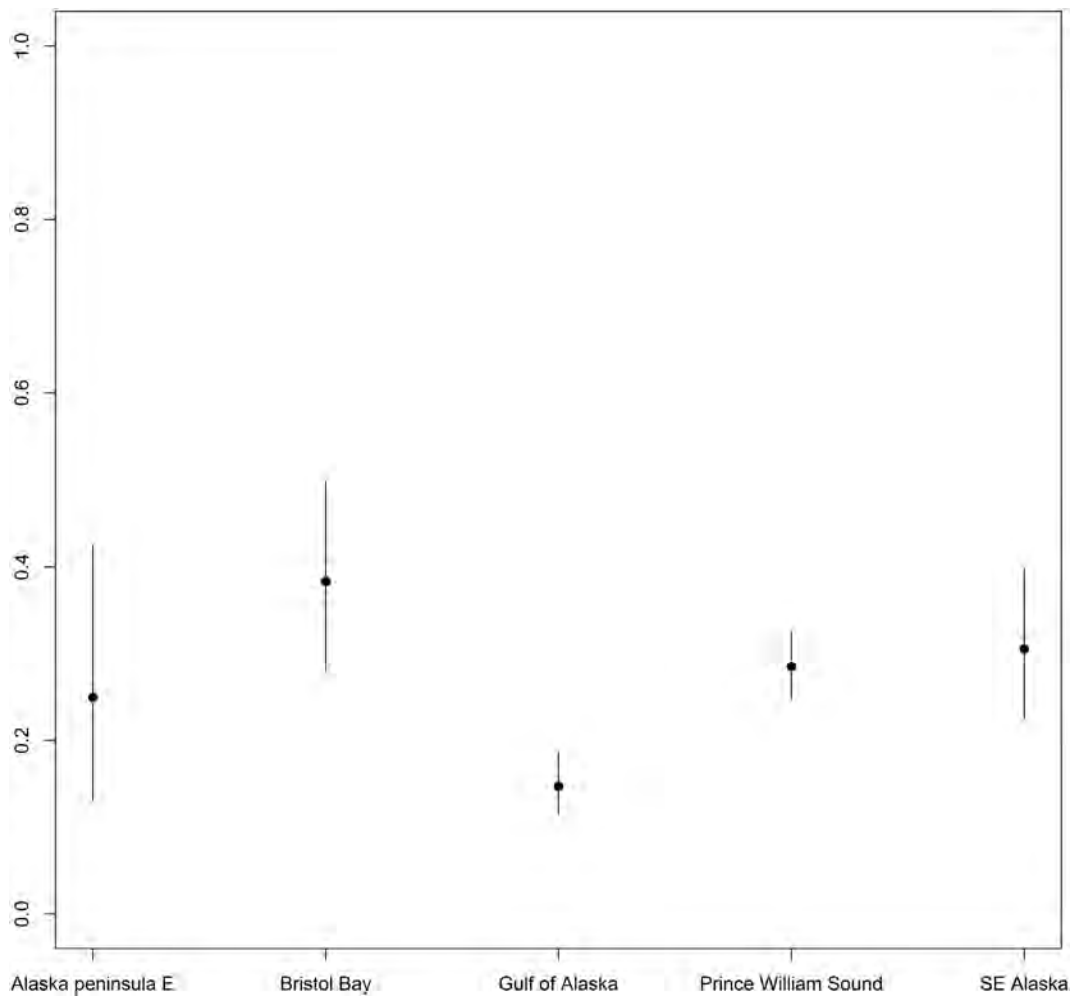

FIGURE 4 | Differences in probability of being seropositive between harbor seals sampled at various regions. Differences in probability of being *Brucella* spp. seropositive between harbor seals sampled at various regions in Alaska. Points indicate predicted mean for all age groups, while error bars indicate 95% confidence intervals.

isolates obtained from eared seals in other studies; four bacteriology positive California sea lion placentas (16, 17), of which two showed signs of inflammation and multifocal acute necrosis (17). Additionally, transplacental transmission of brucellae in California sea lions has been indicated when brucellae strains belonging to the zoonotic ST27 were detected by PCR in three placentas and multiple fetal tissues in parallel (16). Terrestrial brucellae of unknown origin have also been detected by PCR in blood and milk from two apparently clinically healthy wild California sea lions, and marine mammal brucellae were detected in blood and milk from one animal (19). *B. pinnipedialis* has also been detected by PCR in six Northern fur seal placentas, of which one had severe placentitis (18), and in one Northern fur seal spleen with no pathology associated (45). The low number of isolates and PCR-positive cases obtained from eared seals make drawing any conclusions regarding the presence or absence of pathology in these species difficult, however, it is worth noticing that the few cases reported have often been associated with pathology in the reproductive organs (16–18) and that transplacental transmission has been suggested (16). The low seroprevalences detected in eared seals of all ages in the present study suggests a low level of exposure due to possibly a different diet or a greater resistance toward the infection. Considering the reports of pathology in eared seals, morbidity and/or mortality due to infection is also possible. Further studies, including samples suited for bacterial isolation and/or PCR and from a higher number of individuals from different age groups, are needed to determine to what degree the infection is a threat to the Alaskan eared seal populations.

Certain eared seal species are able to host infections with the zoonotic ST27 (16) and terrestrial brucellae (19). There have been three cases of naturally acquired infections in humans with ST27, none of which had been in contact with marine mammals; however, they had been at the coast, eaten raw shellfish (46) or been in contact with raw fish bait (47). Further studies on both marine mammals and other species from the Arctic marine ecosystem are warranted in order to address this important issue, especially as marine mammals and other marine species are used for human consumption. Whether the zoonotic ST27 is present in Alaskan waters is currently unknown and warrants further investigation; however given the ample opportunities for transfer from marine mammals to humans, it appears that if ST27 were present more cases would be known.

In conclusion, the *Brucella* serological pattern is very different for true and eared seals. The infection in true seals seems to be relatively common, yet shown in the present study to be transient and decreasing with increasing age for harbor seals, becoming virtually absent at the age of sexually maturity. Similar patterns were present also for the other true seal species; however, firm conclusions could not be made due to sample size. This suggests that true seals may not be the primary hosts of *B. pinnipedialis*, but rather a spillover host susceptible to infection from other sources in the marine environment. In eared seals, we detected only two seropositive animals which could be explained by a low level of exposure or lack of susceptibility to infection; however, it could also be explained by high susceptibility to *Brucella* infection with mortality removing infected animals from the population. Comparison of true and eared seal *Brucella* isolates with established bacteriological and molecular methods (6) could provide new information about their potential differences and similarities. Furthermore, the pathogenicity of isolates should be compared to already characterized terrestrial *Brucella* strains in established *in vitro* cell (7) and *in vivo* mouse (12) *Brucella* models.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of four Institutional Animal Care and Use protocols approved by two separate committees. These protocols were approved by the Alaska Department of Fish and Game's Institutional Animal Care and Use Committee (protocols #06-16, 0921, #03-0014, 09-08, and #2010-13R) and by the University of Alaska Fairbanks' committee (protocol #98-23).

AUTHOR CONTRIBUTIONS

Acquisition of samples: LQ and KB. Design of study: IN, KB, and JG. Laboratory testing: IN. Statistical analysis: RR. Interpretation of data: IN and RR. Drafting the work or revising it critically for important intellectual content: IN, RR, AL, MT, LQ, KB, and JG. All authors have read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/articles/10.3389/fvets.2018.00008/full#supplementary-material.

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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Resource partitioning between Pacific walruses and bearded seals in the Alaska Arctic and sub-Arctic

L. E. Oxtoby[1,2] · L. Horstmann[1] · S. M. Budge[3] · D. M. O'Brien[4] · S. W. Wang[1,2] · T. Schollmeier[1] · M. J. Wooller[1,2]

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Abstract Climate-mediated changes in the phenology of Arctic sea ice and primary production may alter benthic food webs that sustain populations of Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*). Interspecific resource competition could place an additional strain on ice-associated marine mammals already facing loss of sea ice habitat. Using fatty acid (FA) profiles, FA trophic markers, and FA stable carbon isotope analyses, we found that walruses and bearded seals partitioned food resources in 2009–2011. Interspecific differences in FA profiles were largely driven by variation in non-methylene FAs, which are markers of benthic invertebrate prey taxa, indicating varying consumption of specific benthic prey. We used Bayesian multi-source FA stable isotope mixing models to estimate the proportional contributions of particulate organic matter (POM) from sympagic (ice algal), pelagic, and benthic sources to these apex predators. Proportional contributions of FAs to walruses and bearded seals from benthic POM sources were high [44 (17–67)% and 62 (38–83)%, respectively] relative to other sources of POM. Walruses also obtained considerable contributions of FAs from pelagic POM sources [51 (32–73)%]. Comparison of $\delta^{13}C$ values of algal FAs from walruses and bearded seals to those from benthic prey from different feeding groups from the Chukchi and Bering seas revealed that different trophic pathways sustained walruses and bearded seals. Our findings suggest that (1) resource partitioning may mitigate interspecific competition, and (2) climate change impacts on Arctic food webs may elicit species-specific responses in these high trophic level consumers.

Keywords Fatty acid · Benthic trophic ecology · Compound-specific · Stable isotope analysis · Climate change · *Odobenus rosmarus divergens* · *Erignathus barbatus*

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✉ L. E. Oxtoby
leoxtoby@alaska.edu

[1] Institute of Marine Science, College of Fisheries and Ocean Sciences, University of Alaska Fairbanks, 905 N. Koyukuk Drive, Fairbanks, AK 99775, USA

[2] Alaska Stable Isotope Facility, Water and Environmental Research Center, University of Alaska Fairbanks, 306 Tanana Loop, Fairbanks, AK 99775, USA

[3] Department of Process Engineering and Applied Science, Dalhousie University, PO Box 1000, Halifax, NS B3J 2X4, Canada

[4] Institute of Arctic Biology, University of Alaska Fairbanks, 230 Arctic Health Research Building, Fairbanks, AK 99775, USA

Introduction

Extensive sea ice decline and a temporal shift in seasonal Arctic sea ice retreat have important implications for ice-associated marine mammals, such as Pacific walruses (*Odobenus rosmarus divergens*) and bearded seals (*Erignathus barbatus*). Both species primarily rely on benthic food resources in the Bering, Chukchi, and western Beaufort seas (Lowry et al. 1980; Fay 1982; Fay et al. 1984). Whereas bearded seals are less reliant on sea ice for foraging (Quakenbush et al. 2011), walruses use ice that is available to them within areas of foraging to rest between dives

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(Burns and Frost 1979; Fay 1982; Jay et al. 2010). Projected changes in the sea ice environment may affect access to and availability of benthic prey, potentially affecting resource partitioning between walruses and bearded seals.

Changes in sea ice extent and timing of retreat may impact walruses' access to benthic prey. A reduction in the geographic extent of walrus foraging areas could, in turn, influence resource partitioning between walruses and bearded seals. Walruses utilize ice floes to access food resources on the Beringian shelf (Burns and Frost 1979; Ray et al. 2006). However, in recent years, sea ice retreated north of the shelf break, limiting its availability in shallow areas of the Chukchi Sea where walruses typically forage and rest (Jay et al. 2012). As a result, walruses came ashore to terrestrial haul-out locations in large aggregations that have exceeded 30,000 animals (Jay and Fischbach 2008; Monson et al. 2013). Although walruses have used terrestrial haul-out sites on the Russian coast for several decades (Kryukova et al. 2014), the presence of large walrus herds on the northwest coast of Alaska has been less common, though not unprecedented (Collins 1940). This behavior may have consequences for foraging energetics and prey selection (Costa 1991; Rosen et al. 2007; Noren et al. 2012). At haul-out sites, walruses become central place foragers, a strategy that could risk rapid depletion of local benthic food resources (e.g., Costa 1991; Womble et al. 2009). In years of low ice cover in the Chukchi Sea relative to historical records, walruses foraged more frequently in nearshore locations, some characterized by low caloric density (Jay et al. 2012; Wilt et al. 2014), and swam longer distances to access benthic hotspots or ice floes (Jay et al. 2012). When access to preferred feeding grounds is limited, walruses may also opportunistically consume prey that they encounter in the pelagic realm (e.g., seabirds or seals—Collins 1940; Lowry and Fay 1984; Donaldson et al. 1995). These changes in foraging behavior could increase competition among walruses and between walruses and bearded seals in these coastal locations and decrease competition elsewhere.

Sea ice conditions also influence algal production within the sea ice, under the ice, and in open water, as well as deposition on the seafloor, thus affecting the composition and distribution of benthic invertebrate communities (e.g., Grebmeier et al. 2006a; Arrigo 2013; Boetius et al. 2013). Changes in sea ice and algal phenology appear to differ in direction and magnitude between the Bering and Chukchi seas, however (e.g., Brown and Arrigo 2012), so the overall impact on benthic prey availability in foraging areas used by walruses and bearded seals remains unclear.

In the Bering Sea, sea ice cover varies among years, fluctuating between "warm" and "cold" periods. In cold years (e.g., 2007–2014), sea ice melts in spring and releases sea ice particulate organic matter (sympagic or ice—i-POM), which sinks to the benthos ungrazed, where it provides an important food source to benthic fauna (Grebmeier et al. 2006a). Ice melt also releases nutrients needed to seed a phytoplankton bloom in the water column at the ice edge (pelagic—p-POM) (Sakshaug and Skjoldal 1989; Perrette et al. 2011). I-POM and p-POM deposited on the sediment can also be oxidized by sediment microbial communities, creating a unique phytodetrital POM source (benthic—b-POM) that is available to benthic fauna (Oxtoby et al. 2016). In contrast, warm years (e.g., 2001–2005) are characterized by earlier ice retreat (Stabeno et al. 2012), which may have implications for trophic pathways, including those that sustain benthic biomass. In the Bering Sea, earlier ice melt may result in intensified wind mixing, which prevents stratification and delays the development of pelagic blooms (Hunt et al. 2002, 2008, 2011). When a pelagic bloom occurs later in the season, pelagic algal grazers are abundant and consume the p-POM before it sinks to the benthos (Walsh and McRoy 1986; Huntley and Lopez 1992). An ecological shift in the Bering Sea in which more production is partitioned to the pelagic realm and benthic prey resources concurrently decline (Grebmeier et al. 2006b; Grebmeier 2012) could result in increased competition among benthic-feeding marine mammals, such as walruses and bearded seals.

In the adjacent Chukchi Sea, however, recent observations of under-ice pelagic blooms suggest that export of production to the benthos may continue and possibly increase in this region, given the potentially high biomass and large spatial extent of these early season phytoplankton blooms (Arrigo et al. 2012, 2014; Lowry et al. 2014). Additional research is necessary to anticipate shifts in algal production and deposition in the Bering and Chukchi Seas, and any resulting changes in the benthic ecosystem under future climate scenarios.

Bearded seals and walruses have coexisted for thousands of years on the Beringian shelf, sharing food resources and foraging grounds in the Bering and Chukchi seas (Repenning 1976; Lowry et al. 1980; Harington 2008). Current understanding of ice-associated pinniped feeding ecologies is based on traditional ecological knowledge (TEK) (e.g., Noongwook et al. 2007; Huntington and Quakenbush 2013), stomach content analysis (e.g., Sheffield and Grebmeier 2009; Crawford et al. 2015), stable isotope analyses (e.g., Dehn et al. 2007; Seymour et al. 2014a, b), and, occasionally, direct observations of foraging (e.g., Donaldson et al. 1995; Lovvorn et al. 2010). Fatty acids (FAs) have been used to infer dietary niche separation between walruses and bearded seals (Budge et al. 2007) and to examine possible competition over benthic resources (Cooper et al. 2009; Wang et al. 2015b). More recently, compound-specific stable isotope analysis (CSIA) of FAs has been used as a tool to estimate proportional contributions of algal

FA sources with unique chemical signatures (e.g., i-POM, p-POM, and b-POM) to ice seals (Wang et al. 2016) and to benthic invertebrates (Oxtoby et al. 2016) to describe trophic connectivity in Arctic and sub-Arctic marine food webs (e.g., Budge et al. 2008; Graham et al. 2014; Wang et al. 2015a). When combined with FA analyses, CSIA of FAs can constrain proportional contributions of unique POM sources (i-POM, p-POM, and b-POM) to the diets of higher trophic level consumers.

In this study, we used a multi-proxy analytical approach (FA trophic markers, profiles, and CSIA of FAs), which provided several lines of evidence from which to interpret variation in the diets and trophic pathways that sustain walruses and bearded seals. Our main objectives were to: (1) describe the degree of dietary overlap between walruses and bearded seals, (2) estimate the proportional contributions of sympagic (i-POM), pelagic (p-POM), and benthic (b-POM) production sources to each species using previously published data (Wang et al. 2014, 2015a; Oxtoby et al. 2016), and (3) interpret differences in diet and trophic pathways between walruses and bearded seals by relating the stable carbon isotope composition of their algal FAs to those from benthic invertebrate prey from distinct feeding groups in the Chukchi Sea (this study) and previously published data from the Bering Sea (Oxtoby et al. 2016).

Materials and methods

Sample acquisition

Walrus specimens were opportunistically sampled during spring/summer subsistence harvests in 2009 ($n = 4$), 2010 ($n = 44$), and 2011 ($n = 9$) near the communities of Gambell and Savoonga on St. Lawrence Island, Alaska (Fig. 1; Table 1). Alaskan Native subsistence hunters provided samples for scientific research in collaboration with the U.S. Fish and Wildlife Service (USFWS), U.S. Geological Survey, the Eskimo Walrus Commission, and the North Slope Borough Department of Wildlife Management. Bearded seals, which were analyzed as part of another study (Wang et al. 2016), were collected in 2009 ($n = 10$) and 2010 ($n = 20$) in cooperation with Alaskan Native subsistence hunters from the communities of Savoonga, Little Diomede, and Point Hope (Fig. 1; Table 1) and the Alaska Department of Fish and Game (ADF&G) Arctic Marine Mammal Program. Bearded seal specimens were processed and analyzed using the same protocols and methodologies as described for walruses (additional information concerning bearded seal specimens is described in Wang et al. (2016)).

Subsistence hunters recorded information about individual walruses harvested, including sex, stomach contents,

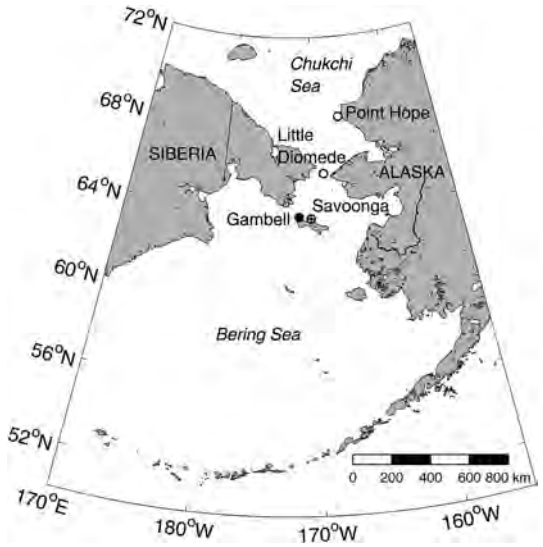

Fig. 1 Locations of Alaskan communities where Pacific walruses (*filled circle*), bearded seals (*open circle*), or both (*circle with cross*) were harvested in 2009–2011. Information on specimens and analyses is provided in Table 1

lactation status, presence of a calf, and body condition of the animals. Estimated ages of walruses were obtained by sectioning teeth to determine the number of growth layer groups in cementum, which correspond to the age of the animal (Mansfield and Fisher 1960) (Matson's Laboratory, Montana, USA). All animals (walruses and bearded seals) included in this study were >4 years of age based on tooth age estimates or hunters' evaluations of morphological and reproductive characteristics of the animals. Blubber samples from muscle to skin were taken from the trunk of the body immediately after death. Due to air temperatures below freezing, samples froze on site and were shipped frozen to UAF, where they were immediately wrapped in aluminum foil, and sealed in plastic bags for storage at −80 °C.

Benthic invertebrate specimens ($n = 160$ from seven taxa) were collected at 14 stations on the Chukchi Sea shelf in August and September 2012 during the Russian–American Long-term Census of the Arctic (RUSALCA), the Chukchi Sea Offshore Monitoring In Drilling Area (COMIDA), and the Arctic Ecosystem Integrated Survey (Arctic EIS) research expeditions. Samples were collected using bottom trawls or van Veen grabs at depths ranging from 34 to 58 m. Benthic prey consisted of an omnivore (the snow crab *Chionoecetes opilio*), a subsurface deposit-feeder (the bivalve *Nuculana radiata*), and suspension/surface deposit-feeders (the bivalves *Liocyma fluctuosa*, *Serripes groenlandica*, *Astarte* spp., *Macoma* spp., and *Ennucula tenuis*). Invertebrate samples were frozen at −20 °C and then freeze-dried in a Virtis Freeze Dryer

Table 1 Sample sizes of Pacific walruses and bearded seals analyzed for fatty acid composition and stable carbon isotope values of fatty acids ($\delta^{13}C_{FA}$)

	Pacific walrus		Bearded seal		
	M	F	M	F	U
FA composition					
2009	4	0	3	5	2
2010	8	11	5	15	0
$\delta^{13}C_{FA}$					
2009	4	0	3	5	2
2010	8	28	5	15	0
2011	1	8	0	0	0

See Fig. 1 for harvest locations

M males, *F* females, *U* unknown sex

(model 52; The Virtis Company, NY, USA) while on board the ship.

Sample preparation and lipid extraction

In preparation for lipid extraction, walrus blubber samples were placed on a sterile glass cutting board, and a sterile knife was used to trim the outermost layer of blubber away to expose inner blubber layers. A longitudinal sample from the exposed muscle to the skin layer (full blubber depth) was removed and weighed (~1 g). Wet weights and dry weights were taken for invertebrate specimens (Mettler 200 analytical balance Greifensee, Switzerland). Invertebrate samples were then homogenized and stored in crimp top vials at −80 °C prior to lipid extraction.

Lipids from full-depth blubber samples were solvent extracted using a modified Folch procedure in a ratio of 8:4:3 of chloroform, methanol, and deionized water (Folch 1957; Budge et al. 2006). Invertebrate samples from the Chukchi Sea, which were collected and analyzed as part of a separate research initiative, were lipid extracted using an accelerated solvent extraction (ASE) system (Dionex ASE 200, CA, USA). Approximately 0.5 g hydromatrix (Dionex, CA, USA) was combined with a subsample of 0.5 g homogenized freeze-dried tissue sample. An 11 ml stainless steel thimble was assembled with two cellulose filters and a thin layer of sand before the hydromatrix and tissue mixture were added. An additional cellulose filter was placed at the top before it was loaded into an ASE system. Dichloromethane (DCM, Fisher Thermo-scientific, Fair Lawn, NJ, USA) with butylated hydroxytoluene (BHT; Sigma Chemical, St. Louis, MO, USA) was added at 100 mg/L to prevent lipid oxidation. The extraction occurred at 85 °C under 1500 psi nitrogen with two static cycles of 5 min each. Fatty acid methyl esters (FAME) for all samples were prepared from lipid extracts using an acidic transesterification procedure according to Budge et al. (2006).

FA analysis and compound-specific stable isotope analysis of FAs

Relative proportions of individual FAs from blubber samples were measured using a Perkin Elmer Autosystem II gas chromatograph (GC) (Perkin Elmer, Boston, MA, USA) with a flame ionization detector (FID) containing a 30 m 0.25 mm i.d. column coated with 50% cyanopropyl polysiloxane (0.25 mm film thickness; J&W DB-23; Folsom, CA, USA). Samples (1 μL of FAME in hexane) were injected in splitless mode and analyzed according to a temperature program detailed in Budge et al. (2006). Each sample was analyzed in duplicate and FA proportions were averaged. FA identities were determined by cross referencing retention times with those from an in house standard (menhaden oil) containing FAs previously identified using GC mass spectrometry (MS) (Thermo Finnigan Polaris Q; Bremen, Germany).

FAs from invertebrate samples were analyzed for their relative concentrations by adding an internal standard (23:0 at 1 mg/20 mg lipid) prior to methylation. Esterified lipid samples were dried (TurboVap) and FAMEs re-suspended in hexane to 20 mg/ml. The FAs 16:1n-7 and 20:5n-3 were identified by comparing peak retention times in gas chromatography (GC-FID, model 6850, Agilent Technologies, Wilmington, DE) to a known FA standard (Supelco, 189-19). The Supelco 189-19 standard was used to create a calibration curve from 0.1 to 1.0 mg for quantification of 16:1n-7 and 20:5n-3. Calibration curves for both FAs were extended utilizing FAME standards (Sigma-Aldrich, Saint Louis, MO, USA) to reach concentrations of 5 mg/ml to encompass the concentration range found in samples. FA areas were corrected using Ackman response factors (Ackman and Sipos 1964), because they vary slightly for different FAs due to the interaction of FAs with the GC flame ionization detector (FID). The Ackman response factor is the recorded areas of the calibration curve divided by the 18:0 area values. The FA 18:0 was used as a baseline reference for Ackman response factors, because it elutes in the middle of the run for most marine samples (Ackman and Sipos 1964). Corrected FA areas were then related back to the area of the internal standard.

$\delta^{13}C$ values of individual FAME from walrus blubber and benthic invertebrate tissues were analyzed at the Alaska Stable Isotope Facility (ASIF) using a GC (Thermo Scientific Trace GC Ultra) linked to an isotope ratio mass spectrometer (IRMS—Thermo Finnigan Delta V) through a combustion interface (IsoLink; http://www.isolink.com). The GC column, temperature program, and mode of injection were the same as for the GC-FID analyses used for

blubber and invertebrate samples. 1 μL of FAME in hexane was injected at a sample concentration of FAME adjusted to generate a voltage of 500–3000 mV for 20:5n-3. We used a FAME standard consisting of 16:0 and 18:0 (Nu-Chek Prep, Inc.; Elysian MN), which we injected throughout sample runs to track analytical error ($n = 20$ injections), which was <0.1‰ and <0.2‰, respectively (expressed as 1 SD of 16:0 and 18:0).

Stable carbon isotope ratios of FAs in a sample are described using the conventional delta (δ) notation in parts per thousand (‰) and are expressed as follows: $\delta^{13}C = [(^{13}C/^{12}C_{standard}/^{13}C/^{12}C_{sample}) - 1] \times 1000$, where the standard is the international reference material Vienna Pee Dee Belemnite (VPDB). We analyzed a standard mixture containing eight calibrated *n*-alkanoic acid esters (Mixture F8, Indiana University Stable Isotope Reference Materials), where r^2 of the known versus expected correlation was >0.99, to calibrate $\delta^{13}C$ values of individual FAs. Expected $\delta^{13}C$ values for FAME from the standard mixture ranged from -23.24 ± 0.01‰ to -30.92 ± 0.02‰. We also corrected $\delta^{13}C_{FA}$ values to account for carbon added during transesterification using the following equation (Eq. 1):

$$\delta^{13}C_{FA} = [(n+1)(\delta^{13}C_{FAME}) - (\delta^{13}C_{methanol})]/n \quad (1)$$

where $\delta^{13}C_{FA}$ is the adjusted value of the FA of interest, *n* is the number of its carbon atoms, $\delta^{13}C_{FAME}$ is the calibrated value of the FAME, and $\delta^{13}C_{methanol}$ is the stable isotope composition of the carbon contributed by the methanol (Abrajano et al. 1994). $\delta^{13}C_{methanol}$ ($\delta^{13}C_{methanol} = -49$‰) was calculated by subtracting the $\delta^{13}C$ value of esterified 16:0 and 18:0 standards from the corresponding $\delta^{13}C$ values of their free FAs (Wang et al. 2014, 2015a).

FAs are expressed using the nomenclature A:Bn-X, where A indicates the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group of a FA. Non-methylene interrupted (NMI) dienoic and trienoic FAs are distinguished from methylene interrupted FAs by the symbol Δ (e.g., 20:2Δ5,11) and double bond positions are given relative to the carboxylic acid functionality. *Iso*- and *anteiso*-methyl branched FAs are further identified by lowercase italicized letters (e.g., *i*-15:0, a FA with 15 carbon atoms, 0 double bonds and a methyl branch on the second to last carbon atom in the chain). FA data (77 individual FAs) for bearded seals and walruses are provided in Online Resource 1.

We report relative proportions and summed relative proportions of FAs considered to be markers of specific sources (reviewed in Parrish 2013). NMI FAs, which are synthesized exclusively by benthic invertebrate taxa (Paradis and Ackman 1977; Joseph 1982; Kawashima 2005; Barnathan 2009; Monroig et al. 2012), were used as "benthic" markers. The sum of *iso*- and *anteiso*-methyl branched FAs with an odd number of carbon atoms (*ai*-15:0, *i*-15:0, *ai*-17:0, *i*-17:0) was used as a marker of bacteria (Volkman et al. 1980; Budge and Parrish 1998). The bacterial marker, which can be elevated in b-POM relative to i-POM and p-POM, can also vary among benthic invertebrate prey from different feeding guilds (Oxtoby et al. 2016). This makes it a useful trophic marker for examining differences in the types of benthic prey consumed (e.g., subsurface deposit-feeder, mobile predator, and suspension-feeder) and POM sources that sustain those prey. The FAs 16:1n-7, 20:5n-3, and 22:6n-3 are considered to be algal in origin (reviewed in Dalsgaard et al. 2003), so they were used as isotopic end members for compound-specific stable isotope multi-source mixing models.

Additional data sets were provided from other studies (i.e., Wang et al. 2014, 2015a, 2016; Oxtoby et al. 2016) to conduct the analyses described in the following section (*Data analyses*). Benthic invertebrate specimens, and POM samples from ice cores (i-POM), seawater (p-POM), and surface sediment scrapes (b-POM) were collected from the Bering Sea in collaboration with the Bering Sea Ecosystem Study (BEST-BSIERP) in 2009 and 2010. These samples were processed using the same lipid extraction and FA transesterification protocols and instrumentation as described for walrus blubber samples. Additional details regarding sample collection, preparation, and analysis of I-POM and p-POM are detailed in Wang et al. (2014, 2015a). Similar details for b-POM and benthic invertebrate analyses are described in Oxtoby et al. (2016).

Foraging grounds for walruses and bearded seals span the Bering and Chukchi seas (e.g., Lowry et al. 1980; Fay 1982; Jay et al. 2012). Evidence from recent tagging studies indicates overlapping and widespread habitat use throughout the Arctic and sub-Arctic seas by individual walruses (Jay et al. 2012; Quakenbush et al. 2016) and bearded seals (Cameron and Boveng 2009; Cameron et al. 2010); therefore, inclusion of invertebrate specimens and POM samples from the Chukchi and Bering seas was appropriate.

Data analyses

Multivariate non-parametric procedures were performed to describe differences in FA and NMI FA profiles based on 77 FAs that were present in proportions >0.1% of the total between species and by sex within species. FA percentage data were transformed using a $\log(X + 1)$ transformation prior to statistical analysis. We measured differences in profiles using a two-factor nested permutational multivariate analysis of variance (PERMANOVA) with species and sex (nested) as factors. Similarity percentage routines (SIMPER) were employed to identify the FAs that contributed most to dissimilarities in the FA and NMI FA profiles. For this analysis, we removed multivariate outliers

($n = 2$) based on an nMDS biplot and on hunters' observations. Multivariate outliers were male walruses harvested in Savoonga, AK in 2009 and 2010. One walrus was severely emaciated with an empty stomach, while the other walrus appeared healthy. PERMANOVA and SIMPER routines were performed in PRIMER (version 6, Primer-E Ltd). Univariate data met assumptions for parametric analysis, so a two-factor nested ANOVA was used to compare the relative proportions of individual NMI FAs (Fig. 3), the sum of NMI FAs, a composite bacterial marker (sum of *ai*-15:0, *i*-15:0, *ai*-17:0, and *i*-17:0), and δ^{13}C values of algal FAs (16:1n-7, 20:5n-3, and 22:6n-3) between species and by sex (nested) within species. Tukey Honest Significant Differences (HSD) test was performed for pairwise comparisons at a 95% significance level ($\alpha = 0.05$) (R version 3.2.2).

We used a Bayesian mixing model (SIAR, R version 3.2.3) (Parnell et al. 2010) to estimate the proportional contributions of FAs from POM sources (i-POM, p-POM, and b-POM) to walruses and bearded seals. Models were based on δ^{13}C values of the algal FA markers 16:1n-7, 20:5n-3, and 22:6n-3, so we ran four models using varying combinations of δ^{13}C values and models with and without concentration dependencies (Online Resource 2) for comparison (as in Wang et al. 2016). Concentration dependencies account for differences in the relative proportions of individual FA markers in POM sources by weighting the linear mixing model accordingly (Phillips and Koch 2002). We assumed an FA trophic enrichment factor of 0 (Budge et al. 2011; Wang et al. 2016). Male and female walruses were analyzed separately, whereas sexes were combined for bearded seals, because there were no significant differences in FA sources for bearded seals between sexes (see "Results"). Results are presented as means (95% credibility interval) (Bayesian confidence interval).

Walruses and bearded seals consume a wide variety of prey taxa, including benthic invertebrates, demersal, and pelagic prey (e.g., Sheffield and Grebmeier 2009; Huntington and Quakenbush 2013; Crawford et al. 2015). Therefore, we did not use a Bayesian mixing model to estimate the proportional contributions of benthic prey groups as the results would not accurately reflect the full breadth of their diets. Instead, we used δ^{13}C values of algal FAs from benthic invertebrate taxa that are examples of varying feeding types to qualitatively interpret differences in their diets. We compared δ^{13}C values of algal FAs from walruses and bearded seals to those from benthic prey from distinct feeding groups from the Chukchi Sea (an omnivore, a subsurface deposit-feeder, and suspension/surface deposit-feeders) (this study) and the Bering Sea (a predator, a head down deposit-feeder, and suspension/surface and subsurface deposit-feeders) (Oxtoby et al. 2016). Benthic invertebrate specimens from the Chukchi Sea were included in this study to extend the geographic coverage of benthic prey from the Bering Sea (Oxtoby et al. 2016) to known summer feeding grounds for Pacific walruses (e.g., Ray et al. 2006). Given the opportunistic nature of sample collection in the Arctic and sub-Arctic and the limited availability of compound-specific datasets, we were unable to account for interannual differences in δ^{13}C values of benthic prey from the Chukchi and Bering seas. However, mean δ^{13}C values of algal FAs (16:1n-7 and 20:5n-3) from *M. calcarea*, *E. tenuis*, and *N. radiata* collected in 2009–2010 from the Bering Sea (Oxtoby et al. 2016) were similar to those reported in 2012 from the Chukchi Sea (this study) (differences between mean values ranged from 0.4 to 0.7‰ and 0.7 to 1.3‰ for 16:1n-7 and 20:5n-3, respectively).

Results

Fatty acid profiles and markers

FA profiles of walruses and bearded seals were significantly different (PERMANOVA, $P < 0.01$) (Fig. 2). FAs that contributed most to differences between walruses and bearded seals were the NMI FAs 20:2Δ5,11, 22:2Δ7,15, and 24:1, 16:3n-3, 20:2n-9, 23:0, and *ai*-15:0 (SIMPER) (Fig. 2). Additional NMI FAs (22:2Δ7,13, 20:2Δ5,13, and 20:3Δ5,11,14) were among the FAs that collectively contributed up to 45% of the variation in FA profiles between species (Fig. 2). Within species, FA profiles differed between male and female walruses

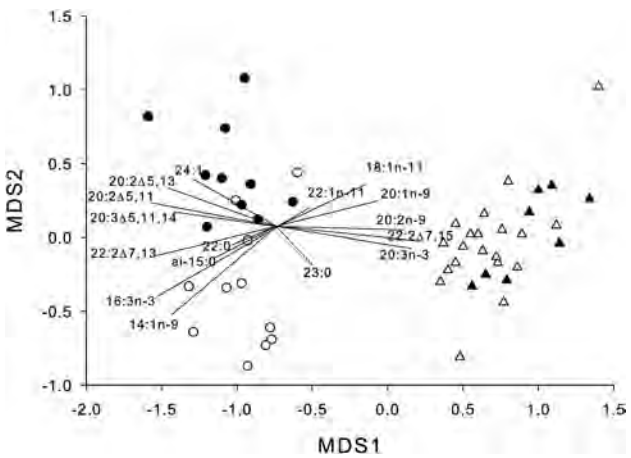

Fig. 2 Non-metric multidimensional scaling plot of male (*filled symbols*) and female (*open symbols*) Pacific walruses (*circles*) and bearded seals (*triangles*). Distances are based on Bray–Curtis similarity matrices using 77 fatty acids occurring in relative proportions >0.1%. Fatty acid vectors displayed are those that contributed most to differences between species and accounted for 45% of the dissimilarity (SIMPER). We set the level of dissimilarity to 45% to include all, but one non-methylene interrupted fatty acid. Vector length and direction correspond to the strength of correlation with nMDS axes. 2D stress = 0.07

(PERMANOVA, $P = 0.03$), but not between male and female bearded seals (PERMANOVA, $P = 0.07$) (Fig. 2).

NMI FA profiles of walruses differed significantly from those of bearded seals (PERMANOVA, $P < 0.01$). Walruses had significantly higher relative proportions of most NMI FAs (20:2Δ5,11, 20:2Δ5,13, 20:3Δ5,11,14, 22:2NMID, and 22:2Δ7,13) than bearded seals (two-factor nested ANOVA, $P < 0.01$) with the exception of 22:2Δ7,15, which was greater in bearded seals (two-factor nested ANOVA, $P < 0.01$) (Table 2; Fig. 3). Due to large relative proportions of 22:2Δ7,15 in bearded seals, the sum of the relative proportions of NMI FAs did not differ between species (two-factor nested ANOVA, $P = 0.10$) (Table 2). Sex-specific differences in NMI FA profiles were detected among walruses (PERMANOVA, $P < 0.01$), but not among bearded seals (PERMANOVA, $P = 0.21$). No significant differences were detected between sexes for either species for five of the six NMI FAs (two-factor nested ANOVA, $P > 0.10$) (Table 2). A significant difference was detected for 22:2NMID between male and female walruses (Tukey HSD test, $P < 0.01$) (Table 2).

The relative proportion of the composite bacterial FA marker was significantly higher in walruses relative to bearded seals (two-factor nested ANOVA, $P < 0.01$) (Table 2); no differences between sexes were detected for either species (two-factor nested ANOVA, $P = 0.90$).

Stable carbon isotope analysis of fatty acids

$\delta^{13}C$ values of algal marker FAs (16:1n-7, 20:5n-3, and 22:6n-3) were higher in bearded seals relative to walruses (two-factor nested ANOVA, $P < 0.01$), but did not vary between sexes in either species (two-factor nested ANOVA, $P > 0.10$) (Fig. 4). Mean $\delta^{13}C$ values of algal FAs ranged from -28.9 ± 1.2‰ (20:5n-3) to -26.6 ± 1.1‰ (22:6n-3) in walruses (mean ± 1SD,

Table 2 Relative proportions (% total) of benthic fatty acid markers in Pacific walruses and bearded seals, including individual non-methylene interrupted (NMI) fatty acids, their sum, and a composite bacterial fatty acid marker

	Pacific walrus	Bearded seal
20:2Δ5,11	0.29 (0.08)[a]	0.02 (0.02)[b]
20:2Δ5,13	0.16 (0.06)[a]	0.08 (0.02)[b]
20:3Δ5,11,14	0.06 (0.02)[a]	0.03 (0.01)[b]
22:2NMID*	0.08 (0.04)[a]	0.06 (0.02)[b]
22:2Δ7,13	0.20 (0.08)[a]	0.11 (0.06)[b]
22:2Δ7,15	0.08 (0.03)[a]	0.49 (0.11)[b]
Sum (NMI)	0.87 (0.23)[a]	0.78 (0.13)[a]
Sum (Bacterial)	0.97 (0.21)[a]	0.80 (0.09)[b]

Letters a, b indicate significant differences between mean values for each species (two-factor nested ANOVA, $P < 0.01$). Values are means (1SD) with sexes pooled. * 22:2NMID varied significantly between male and female walruses (*NMID* non-methylene interrupted dienoic fatty acid). The composite bacterial marker consists of the sum of the fatty acids *ai*-15:0, *i*-15:0, *ai*-17:0, and *i*-17:0

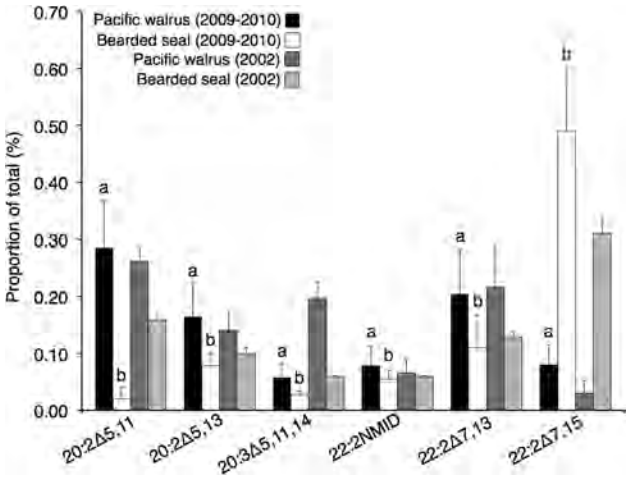

Fig. 3 Relative proportions (% total) of non-methylene interrupted fatty acids in Pacific walruses and bearded seals. Values are means + 1SD, with sexes pooled from 2009 to 2010 (this study) and from 2002 (Budge et al. 2007; Cooper et al. 2009). Letters *a*, *b* indicate significant differences between species (two-factor nested ANOVA, $P < 0.01$). All NMI FA were significantly higher in Pacific walruses than in bearded seals in 2009–2010, with the exception of 22:2D7,15

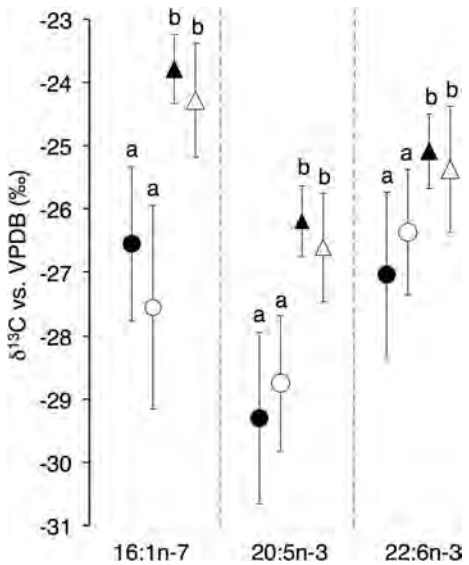

Fig. 4 $\delta^{13}C$ values (‰) for algal marker fatty acids from male (*filled symbols*) and female (*open symbols*) Pacific walruses (*circles*) and bearded seals (*triangles*). Algal marker fatty acids are 16:1n-7, 20:5n-3, and 22:6n-3 (mean ± 1SD). Letters *a*, *b* indicate significant differences between species and sexes (two-factor nested ANOVA, $P < 0.01$). $\delta^{13}C_{FA}$ values were consistently higher in bearded seals than in Pacific walruses

$n = 41$, sexes pooled) and from -26.5 ± 0.8‰ (20:5n-3) to -24.1 ± 0.8‰ (16:1n-7) in bearded seals (mean ± 1SD, $n = 28$, sexes pooled).

We present a range of estimates generated from Bayesian multi-source FA stable isotope mixing models that incorporated various combinations of algal marker FAs and their concentration dependencies (Tables 3; see Online Resource 3 for additional information about sources). The model that used 20:5n-3 and 22:6n-3 provided the most reliable estimates due to greater sample sizes compared with models that included 16:1n-7, which was not always measurable due to GC coelution of monounsaturated and saturated FAs containing 16 carbon atoms. Samples in which coelution occurred were removed from the data set prior to analysis, so model results that included 16:1n-7 had smaller sample sizes (fewer modeled individuals) relative to models without 16:1n-7. Through their diets, walruses and bearded seals obtained substantial contributions of FAs from b-POM [ranging from 44 (17–67)% in walruses to 62 (38–83)% in bearded seals] (Table 3). Bearded seal diets contained lower contributions from p-POM [10 (1–22)%] relative to walruses [51 (32–73%)] (Table 3). Proportional

Table 3 Estimates of the proportional contributions (%) of sympagic, pelagic, and benthic particulate organic matter (i-POM, p-POM, and b-POM, respectively) to consumer diets

i-POM	Pacific walrus (M)	Pacific walrus (F)	Pacific walrus (pooled)	Bearded seal (pooled)
Without				
16:1n-7, 20:5n-3, 22:6n-3	14 (0–28)	19 (0–36)	14 (0–26)	44 (36–52)
16:1n-7, 20:5n-3	26 (4–45)	19 (0–41)	23 (2–40)	44 (33–55)
16:1n-7, 22:6n-3	15 (0–29)	23 (2–42)	15 (2–28)	47 (38–56)
20:5n-3, 22:6n-3	10 (0–23)	17 (4–29)	13 (2–23)	40 (31–49)
With				
16:1n-7, 20:5n-3, 22:6n-3	8 (0–25)	15 (0–37)	14 (0–26)	31 (16–48)
16:1n-7, 20:5n-3	12 (0–36)	13 (0–35)	23 (2–40)	20 (1–64)
16:1n-7, 22:6n-3	15 (0–34)	26 (1–47)	15 (2–28)	18 (8–31)
20:5n-3, 22:6n-3	**7 (0–20)**	**8 (0–19)**	**13 (2–23)**	**27 (15–42)**
p-POM				
Without				
16:1n-7, 20:5n-3, 22:6n-3	37 (6–66)	49 (25–75)	45 (23–70)	5 (0–12)
16:1n-7, 20:5n-3	38 (8–69)	52 (26–80)	53 (27–81)	4 (0–10)
16:1n-7, 22:6n-3	21 (0–42)	37 (10–62)	27 (7–46)	5 (0–13)
20:5n-3, 22:6n-3	67 (41–91)	60 (44–74)	67 (54–80)	15 (3–27)
With				
16:1n-7, 20:5n-3, 22:6n-3	36 (10–60)	46 (20–73)	42 (21–61)	8 (0–17)
16:1n-7, 20:5n-3	42 (14–68)	50 (25–78)	49 (30–68)	5 (0–14)
16:1n-7, 22:6n-3	22 (2–44)	35 (7–62)	23 (4–43)	10 (0–24)
20:5n-3, 22:6n-3	**58 (31–90)**	**47 (27–69)**	**51 (32–73)**	**10 (1–22)**
b-POM				
Without				
16:1n-7, 20:5n-3, 22:6n-3	49 (15–83)	32 (3–57)	41 (13–67)	51 (39–62)
16:1n-7, 20:5n-3	35 (0–69)	28 (0–54)	23 (0–50)	53 (39–66)
16:1n-7, 22:6n-3	64 (35–93)	40 (9–70)	58 (33–83)	48 (35–60)
20:5n-3, 22:6n-3	23 (0–48)	24 (6–41)	20 (5–36)	45 (30–60)
With				
16:1n-7, 20:5n-3, 22:6n-3	56 (28–86)	39 (6–68)	54 (32–76)	61 (37–81)
16:1n-7, 20:5n-3	46 (17–76)	37 (4–63)	47 (26–67)	75 (28–97)
16:1n-7, 22:6n-3	63 (28–94)	39 (4–74)	63 (30–92)	72 (48–90)
20:5n-3, 22:6n-3	**35 (1–62)**	**45 (17–71)**	**44 (17–67)**	**62 (38–83)**

Values are based on stable isotope mixing models run without and with concentration dependencies (Online Resource 3) [means (95% credibility intervals)]. Male (M) and female (F) Pacific walruses were analyzed separately and combined, whereas sexes were combined for bearded seals. We posit that model estimates in bold type are the most reliable estimates based on sample size and incorporation of concentration dependencies

contributions of POM sources were similar between male and female walruses (Table 3). However, Bayesian credibility intervals were larger for walruses relative to bearded seals for POM sources, reflecting greater variation in their $\delta^{13}C$ values of algal marker FAs (Fig. 4).

The stable carbon isotope composition of algal FAs 16:1n-7 and 20:5n-3 from male and female walruses was similar to algal FAs from suspension/surface deposit-feeding bivalves and from an example of a subsurface deposit-feeder (the bivalve *N. radiata*) from the Chukchi and Bering seas (Fig. 5). In contrast, $\delta^{13}C$ values of algal FAs from male and female bearded seals clustered more closely to *Nephtys* spp., a predatory polychaete and *C. opilio*, an epibenthic omnivore (Fig. 5).

Discussion

Walruses and bearded seals collected from 2009 to 2011 had distinct diets consistent with earlier studies (Budge et al. 2007; Cooper et al. 2009). Interspecific dietary differences were revealed by variation in benthic prey taxa, as evidenced by differences in individual benthic FA markers. However, there was no evidence of difference in the sum of all benthic marker FAs between walruses and bearded seals, indicating a similar general reliance on benthic food resources. Benthic POM (b-POM) sources contributed the majority of FAs to the prey taxa that supported walruses and bearded seals; pelagic POM sources contributed an additional substantial source of FAs to the prey resources that supported walruses. We posit that differences in the diets and trophic pathways that sustain walruses and bearded seals resulted from higher predation on surface and subsurface deposit-feeding bivalves by walruses and from higher consumption of predatory and epibenthic omnivorous prey by bearded seals. Relative proportions of benthic marker FAs from this study are consistent with the patterns reported from 2002 (Budge et al. 2007; Cooper et al. 2009), suggesting that resource partitioning between species has not changed over time despite interannual variability documented in bearded seal diets based on their benthic marker FAs (Cooper et al. 2009; Wang et al. 2015b).

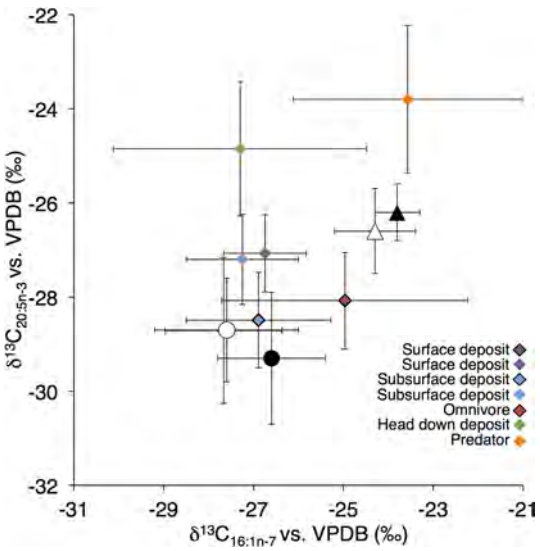

Fig. 5 $\delta^{13}C$ values (‰) of algal marker fatty acids (16:1n-7 and 20:5n-3) from male (*filled symbols*) and female (*open symbols*) Pacific walruses (*circles*) and bearded seals (*triangles*), and benthic invertebrate prey from distinct feeding groups. Prey taxa were collected from the Chukchi Sea (*symbols outlined in black*, this study) and Bering Sea (Oxtoby et al. 2016). Surface deposit = suspension/surface deposit-feeding bivalves *Liocyma fluctuosa*, *Serripes groenlandica*, *Astarte* spp., *Macoma* spp., and *Ennucula tenuis* (this study, covered by female walrus symbol), Surface deposit = *Macoma calcarea* and *Ennucula tenuis* (Oxtoby et al. 2016), subsurface deposit = subsurface deposit-feeding bivalve *Nuculana radiata*, Omnivore = omnivorous crab *Chionoecetes opilio*, Head down deposit = head down deposit-feeding polychaete *Leitoscoloplos pugettensis*, predator = predatory polychaete *Nephtys* spp.

Interspecific and intraspecific variation in diet

Walruses are gregarious animals that travel in large herds and occasionally congregate en masse at terrestrial haulout sites (Fay 1982; Jay and Fischbach 2008), whereas bearded seals tend to be solitary (Cleator et al. 1989; Simpkins et al. 2003). Increased foraging pressure on localized food resources by walrus herds could oblige individual walruses to consume different prey from one another. In contrast, bearded seals, as solitary individuals, might have a greater ability to forage on a similar variety of preferred food resources, assuming that similar prey distributions are available where individuals forage. We documented higher variation in the stable isotope composition of algal FAs from walruses relative to bearded seals. Differences in dietary breadth do not explain the higher variation, because evidence from stomach content analyses indicates that diets of walruses and bearded seals are characterized by similar breadth (Sheffield and Grebmeier 2009; Quakenbush et al. 2011). Instead, we posit that individual walruses consistently targeted different prey from one another whereas individual bearded seals consistently foraged for similar mixtures of prey relative to one another. These foraging patterns would account for variable $\delta^{13}C$ values of FAs from walruses despite having similar dietary breadth to bearded seals.

Differences in some FA dietary proxies between male and female walruses suggested sex-specific differences in diet, whereas there were no differences in FA dietary proxies between male and female bearded seals. Sexual segregation in summer likely explains dietary differences between male and female walruses (Jay and Hills 2005; Ray et al. 2006). In summer, females and calves migrate northward to the Chukchi Sea, while males migrate south to Bristol Bay

and locations along the Russian coast including the Gulf of Anadyr to forage (Fay 1982; Ray et al. 2006). We suggest that sex-specific differences in FA profiles can be attributed to the incorporation of prey FAs during sexual segregation in the previous summer. Male Walruses feed minimally during the winter reproductive period when females are present, so their blubber may preserve a summer foraging signal (Ray et al. 2006). This would imply a high accumulation of dietary fatty acids during summer months and a slow FA turnover rate, such that dietary differences could be detected during the spring harvest (i.e., up to 6 months after sexual reintegration in late fall). Blubber turnover rates have not been calculated for Pacific walruses or bearded seals. However, blubber turnover rates estimated for adult harp seals (*Phoca groenlandica*) (Kirsch et al. 2000) and newly weaned juvenile harbor seals (*Phoca vitulina richardsi*) (Nordstrom et al. 2008) reveal that turnover is nonlinear and likely occurs on the order of months. A minimum of 3 months was determined for juvenile harbor seals, which is likely an underestimate for adult walruses or bearded seals given the rapid growth characteristic of newly weaned juveniles (Nordstrom et al. 2008).

Differences in diet between sexes could also result from the ability of male walruses to obtain "atypical" prey [e.g., ringed (*Pusa hispida*) and bearded seals] (Lowry and Fay 1984; Huntington and Quakenbush 2013; Seymour et al. 2014b) and from selective foraging by reproductive females for high lipid content prey to support high energetic demands (Noren et al. 2012, 2014). Differential metabolism among males and females may affect FA profiles and $\delta^{13}C$ values of FAs. However, Pacific walruses and bearded seals are unlike certain phocid species that undergo dramatic fluctuations in body condition due to extreme fasting during life history stages like breeding, lactating, and molting. During breeding and molting (March through mid-July for bearded seals and April–August for Pacific walruses), sexes of both species employ a facultative feeding strategy (Fay 1982; Kovacs and Lavigne 1992; Cameron et al. 2010). As a result, we assume the influence of FA catabolism on FA dietary proxies to be minor compared to the influence of diet given intermittent foraging and a dietary signature representative of past foraging recorded in their blubber.

Although walrus and bearded seal diets were characterized by similar proportional contributions of benthic POM, their diets were distinct. FAs from walruses were isotopically similar to those from bivalves from the Chukchi (this study) and Bering seas (Oxtoby et al. 2016), which have been shown to consume organic matter available in surface sediments, irrespective of its origin (Oxtoby et al. 2016). Furthermore, a strong pelagic signal in primary consumers, such as suspension/surface deposit-feeders and subsurface deposit-feeders in the benthic environment, likely results from the dominance of pelagic production to total annual primary production in the Arctic and sub-Arctic marine ecosystem (McRoy and Goering 1976; Gosselin et al. 1997). We attribute dietary differences, including higher proportional contributions of p-POM to walruses relative to bearded seals, to greater consumption of suspension/surface and subsurface deposit-feeding bivalves.

Prey consumed by bearded seals contained lower proportional contributions of pelagic POM relative to those consumed by walruses. Of the prey species described, bearded seals were most isotopically similar to *Nephtys* spp., a mobile predator, and *C. opilio* (snow crab), an omnivore. These species have also been identified as major prey taxa in bearded seal diets based on stomach content analyses (Lowry et al. 1980). Previous research attributed high stable carbon isotope values of FAs ($\delta^{13}C_{FA}$) characteristic of *Nephtys* spp. from the Bering Sea to indirect consumption of i-POM through their prey based on the multi-proxy approach used in this study (Oxtoby et al. 2016). *C. opilio* consumes a broad range of benthic taxa, including bivalves, gastropods, polychaetes, amphipods, and other crustaceans (Kolts et al. 2013a, b; Divine et al. 2015). Carbon isotope ratios of snow crabs (total organic carbon—TOC) were high compared with bivalves (Kolts et al. 2013b), a pattern which is similar to our compound-specific results. However, whether these values reflect an ice algal or benthic signature is unclear. We attribute differences in the proportional contributions of POM sources to bearded seals relative to walruses to higher consumption of predatory and omnivorous invertebrates.

Demersal and pelagic fishes are also important food resources for bearded seals (Lowry et al. 1980; Quakenbush et al. 2011; Crawford et al. 2015). The frequency of occurrence of certain forage fish species, including Arctic Cod (*Boreogadus saida*) and walleye pollock (*Gadus chalcogrammus*), in bearded seal stomachs was greater in recent years (2003–2012) than during a historical period (1975–1984) (Crawford et al. 2015). However, there were no available data sets of $\delta^{13}C_{FA}$ values from adult fishes to elucidate dietary differences that may have resulted from consumption of fishes. Certain forage fish species (e.g., Arctic cod, Canadian eelpout—*Lycodes polaris*, and Longear eelpout—*Lycodes seminudus*) typically have a ratio of 20:1n-9 to 22:1n-11 greater than 1 (Iverson et al. 2002; Falk-Petersen et al. 2004; Dissen 2015), which was characteristic of bearded seals included in this study. Consequently, it is likely that these, and possibly other fish species, may account for part of the dietary signature in bearded seals. Compound-specific stable isotope analysis of additional prey taxa could offer further insight into the relative importance of prey from different feeding groups to the diets of walruses and bearded seals; for example,

shrimp, polychaetes, fish, and bivalve species such as *Mya* spp. that are dominant prey items in bearded seals and walrus stomachs (Lowry et al. 1980; Dehn et al. 2007; Sheffield and Grebmeier 2009).

Proportional contributions of i-POM to bearded seals are lower than recent estimates that also used FA stable isotope mixing models to apportion POM sources to bearded seals (Wang et al. 2016). Our model included a benthic source (b-POM), which is characterized by $\delta^{13}C$ values for 20:5n-3 comparable to those of i-POM, possibly due to isotopic fractionation associated with microbial degradation of algal material (see Sun et al. 2004; Oxtoby et al. 2016 for further discussion of isotopic fractionation of b-POM). Without consideration of differences among FA profiles and individual FA markers which differentiate i-POM and b-POM, a model containing only two sources (i-POM and p-POM) would allocate any contributions from b-POM to i-POM. Indeed, the sum of i-POM and b-POM contributions from our model was roughly equal to the contribution estimated from i-POM alone to bearded seal diets in 2009 and 2010 (Wang et al. 2016).

We interpreted contrasting diets as evidence of resource partitioning, but this conclusion relies on the premise that walrus and bearded seal share the same foraging area. Although specimens were harvested in geographically distinct areas, there is no evidence to suggest that movements of walruses or bearded seals would be confined to harvest locations. Aerial surveys and telemetry data from individuals corroborate ship-based observations and the traditional ecological knowledge describing seasonal migrations and habitat use (Lowry et al. 1980; Fay 1982; Huntington and Quakenbush 2013; Huntington et al. 2016). These studies document widespread habitat use by walruses (Jay et al. 2012; Quakenbush et al. 2016) and bearded seals (Cameron and Boveng 2009; Cameron et al. 2010) in the Bering and Chukchi seas. Factors that may explain patterns in habitat use for walruses include prey availability, bathymetry, and summer ice availability (Jay et al. 2012, 2014). Seasonal movements are similar for bearded seals; however, bearded seals are less reliant on sea ice as a haul-out (Cameron et al. 2010 and references therein).

Conclusions

Walruses and bearded seals had distinct diets in the recent study years. The sum of benthic prey markers did not differ between walruses and bearded seals, supporting the idea that they rely on benthic food resources to a similar extent. However, differences in individual benthic markers indicated that they relied on distinct benthic prey. Walruses and bearded seals were also sustained by two distinct trophic pathways characterized by different contributions of algal organic matter sources to their respective prey; specifically, higher predation on surface and subsurface deposit-feeding bivalves by walruses and higher predation on predatory and epibenthic omnivorous prey by bearded seals. Resource partitioning of benthic invertebrate prey may facilitate the co-occurrence of these two species. Given that the dominant trophic pathways supporting each consumer are distinct, climate-induced changes in algal production in the Arctic could affect walruses and bearded seals differently.

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Author contribution statement LEO wrote the manuscript. SMB, MJW, and LEO formulated the concept. SMB developed methodology for the fatty acid analysis. TS conducted the fieldwork for invertebrate sample collection. LEO and TS performed the fatty acid and compound-specific stable isotope analyses. LEO performed the data analysis based on helpful suggestions from LH, SMB, and DOB. LH, DOB, SWW, TS, and MJW provided substantive editorial advice.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All applicable institutional and/or national guidelines for the care and use of animals were followed.

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Viral emergence in marine mammals in the North Pacific may be linked to Arctic sea ice reduction

E. VanWormer[1,2], J. A. K. Mazet[1], A. Hall[3], V. A. Gill[4,5], P. L. Boveng[6], J. M. London[6], T. Gelatt[6], B. S. Fadely[6], M. E. Lander[6], J. Sterling[6], V. N. Burkanov[6], R. R. Ream[6], P. M. Brock[7], L. D. Rea[8,9], B. R. Smith[1], A. Jeffers[10], M. Henstock[11], M. J. Rehberg[8], K. A. Burek-Huntington[12], S. L. Cosby[10], J. A. Hammond[11] & T. Goldstein[1*]

Climate change-driven alterations in Arctic environments can influence habitat availability, species distributions and interactions, and the breeding, foraging, and health of marine mammals. Phocine distemper virus (PDV), which has caused extensive mortality in Atlantic seals, was confirmed in sea otters in the North Pacific Ocean in 2004, raising the question of whether reductions in sea ice could increase contact between Arctic and sub-Arctic marine mammals and lead to viral transmission across the Arctic Ocean. Using data on PDV exposure and infection and animal movement in sympatric seal, sea lion, and sea otter species sampled in the North Pacific Ocean from 2001–2016, we investigated the timing of PDV introduction, risk factors associated with PDV emergence, and patterns of transmission following introduction. We identified widespread exposure to and infection with PDV across the North Pacific Ocean beginning in 2003 with a second peak of PDV exposure and infection in 2009; viral transmission across sympatric marine mammal species; and association of PDV exposure and infection with reductions in Arctic sea ice extent. Peaks of PDV exposure and infection following 2003 may reflect additional viral introductions among the diverse marine mammals in the North Pacific Ocean linked to change in Arctic sea ice extent.

Climate change and natural variability are rapidly reshaping Arctic environments[1,2], where circumpolar declines in sea ice and rising water and air temperatures have the potential to affect diverse species of marine wildlife[3,4]. Reduction in sea ice extent and thickness impacts habitat availability, species distributions and interactions, as well as the breeding and foraging ecology of Arctic marine mammals[3,5,6]. Arctic climate change may also play an important role in marine mammal health[7]. In addition to influencing animal nutrition and physiological stress, environmental shifts may drive exposure to new pathogens in Arctic marine mammals[8]. By altering animal behavior and removing physical barriers[3], loss of sea ice may create new pathways for animal movement and introduction of infectious diseases into the Arctic[8,9]. Although the remoteness of Arctic marine mammals creates challenges for monitoring their health, pathogen surveillance in the North Atlantic and North Pacific Oceans provides clues to polar and circumpolar infectious disease dynamics. Phocine distemper virus (PDV), a pathogen

[1]Wildlife Health Center, One Health Institute, 1089 Veterinary Drive, School of Veterinary Medicine, University of California, Davis, CA, 95616, USA. [2]School of Veterinary Medicine and Biomedical Sciences, School of Natural Resources, University of Nebraska, Lincoln, NE, 68583, USA. [3]Sea Mammal Research Unit, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife, KY16 8LB, UK. [4]U.S. Fish and Wildlife Service, Marine Mammals Management, 1011 East Tudor Road, Anchorage, AK, 99503, USA. [5]NOAA Fisheries, 222W. 7th Ave, Rm 552, Anchorage, AK, 99513, USA. [6]Alaska Fisheries Science Center Marine Mammal Laboratory, NMFS, 7600 Sand Point Way N.E., Seattle, Washington, 98115, USA. [7]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, G61 1QH, UK. [8]Steller Sea Lion Research Program, Alaska Department of Fish and Game, Division of Wildlife Conservation, 1300 College Road, Fairbanks, AK, 99701, USA. [9]Institute of Northern Engineering, 1764 Tanana Loop, ELIF Suite 240, University of Alaska Fairbanks, Fairbanks, Alaska, 99775, USA. [10]Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Medical Biology Centre, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL, UK. [11]The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 0NF, UK. [12]Alaska Veterinary Pathology Service, 23834 The Clearing Dr., Eagle River, AK, 99577, USA. *email: tgoldstein@ucdavis.edu

responsible for extensive mortality in European harbour seals (*Phoca vitulina vitulina*) in the North Atlantic, was identified in northern sea otters (*Enhydra lutris kenyoni*) in Alaska[8]. This finding raised the possibility that increased contact between Arctic and sub-Arctic marine mammals could result from climate change-associated reductions in Arctic sea ice extent which could alter animal movement allowing for disease transmission across the Arctic Ocean.

Phocine distemper virus was recognized as an important pathogen of phocid seals in 1988 when an outbreak caused mass mortality among European harbour seals in the North Atlantic Ocean. An early hypothesis suggested that harp seals (*Pagophilus groenlandicus*) from the Arctic may have been the source and reservoir of infection for other seal species in the Atlantic, and sympatric grey seals (*Halichoerus grypus*) may provide the link for viral transmission from Arctic species to harbour seals[10,11]. A second PDV epidemic occurred among European harbour seals from May to November in 2002, leading researchers to question whether the virus had persisted in the region in marine or terrestrial hosts, or if it was reintroduced[12]. Differences between the 1988 and 2002 PDV isolates identified through phylogenetic analysis supported introduction of a new virus. However, a PDV isolate from a harbour seal infected during a 2006 outbreak on the US Atlantic coast showed more similarity (99.3% across the genome, Genbank accession numbers NC_028249, KY229928) to the 1988 isolate, suggesting the possibility of multiple viral lineages circulating in Arctic and Atlantic seal species[13]. While Atlantic harbour seals were highly susceptible to these three strains of PDV and suffered large population losses associated with infection[14], sympatric species, including grey seals and Arctic species such as harp and hooded (*Cystophora cristata*) seals, appear to have varying susceptibility. Although sporadic deaths do occur in these species, they are not on the order of magnitude seen with harbour seals (as reviewed in[9]).

In contrast to the North Atlantic, outbreaks of PDV have not been observed in the North Pacific Ocean. Although a small number of seropositive Kuril harbour seals (*Phoca vitulina stejnegeri*), Steller sea lions (*Eumetopias jubatus*), and spotted seals (*Phoca largha*) were reported in 1994–1999 near Japan[15], PDV was not molecularly confirmed in these animals and seropositive results may reflect exposure to the closely related canine distemper virus (CDV). Ringed seals (*Phoca hispida*) that were seropositive for PDV were detected along Canada's Northwest Territories near northern Alaska in 1993 and 1994[10], but serologic surveys of Pacific harbour seals (*Phoca vitulina richardsii*), Steller sea lions, and northern sea otters off Alaska prior to 2000 showed little evidence of exposure to distemper viruses, and PDV had not been identified as a cause of illness or death[16–18]. PDV was not confirmed in the North Pacific Ocean until virus was detected in northern sea otters sampled in 2004, which raised a number of questions regarding the timeline of introduction into the North Pacific, how the virus reached sea otters, and what role the virus may play in North Pacific marine mammal illness and mortality.

Nomadic Arctic seals with circumpolar distributions (e.g. ringed and bearded, *Erignathus barbatus*, seals) and geographic ranges that intersect with those of harp seals, may be carriers of PDV to the North Pacific. Overlapping distributions of marine mammal species may then allow for transmission of the virus to other ice seal species (spotted seals and ribbon seals, *Histriophoca fasciata*) and sub-Arctic species such as Steller sea lions, northern fur seals (*Callorhinus ursinus*), and northern sea otters. We hypothesized that reduction in Arctic Ocean sea ice increased contact among Arctic seal species, leading to PDV introduction into the North Pacific Ocean and infection in sub-Arctic species. We evaluated exposure to and infection with PDV in sympatric ice-associated seals, northern fur seals, Steller sea lions, and northern sea otters sampled in the North Pacific Ocean from 2001–2016 to explore the timing of PDV introduction, patterns of transmission following introduction, and environmental and other risk factors associated with the emergence of PDV across the North Pacific Ocean. We incorporated satellite telemetry data from ongoing ecological studies of seals and Steller sea lions, which provided a unique opportunity to combine animal movement and epidemiologic data to understand the potential spread of PDV.

Methods

Marine mammal capture and sampling. Ice-associated seals (bearded seals, ribbon seals, spotted seals, and ringed seals), northern fur seals, Steller sea lions, and northern sea otters were live-captured and manually restrained, sedated, or anesthetized using established methods during ongoing field studies[19–24]. From 2001–2016, paired blood and nasal swab samples were collected when possible[25,26]. Blood and tissues were also collected from dead animals harvested for subsistence[27] or stranded and recovered on beaches.

Ethics statement. All animal sampling and testing methods were carried out in accordance with relevant guidelines and regulations. Animals were sampled under experimental protocols approved by the US Fish and Wildlife Service's Marine Mammals Management Office (Permit MA041309-1 for northern sea otters) and the Alaska Fisheries Science Center's Marine Mammal Laboratory (Permits 14327-01 for northern fur seals, 782-1532 for Steller sea lions, and 782-176 and 782-1676 for ice-associated seals). Experimental protocols for Steller sea lion samples collected by Alaska Department of Fish and Game (ADF&G) were authorized under MMPA permits 358-1564, 358-1769, 358-1888, 14325, and 18537 and under ADF&G IACUC protocols 03-0002, 06-07, 09-28, 2011-025 and 2013-30. Samples were tested under the Biological Use Authorization BUA# R2407 approved by the University of California Davis Institutional Biological Committee.

Serologic and molecular analysis. Serology to detect antibodies to PDV (n = 1,227 animals tested 2001–2013) was performed by micro neutralisation[28]. Briefly, 1 in 10 dilutions of serum were made followed by 2-fold dilutions in high glucose DMEM medium (Gibco, USA). Cell monolayers were examined for cytopathic effect and end point neutralisation titres determined by the Reed and Muench method. Sera were tested against PDV/USA2006 and PDV/DK/2002 strains. As the earliest molecularly confirmed PDV infection in the North Pacific was detected in 2004[8], a subset of sera from Steller sea lions (n = 80) sampled 2001–2004 in Russia, the Aleutian Islands, the Gulf of Alaska, and Southeast Alaska was tested by micro neutralization against the related morbillivirus, canine distemper virus (CDV/Snyder Hill/wild type strain).

Serologic titres were log transformed and cumulative percent was plotted against the log(10) titre to determine the appropriate cut-off to classify positive animals for further analyses (i.e. the geometric mean). The cut-off for PDV seropositive animals used was ≥1:32 (log10 = 1.5, Supplementary Fig. 1). This threshold level was used to minimize the likelihood of false positives, providing a robust estimate of seropositivity. Steller sea lions tested for both PDV and CDV were classified as seropositive for one virus based on the highest titre measured in each sample.

For molecular analysis, total RNA was extracted using Tri Reagent™ (Sigma) and complementary DNA transcribed (Superscript III, Invitrogen) with random nonamers from nasal swabs, blood, and tissues from animals sampled 2001–2002 and 2004–2016 (n = 1,994). Samples were screened for a fragment of the morbillivirus phosphoprotein (P) gene using a Real-Time Quantitative PCR designed for the study (Forward primer (RT-P2 s): 5′-CAT GCT AAT GGA GGA AGA GTT GAC T-3′; Reverse Primer (RT-P2 as): 5′-GTT CTC CCA TCC CTC TTT TGG-3′; Probe (PDV P2): 5′d FAM-CTC TGC TTG GCA CAG GCC ACA ATG-BHQ-1 3′) to quantify viral load or a heminested PCR with universal morbillivirus primers followed by PDV and CDV-specific primers to obtain products for sequencing[29]. Heminested PCR products were visualized by agarose gel electrophoresis and products of the expected size were cloned before sequencing. Sequences were edited manually in Geneious Pro v9.1.3 (Biomatters Ltd., Auckland, NZ) and compared with known sequences in the Genbank database. Viral nucleotide sequences were aligned with ClustalW and MUSCLE, and phylogenetic trees constructed comparing the corresponding P-gene fragments (389 bp) of known morbilliviruses using Bayesian (Mr. Bayes) algorithms v3.2.6[30]. A subset of duplicate samples (n = 60 northern fur seal nasal swabs) was submitted to The Pirbright Institute, Non-Vesicular Disease Reference Laboratory in the UK for confirmatory testing. Nasal swab, blood, or tissue samples with a $C_t < 37$ and/or confirmed by sequencing were considered positive.

Assessing Arctic Ocean sea ice extent and open water routes from the north Atlantic to north Pacific Oceans. Sea ice in the Arctic Ocean reaches its minimum annual extent, which varies in size (area covered) and shape (geographic extent), during August to September. In some years, open water routes are created along its edges connecting the northern Atlantic and Pacific Oceans. Additionally, a trend in reduced minimum extent of the Arctic sea ice has been linked to long-term climate change[2]. Two potential routes of contact near the sea ice edge exist across the Arctic Ocean between the Atlantic and Pacific – (1) along the northern Russian coast from the Barents Sea and eastern North Atlantic and (2) along the coast and islands of northern Canada from the western North Atlantic (Fig. 1). Contact with potential PDV reservoir species is possible on either route, as harp seals breed in the Barents Sea, the coast of Greenland, and the eastern coast of Canada[31]. To evaluate associations between climate change-related reductions in Arctic sea ice extent and PDV exposure or infection, we assessed presence of Russian and Canadian open water routes through the Arctic Ocean in August-September of the year prior to animal sampling (e.g., ice extent in August-September 2002 was used for animals tested for PDV in 2003). Presence of open water routes in a given year (2000–2015) was manually assessed in ArcGIS v10.3.1 (ESRI, Redlands, California, USA) using satellite imagery of waters bordering northern Russia (from the Barents Sea to the Chukchi Sea) and northern Canada (from the eastern Canadian Arctic to the Canadian Northwest Territories) from publicly available, monthly Arctic sea ice extent shapefiles archived 1979-present by the National Snow and Ice Data Center[32]. These shapefiles classify areas of the Arctic Ocean with sea ice concentration less than 15% as open water.

Phocine distemper virus prevalence and risk factor analyses. PDV seroprevalence and prevalence of viral infection (PCR detection of viral nucleic acid) with 95% exact confidence intervals were calculated for each species and all species combined by year of sample collection. Observed prevalence was calculated as the number of positive (seropositive or PCR positive) animals divided by the total number of animals tested. To evaluate infection annually, we limited calculations for prevalence of viral infection to animals actively shedding PDV (PCR detection of PDV in nasal swabs from live captured and subsistence harvested animals). Prevalence calculations and statistical analyses described below were performed using R v3.3.2[33] with a significance level of $\alpha = 0.05$.

Associations between PDV exposure (seropositive titre) or viral infection (PCR positive) and demographic variables (animal group (ice-associated seals, Steller sea lions, northern fur seals, and northern sea otters), age class, and sex), disposition (live captured or subsistence harvested vs. found dead), and presence of an open water route through Arctic Ocean sea ice were assessed in mixed effects logistic regression models using the lme4 package[34]. We evaluated three open water route variables: (1) presence of an open water route along the Russian coast, (2) presence of an open water route along the Russian coast in a year following closed sea ice, and (3) presence of an open water route along the Canadian coast. Sampling region (Bering Sea, Pribilof Islands, Southeast Alaska, Eastern Gulf of Alaska, Central Gulf of Alaska, Western Gulf of Alaska, Eastern Aleutian Islands, Central Aleutian Islands, Western Aleutian Islands, or Russia) was determined based on individual animal sampling locations and included as the random effect in mixed effects models. Risk factors associated with PDV exposure or viral infection in univariable regression models ($P \leq 0.20$) were incorporated into multivariable models. Following inclusion of statistically ($P \leq 0.05$) and biologically significant variables, multivariable models were compared using Akaike's Information Criterion (AIC) to select the most parsimonious model. Adjusted odds ratios with respective 95% confidence intervals were calculated for levels of each risk factor.

Satellite tag data and spatial analyses. Telemetry databases maintained by the Alaska Fisheries Science Center's Marine Mammal Laboratory and ADF&G were queried retrospectively for bearded seals, northern fur seals, and Steller sea lions tagged at times and locations that coincided with the geographic area and, when possible, years of PDV sample collection. Location data received from Service Argos were filtered[35] using a swim speed of 2 m/s for bearded seals (n = 7), northern fur seals (n = 136), and Steller sea lions (n = 77). Filtered data were processed with a continuous-time correlated random walk (CTCRW) model to predict animal locations for time

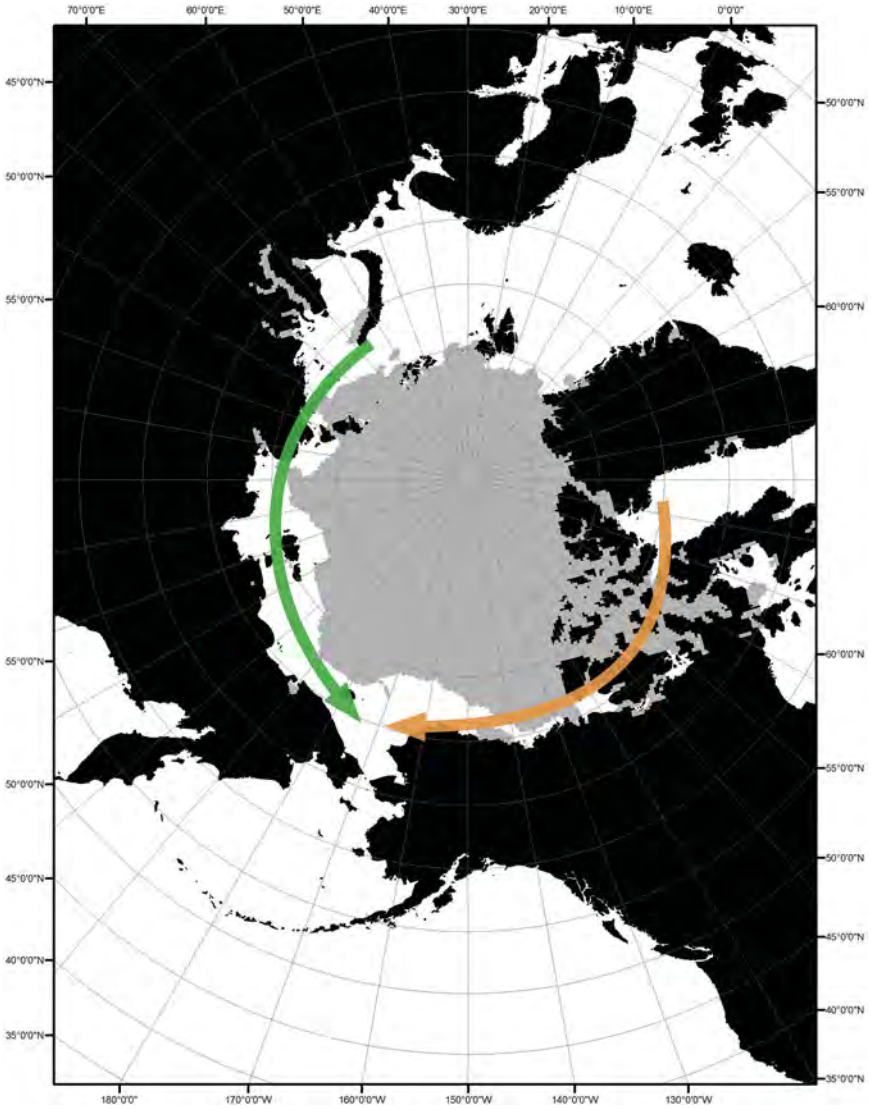

Figure 1. Potential routes for movement of seals infected with PDV through the Arctic Ocean opened by reductions in sea ice extent. Routes along northern Russia (green) and northern Canada (orange) are shown with August 2002 sea ice extent. Changes in historic sea ice barriers may facilitate Arctic and sub-Arctic seal movement and contact that was not possible in years prior to PDV detection, allowing for introduction of PDV into the Northern Pacific Ocean.

intervals (e.g. 20 min or 1 hour) corresponding to the timing of conductivity readings (i.e. wet or dry) collected for each species[36,37]. This model provided swim speed estimates (m/s) between sequential predicted locations, which were used to obtain the average median swim speed (m/s) for each species at sea.

The duration of viral shedding for PDV in pinniped species is unknown, but previous estimates of the combined latent (post-exposure, but not shedding virus) and infectious (shedding virus) periods in European outbreaks range from 11–18 days in harbour seal outbreak models[38]. Mean latent periods of 6–10 days and mean infectious periods of 9–13 days were used to model CDV transmission among wild canids in terrestrial systems[39]. A modeling study of an outbreak of another marine morbillivirus, Dolphin morbillivirus, estimated a mean infectious period of 8 days, with an upper bound of 24 days[40]. To capture variability in marine mammal latent and infectious periods and to assess the geographic distances that an animal infected with PDV could move and potentially transmit the virus (i.e. movement during latent and infectious periods), the distance moved by a PDV-infected individual of a given species was estimated for 1, 2, and 4 week periods (calculated as the median speed (m/s) of movement multiplied by the number of hours in the time period). The resulting estimated 1, 2, and 4 week movement distances in kilometers (km) were used to spatially buffer known animal sampling locations in ArcGIS v10.3.1 to map the distance the virus could be transported via animal movement. A subset of animals with PDV serologic results (seropositive and seronegative animals) and/or PCR testing results (only PCR negative animals) had telemetry data available. For seropositive animals, 1, 2, and 4 week post-sample collection movement tracks were overlaid on the spatial distance buffers to compare observed and expected movement of the virus with host movement.

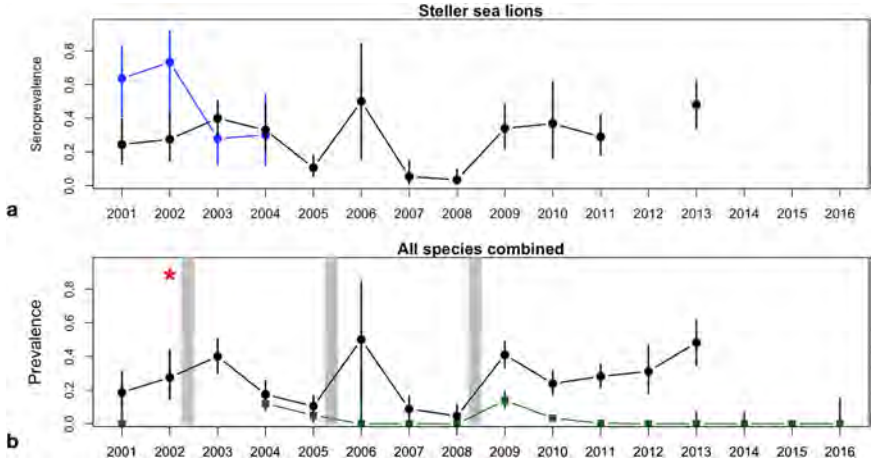

Figure 2. (**a**) Yearly seroprevalence for antibodies to PDV in Steller sea lion pups, juveniles, and subadults (black dots) with seroprevalence of canine distemper virus antibodies (blue dots) measured in a subset of Steller sea lions (n = 80); (**b**) PDV seroprevalence (black dots) and viral infection prevalence (PDV nucleic acid detected from nasal swabs; green squares) for all species combined (ice-associated seals, Steller sea lions, northern fur seals, and northern sea otters) from 2001–2016. Error bars represent 95% exact confidence intervals (CI). A 95% CI was not included for viral infection prevalence in 2008 as only one animal was tested. Presence of an open water route through Arctic sea ice along the northern Russian coast following a year of closed sea ice (grey bars) was significantly associated with animals testing seropositive or PCR positive for PDV. The strain of PDV responsible for an outbreak in harbour seals in the North Atlantic Ocean during 2002 (red star) was detected in PCR positive animals in the North Pacific Ocean throughout the study period.

Results

Samples were collected from 2,530 live and 165 dead ice-associated seals (bearded, ringed, ribbon, and spotted seals), Steller sea lions, northern fur seals, and northern sea otters in the North Pacific Ocean between 2001 and 2016; not all species were sampled in all years (Supplementary Table 1). Sampling locations ranged from Southeast Alaska to Russia along the Aleutian Islands as well as the Bering, Chukchi, and Beaufort Seas.

Steller sea lion samples for serology and/or PCR testing were available for the majority of the study period (13 of 16 years) and suggest peaks in PDV exposure and infection beginning in 2003. Animals sampled earlier in the study period showed greater evidence of exposure to CDV. Comparisons of seroprevalence of PDV and CDV in a subset of 80 Steller sea lions tested for both viruses 2001–2004 suggest exposure to CDV in 2001 (64%; 14 of 22 animals tested) and 2002 (73%; 11 of 15 animals tested, Fig. 2a), shifting to exposure to PDV in 2003 (72%; 18 of 25 animals tested) and 2004 (70%; 14 of 20 animals tested).

Following reduced Arctic sea ice extent and presence of an open water route along Russia in August 2002, serologic and PCR results demonstrated widespread exposure to and infection with PDV in Steller sea lions in the North Pacific Ocean in 2003–2004. Seropositive sea lion pups (2–11 months of age) and juveniles were present at rookery and haul-out locations in the western Pacific/eastern Russia (Koslov Cape and Medny Island), Aleutian Islands (Ugamak Island), Gulf of Alaska (Chowiet Island, Glacier Island, Perry Island, Seal Rocks), and Southeast Alaska (Benjamin Island, Gran Point, Little Island, Southwest Brothers Island) in 2003–2004 (Fig. 3), with over 30% of animals seropositive in both years (38 positive/95 tested in 2003 and 16 positive/48 tested in 2004, Supplementary Table 2). Although no PCR samples were available for 2003, 21 Steller sea lions (12.3% of 171 tested) were PCR positive for PDV (viral RNA detected in nasal swab samples from live-captured animals) in 2004. The PCR-positive animals were 2–3 month old pups from widespread locations across the North Pacific Ocean (Fig. 3) including Russia (Medny and Yamsky Islands), the Aleutian Islands (Adugak and Yunaska Islands), and the Gulf of Alaska (Sugarloaf Island).

After the widespread PDV exposure and infection in 2003–2004, seroprevalence and the proportion of PCR positive Steller sea lions decreased in 2005 (10.6% and 5.3%). A higher seroprevalence (50%) was detected among a small sample (n = 8) of 9-month old pups captured at one location in the Gulf of Alaska in March-April of 2006. These animals were in the 2005 birth cohort, and may have been exposed to the virus in late 2005 or early 2006 following the decline of maternal antibodies. Seroprevalence decreased further in 2007 and 2008 (5.4% and 3.5%, respectively), and all Steller sea lions tested negative by PCR from 2006–2008 (n = 27). Similarly, PDV titres decreased from 2005–2008 (Supplementary Fig. 3a). However, Steller sea lion seroprevalence rose 10-fold from 2008 to 34% in 2009, remaining high in 2010, 2011, and 2013 (36.8%, 29%, and 48.1%; Fig. 2a).

Patterns of seroprevalence and viral infection (PCR) in all sampled species support the second peak of PDV exposure and infection in 2009 (Fig. 2b, Supplementary Table 2). The high seroprevalence (68.7%) observed in ice-associated seals of all ages in 2009 decreased significantly in 2010 (6.3%). Serologic titres also decreased from 2009–2010 (Supplementary Fig. 3b), and the proportion of PCR positive ice-associated seals decreased, from 16% (10 positive/62 tested) in 2009 to 0% (43 tested) in 2010. Seroprevalence and serological titres increased from 2009–2012 in northern fur seals (Supplementary Fig. 3c), whereas the proportion of PCR positives decreased from 11.6% (7 positive/60 tested, confirmed by testing a subset of samples at The Pirbright Institute,

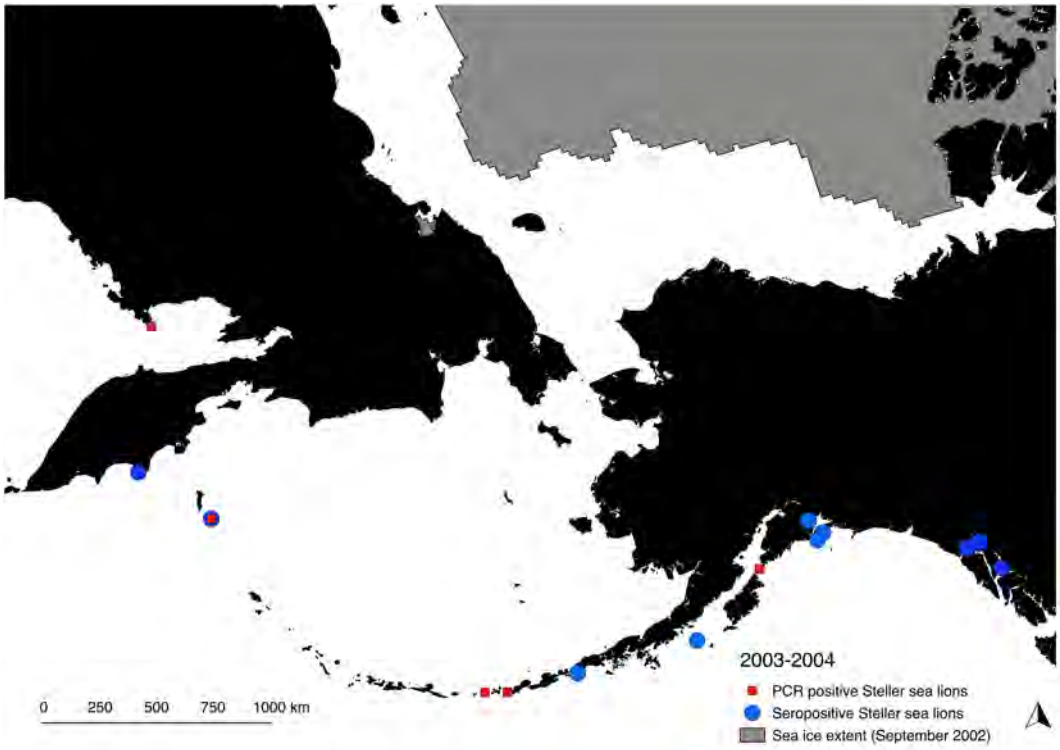

Figure 3. Locations of PDV seropositive and PCR positive Steller sea lions detected from Southeast Alaska to eastern Russia in 2003–2004. Sea ice is shown at its minimum extent in September 2002 prior to widespread detection of PDV in the North Pacific Ocean beginning in 2003.

Non-Vesicular Disease Reference Laboratory) in 2009 and 4.3% (12 positive/280 tested) in 2010 to zero in 2011 and 2012 (57 and 250 animals tested).

Prevalence of PDV viral shedding for all species combined peaked in 2004 and 2009 (no PCR data were available for 2003; Fig. 2b, Supplementary Table 2). Controlling for animal group, age class, and disposition (live vs. dead), the odds of viral infection were 9.2 times higher (95% CI: 5.1–16.8) in animals sampled in 2004 and 2009 relative to other years when animals were tested by PCR (Table 1, model 2). Detection of virus in nasal swabs from live or subsistence harvested animals, suggesting active viral shedding, indicated recent infection. Similar to the decrease detected from 2004 to 2005 (Fig. 2b), viral shedding in all species fell four-fold from 2009–2010 and remained low from 2011–2016. Viral presence was also detected in tissues from dead northern sea otters in 2006 (3 positive/30 tested), 2007 (4/22 tested), 2008 (4/52 tested), and in 2009 (2/9 tested), and from dead SSL pups and fetuses in 2005 (1/8 tested) and in 2012 (2/5 tested). Detection of viral nucleic acid in tissues from dead animals demonstrates that virus is present but doesn't necessarily indicate viral replication and shedding. Therefore, determining the timing of infection for dead animals that test PCR positive for PDV in their tissues is challenging. Sequences of the short fragment of the P-gene from PCR-positive animals of all species and across years and locations clustered together and were most similar to the PDV2002 strain isolated from European harbour seals infected during the 2002 PDV outbreak (Supplementary Fig. 2).

Increased levels of PDV exposure or infection in the sampled animals were associated with the presence of an open water route along the Russian coast. Reductions in sea ice extent created open water routes to the Pacific Ocean along the northern Russian coast (Fig. 1) in August and/or September of 2000, 2002, 2005-2006, and 2008–2015, with the open water routes in 2002, 2005, and 2008 following a year in which sea ice blocked passage through at least part of the Arctic Ocean bordering Russia's coast. When controlling for animal group and age class, presence of an open water route along the northern Russian coast following a year in which the Arctic sea ice along the Russian coast was closed was significantly associated with PDV exposure or infection (Table 1, model 1). The odds of PDV exposure or infection were 3.1 times higher (95% CI: 2.2–4.2) in animals sampled in a year following one of these complete openings in the Russian coast sea ice in August–September of the previous year. In contrast, presence of an open water route along the Canadian coast, which existed in August and/or September of 2006–2007, 2010–2012, and 2015, was negatively associated with PDV exposure or infection (OR: 0.7; 95% CI: 0.5–0.9).

Infected pinnipeds shedding PDV have the potential to reach nearby rookeries and haul-outs as well as more distant areas inhabited by conspecifics or other species (Fig. 4a). Median speeds of travel calculated from satellite-tagged bearded seals, northern fur seals, and Steller sea lions were 0.36 m/s, 0.89 m/s, and 0.32 m/s, respectively. Bearded seal speeds were used as a conservative movement estimate for wide-ranging spotted seals. Estimated movement distances for the 1, 2, and 4-week periods were 210 km, 420 km, and 840 km for bearded and spotted seals; 535 km, 1,070 km, and 2,140 km for northern fur seals; and 194 km, 388 km, and 776 km for Steller sea lions. Recorded movement tracks from one PDV seropositive bearded seal sampled in 2009 and one

Model Number: dataset	Risk Factor (reference category)	Adjusted Odds Ratio	95% Confidence Interval	P-value
1: Viral exposure or infection (serologic or PCR) status	**Open water along northern Russia following a closed ice year**			
	(No: 2000-1, 2003-4, 2006-7, 2009-15)	1	—	—
	Yes: 2002, 2005, and 2008	3.1	(2.2–4.2)	<0.01*
	Open water along northern Canada			
	(Ice present along northern Canada)	1	—	—
	Open water along northern Canada	0.7	(0.5–0.9)	<0.01*
	Age			
	(Adult)	1	—	—
	Juveniles and Subadults	0.5	(0.3–0.7)	<0.01*
	Fetuses, Pups, Young of Year	0.5	(0.3–0.9)	0.01
	Marine mammal group			
	(Steller sea lion)	1	—	—
	Ice-associated seals	1.6	(0.6–4.4)	0.38
	Northern fur seals	0.5	(0.2–1.6)	0.26
	Northern sea otters	0.5	(0.3–0.9)	0.02*
2: Viral infection (PCR) status	**Peak year**			
	No: 2001, 2002, 2005-8, 2010-16	1	—	—
	Yes: 2004 and 2009	9.2	(5.1–16.8)	<0.01*
	Age			
	(Adult)	1	—	—
	Juveniles and Subadults	0.4	(0.2–0.9)	0.04*
	Fetuses, Pups, Young of Year	1.6	(0.7–3.8)	0.28
	Marine mammal group			
	(Steller sea lion)	1	—	—
	Ice-associated seals	2.2	(0.6–8.3)	0.26
	Northern fur seals	3	(0.7–12.7)	0.14
	Northern sea otters	1.2	(0.3–5.3)	0.79
	Sampling status			
	(Healthy: live capture or subsistence harvest)	1	—	—
	Dead stranded	6.2	(1.9–20.6)	< 0.01*

Table 1. Risk factors for PDV exposure or viral infection (Model 1) and viral infection (Model 2) in marine mammals sampled in the North Pacific Ocean 2001–2016. *Statistically significant association with PDV exposure or infection, $\alpha = 0.05$.

seropositive northern fur seal sampled in 2010 occurred within the estimated 1, 2, and 4-week movement distances for each species (Fig. 4b). Movement tracks for three additional PDV seropositive animals (two bearded seals and one northern fur seal) sampled in 2009 also fell within their species-specific 1, 2, and 4-week estimated movement zones. PCR positive spotted seals, ribbon seals, and northern fur seals sampled 2009–2010 were detected in close proximity to bearded seal or northern fur seal movement tracks (Fig. 4b).

Discussion

Serologic, PCR, and sequencing results provide evidence for (1) widespread exposure to and infection with PDV across the North Pacific Ocean beginning in 2003; (2) viral transmission across multiple marine mammal species; and (3) decline of viral infections following peaks of exposure in 2003 and 2009, with sporadic detection of PCR positive animals from 2005–2008 and 2011–2016. Peaks of PDV exposure and infection followed reductions in Arctic sea ice extent, potentially linking climate change and interannual variability with introduction of PDV into the North Pacific Ocean.

During August and September of 2002, sea ice extent in the Arctic Ocean reached a new minimum[32]. Open water along most of northern Russia linked the North Pacific Ocean to the eastern North Atlantic Ocean (Fig. 1), where European harbour seals were dying in an epidemic of PDV that began in May 2002. Reduction in the sea ice that previously created a barrier to animal movement across the Arctic Ocean may have allowed for increased contact between harp seals, the proposed Arctic reservoir of PDV[10,11,41], and circumpolar seal species (including bearded seals and ringed seals). As the strain of PDV detected in North Pacific marine mammals is most similar to the strain isolated during the 2002 outbreak in European harbour seals, seals may have moved it from the North Atlantic outbreak or they may share a common source. If the 2002 PDV strain was circulating in harp seals that breed in the Barents Sea or near the coast of Greenland, it could have been carried south to grey seals and/or sympatric European harbour seals in early 2002 and across the Arctic Ocean to the North Pacific Ocean in August-September. Evidence of widespread PDV exposure in the North Pacific was first detected in Steller sea lions in our study in 2003, with 20 two month-old pups, 10 four to five month-old pups, and 8 animals 16 months

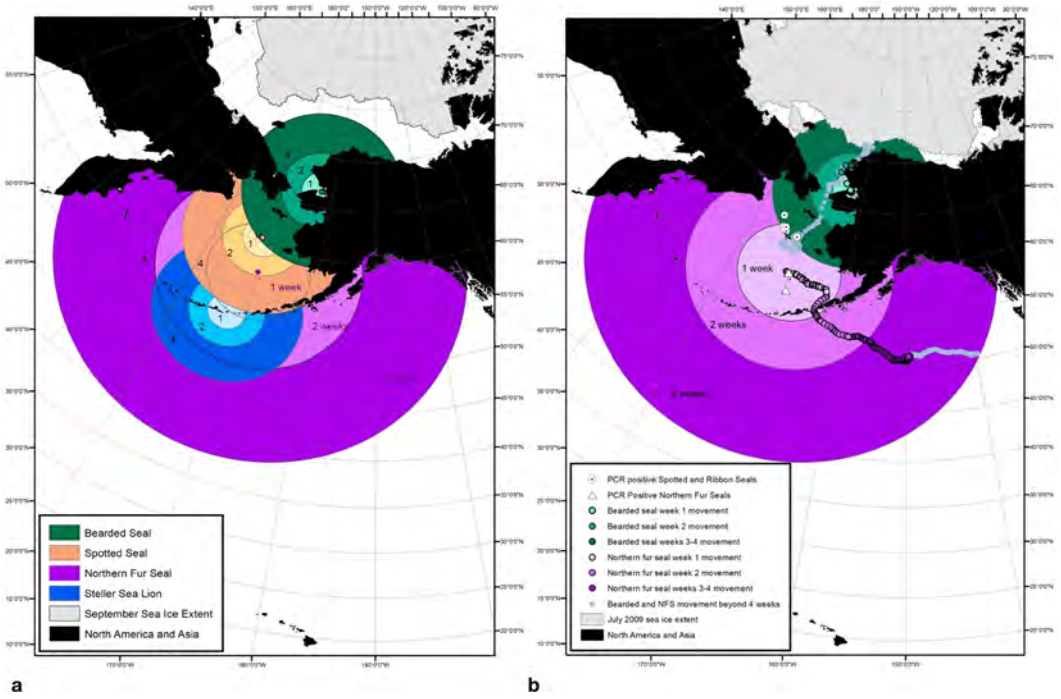

Figure 4. (**a**) Estimated distances animals can travel during the PDV latent and infectious period (1 week, 2 weeks, and 4 weeks) illustrating the areas where viral transmission could occur, based on median travel speeds calculated for satellite-tagged bearded seals (green circles), spotted seals (orange), Steller sea lions (blue), and northern fur seals (purple). (**b**) Recorded tracks of a PDV seropositive bearded seal followed in July 2009 and a seropositive northern fur seal followed in November 2010 shown with sympatric PCR positive spotted seals, ribbon seals, and northern fur seals sampled 2009–2010. Sea ice is shown at its minimum extent in September (panel a) and retreating the following July after reaching a maximum winter extent (panel b).

of age or older testing seropositive. Two month-old pups likely have maternal antibodies reflecting their mothers' prior exposure[42], which creates a challenge for identifying the timing of exposure. However, the seropositive four and five month-old pups identified in 2003 suggest that sea lions in the 2003 birth cohort were exposed to circulating PDV.

Sporadic reports suggest morbillivirus exposure may have occurred prior to 2003 in Japan[15] and Alaska[18] (2 of 160 harbour seals in Alaska tested positive for PDV and were identified as likely false positives). Seropositive Stellar sea lions detected in our study in 2001 and 2002 suggest likely exposure to CDV not PDV as titres to CDV were highest in those years. While it is possible that PDV infection occurred in some sites in the North Pacific Ocean prior to 2003, exposure and molecularly-confirmed infection in Steller sea lion pups sampled from Southeast Alaska to Russia in 2003–2004 (Fig. 3) and PDV seropositive Kuril harbour seals near Japan in 2004[15] suggest that widespread PDV infection in the North Pacific began after 2002 and that the virus rapidly reached geographically distant populations across the North Pacific Ocean after introduction.

Northern sea otters, with range overlap with Steller sea lions, tested positive by serology and PCR beginning in 2004, providing early evidence for cross-species transmission in the North Pacific Ocean. Detection of PDV exposure and infection in northern fur seals and four species of ice-associated seals during the second peak of infection in 2009 suggests additional cross-species transmission occurred. In general, morbilliviruses are transmitted through respiratory droplets or by contact such as through fluids[43] when animals are in close proximity at locations like rookeries. While some pinniped species in Alaska use separate rookeries or haul-outs, cross-species mixing has been observed at ice and land-based locations. Cross-species PDV transmission may also be possible among animals feeding and/or surfacing in close proximity in open water. Telemetry studies and field observations have demonstrated likely overlap in foraging areas such as the prey-rich waters along the Bering Shelf (J. London, pers. obs.).

Given the limited serologic data for species other than Steller sea lions and the sparse PCR data for each species across the study period, it is difficult to determine if PDV was reintroduced in association with changing sea ice extent following the 2003–2004 peak of exposure and infection in the North Pacific Ocean, or whether viral transmission was maintained among marine mammal species. While we may not have detected PDV infection in certain years with smaller sample sizes (Supplementary Table 2), the combined serologic and PCR data provide support for a second peak of infection in 2009. The short sequence fragments of PDV amplified in infected North Pacific marine mammals throughout our study are similar to each other and to the 2002 UK outbreak strain. Strains from the 1988 European outbreak and the 2006 outbreak along eastern North America differ from the 2002 European outbreak and the North Pacific sequences, suggesting that multiple viral strains may be circulating in Arctic reservoir hosts. Sequences most similar to the 2002 PDV isolate in North Pacific species could be due to reintroduction from Arctic hosts or endemic transmission within the North Pacific. In the North Atlantic Ocean,

outbreaks of PDV in European harbour seals appear to sweep through the population within a single year and then fade out until the virus is reintroduced likely through cross-species contact with grey seals or harp seals[41]. In combination with the proportion of susceptible marine mammals in the population at a given point in time, viral reintroduction via contact between Arctic reservoir species may also shape cycles of exposure and infection in the North Pacific Ocean. As seropositive ringed seals in the Northwest Territories of Canada were reported in the 1990's[10], Canadian circumpolar seals could potentially serve as a source of PDV to North Pacific marine mammal species. However, sea ice extent and genetic data from our study support introduction along the Russian route. Openings in the sea ice along the Russian coast were linked to the increased likelihood of PDV exposure or infection (Table 1), and peaks of viral exposure in 2003 and 2009 occurred after a year with low sea ice extent and open water along the Russian coast, suggesting that multiple introductions may have occurred.

Alternatively, cross-species transmission resulting in PDV circulation among ice-associated seals, Steller sea lions, northern fur seals, and northern sea otters might also result in peaks of infection following introduction. The estimated number of Steller sea lions, northern fur seals, and ice-associated seals in Alaskan stocks[44] exceeds the critical community size estimated to be necessary to sustain endemic PDV transmission in European harbour seals (~300,000 animals)[38]. The probability of long-term pathogen persistence is likely to increase in systems with multiple hosts capable of cross-species disease transmission[45]. Differences in the density and spatial distribution of a single species may also enhance the potential for disease persistence. Although species like Steller sea lions and northern sea otters are distributed across the North Pacific, the patchy distribution of haul outs and rookeries could prolong the duration of viral transmission among species following PDV introduction[38]. Low density, patchy groups of terrestrial carnivores spread across a large landscape can maintain transmission of CDV[39]. Population age and sex structure, seasonal patterns of movement and intra-and inter-specific contact, and variation in host susceptibility to viral infection and disease severity could also contribute to the potential for endemic viral transmission. Spatially explicit epidemiologic modeling integrating movement data from satellite-tagged marine mammals with seroprevalence and viral infection prevalence trends may help to elucidate whether circulation among species or reintroduction associated with ice change is driving viral transmission in the North Pacific.

Linking movement data from satellite-tagged marine mammals with biological information on viral shedding illustrates that exposed animals have the potential to carry PDV long distances. Movement of PDV seropositive bearded seals and northern fur seals occurred within their species-specific predicted viral transmission distances and in close proximity to known locations of PCR positive individuals from all species tested. These data demonstrate the potential for animals exposed to PDV to carry the virus to areas with conspecifics and sympatric species. Data from satellite-tagged Steller sea lions suggest infectious animals could move over 100 km in one week, reaching nearby and distant rookeries (Fig. 4a). Bearded seals and northern fur seals have the potential to move over 200 km and 500 km in one week, respectively. Spotted seals and ribbon seals may bridge the gap between northern ice-associated seals and Steller sea lions and northern fur seals living in the southern Bering Sea (Fig. 4a). The ability to move long distances and timing of movements associated with life history cycles likely influence transmission patterns in the North Pacific Ocean and the potential to transmit PDV to species living in southern habitats.

Finally, the animal health impacts of PDV in North Pacific species are unknown, but may be quite different from the outbreaks in the Atlantic Ocean where the virus has caused extensive mortality in harbour seals[46]. Although no mortality events have been documented with PDV infection in Pacific species, the virus may have contributed to sporadic northern sea otter deaths, including those that occurred during an unusual mortality event from 2004–2006[8], as well as to sporadic Steller sea lion deaths (PCR positive tissues in dead animals in this study). We found evidence of PDV viral infection in apparently healthy Steller sea lions, northern fur seals, and ice-associated seals sampled live or through subsistence harvest throughout the study. As with grey seals and Arctic seal species in the North Atlantic, PDV may be able to persist in Arctic and some sub-Arctic species in the North Pacific without causing widespread disease. The current impacts of PDV associated disease and deaths in marine mammal species in the North Pacific Ocean are unknown, but the potential for outbreaks in sensitive species highlights the importance of understanding spatio-temporal PDV transmission in this environment. Climate change-driven reductions in sea ice extent in the Arctic Ocean are projected to increase[47] and open water routes along the northern Russian coast have occurred every August and/or September since 2008. The health impacts of this new normal in the Arctic are unknown, but association of open water routes through Arctic sea ice with increased PDV exposure or infection suggest that opportunities for PDV and other pathogens to cross between North Atlantic and North Pacific marine mammal populations may become more common.

Data and code availability

Sequences obtained in this study were submitted to Genbank with the following accession numbers:

FW07098/Brain/Kodiak/2007_PDV (MH392513)
HF2010-2019/Nasal/Bering_sea/2010_PDV (MH392514)
NFS_G105Y/Nasal/St_George/2010_PDV (MH392515)
SSL_Y389/Nasal/Yamsky/2004_PDV (MH392516)
NFS_SAM81/Nasal/St_Paul/2012_PDV (MH392517)
PL2009-2016/WBC/Bering_sea/2009_PDV (MH392518)
SSL_2012-032/Placenta/Kodiak/2012_PDV (MH392519)
SSL_YUN04-19/Nasal/Yanuska/2004_PDV (MH392520)

Animal species, location, and PDV serology and PCR testing data are summarized in the electronic supplementary material. Sea ice extent data are publicly available from the National Snow and Ice Data Center (NSIDC)[32] at https://doi.org/10.7265/N5736NV7. Mixed effects logistic regression models were run in R using code from the lme4 package[34].

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Author contributions

T. Goldstein conceived of and designed the study, collected field data, performed diagnostic testing, participated in statistical analysis, performed sequence alignments and phylogenetic analyses, and drafted the manuscript; A.H. conceived of and designed the study, participated in statistical analysis, and helped draft the manuscript; J.A.H. conceived of and designed the study, performed molecular diagnostic testing, and helped draft the manuscript; E.V. designed the study, performed statistical analyses, and drafted the manuscript; J.A.K.M. designed the study, participated in data analysis, and helped draft the manuscript; P.M.B. participated in data analysis and helped draft the manuscript; M.E.L., J.S. and J.M.L. collected field data, provided satellite telemetry data and median speed estimates, and helped draft the manuscript; V.A.G., P.L.B., T. Gelatt, B.S.F., V.N.B., R.R.R., L.D.R., M.J.R. and K.A.B.-H. collected field data and reviewed the manuscript; and B.R.S., A.J., M.H. and S.L.C. performed serologic and molecular diagnostic testing. All authors gave approval for publication.

Competing interests

The authors declare no competing interests.

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Bearded seal productivity in Alaska using harvest-based monitoring; 1960s, 1970s, 2000s, and 2010s

Ryan Adam, Anna Bryan, Lori Quakenbush, Justin Crawford, and Louise Biderman

Alaska Department of Fish and Game, Arctic Marine Mammals Program, Fairbanks, Alaska, ryan.adam@alaska.gov

Introduction

Declines in Arctic sea ice extent, thickness, and duration are projected to negatively impact bearded seals (*Erignathus barbatus*) by reducing their time to rest, pup, nurse, and molt on sea ice. Existing population estimates for bearded seals in Alaska cannot be used to detect trends; however, the Alaska Department of Fish and Game works with Alaska Native hunters to collect data from the subsistence harvest that are used to determine several population health indices.

Methods

- Bearded seals were sampled from subsistence harvests in 15 villages along the Beaufort, Bering, and Chukchi sea coasts during 1963–1979 and 2002–2016.
- Female reproductive tracts and canine teeth were collected.
- Age of seals was determined by counting annuli in the dentine or cementum layers of sectioned teeth.
- Reproductive tracts were examined for sexual maturity and reproductive condition.
- Data are grouped by decade 1960s (1963–1969), 1970s (1970–1979), 2000s (2000–2009), and 2010s (2010–2016).

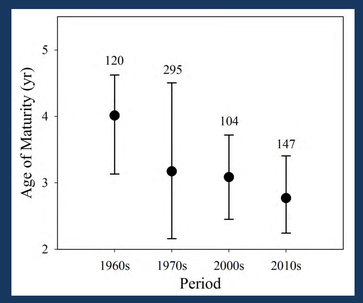
Villages in the Beaufort, Bering, and Chukchi seas where harvested bearded seals were sampled.

Subsistence harvested bearded seal. Point Hope, AK
Photo by: Lori Quakenbush

Age of Maturity

Age of maturity was estimated as the age at which 50% of females had ovulated at least once (DeMaster, 1978). Data was analyzed using a probit regression.

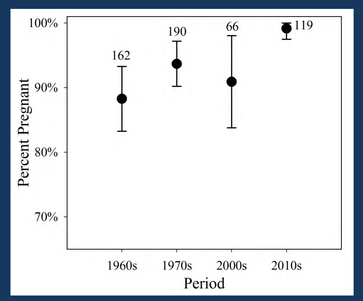
- Seals are maturing at a significantly younger age during the 2010s than during the 1960s ($p<0.05$).



Average age of maturity by decade.

Pregnancy Rate

Pregnancy rate was defined as the proportion of mature females that were pregnant in the year of harvest. Average pregnancy rate was estimated and evaluated for differences among time periods using a logistic regression model.

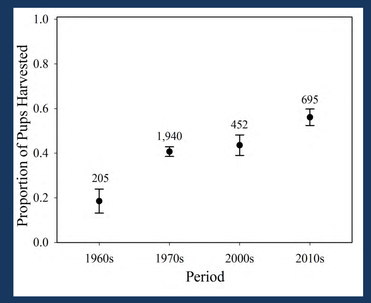


Average pregnancy rate by decade.

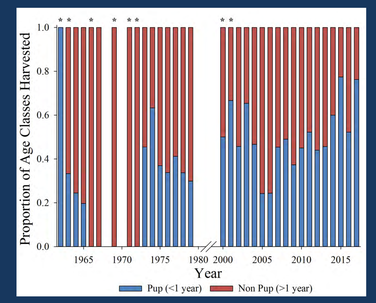
- Pregnancy rate in the 2010s is significantly higher than all other decades ($p<0.05$).

Proportion of Pups Harvested

The proportion of pups (<1 year of age) in the sampled harvest is representative of their presence in the population. If pups were not surviving past weaning, their presence in the harvest would decrease.



Proportion of pups harvested by decade. Sample size is listed above the error bars.



*Annual proportions of age classes harvested. *Sample size in these years were <10 seals.*

- The proportion of pups in the sampled harvest was significantly lower in the 1960s than all other decades and significantly higher in the 2010s than all other decades ($p<0.05$).

Conclusions

- **Indices of seal productivity and weaning have improved in recent years and are not negatively affected by current environmental changes.**
  - Seals are maturing at a younger age.
  - Pregnancy rate is higher.
  - Proportion of pups in the sampled harvest is higher.
- **Continued monitoring is important as environmental conditions change.**

References

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Acknowledgments

This project would not be possible without the willingness of hunters to contribute samples from their harvest, the support of their communities, local governments, and Tribal Councils. We appreciate the support from the North Slope Borough (NSB) and the Ice Seal Committee. John Burns, Kathy Frost, and Lloyd Lowry were instrumental in the early development of this monitoring. We thank Mark Nelson, Gay Sheffield, and our college interns for assistance in field collections and sample processing. Research was funded primarily by NOAA, but has also been supported by NSF, NPRB, and NSB. Research was conducted under NMFS Permits 358-1585, 358-1787, and 15324.

Age structure of subsistence harvested ice seals in Alaska 2000–2018

Ryan Adam, Anna Bryan, Lori Quakenbush, Justin Crawford, and Louise Biderman

Alaska Department of Fish and Game, Arctic Marine Mammals Program, Fairbanks, Alaska, ryan.adam@alaska.gov

Introduction

Age distribution is an important component for understanding population dynamics. Since 2000, the Alaska Department of Fish and Game has used cementum annuli to age teeth from harvested ice seals: ringed seals (*Pusa hispida*), bearded seals (*Erignathus barbatus*), spotted seals (*Phoca largha*), and ribbon seals (*Histriophoca faciata*). Samples from seals were collected in collaboration with Alaska Native hunters as part of a subsistence harvest bio-monitoring program.

Methods

- Canine teeth were collected from subsistence harvested ice seals in 16 villages along the Bering, Chukchi, and Beaufort seas during the period of 2000–2018.
- Seal age was determined by counting annuli in the cementum layers of sectioned teeth.
- Age classes were determined based on sexual maturity for each seal species (Table 1) (Crawford et al., 2015; DeMaster, 1978).

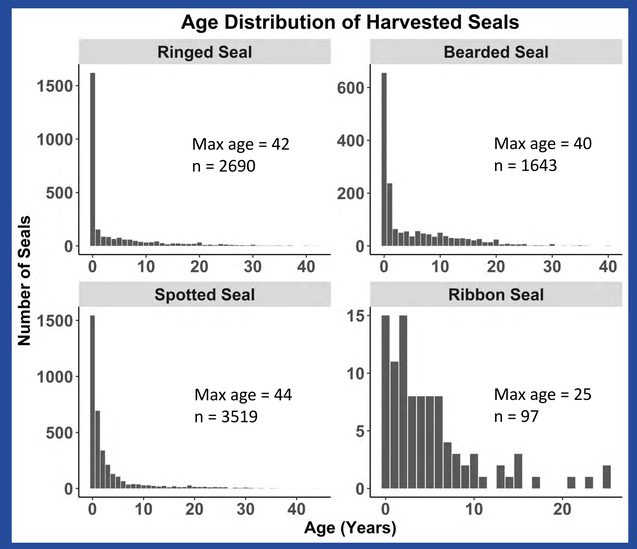
	Pup	Subadult	Adult
Ringed	<1	1-4	≥5
Bearded	<1	1-6	≥7
Spotted	<1	1-3	≥4

Table 1. Year ages used to define age classes.

- Few ribbon seals are harvested in Alaskan waters; therefore, they were not included in the age class analysis.

Subsistence harvested ice seals in Teller, AK. Photo by: Letty Hughes

Age Distribution

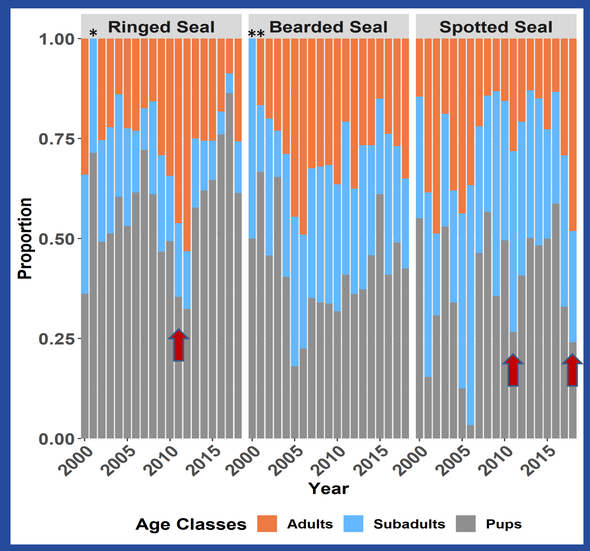


Age distribution of sampled seals by species during 2000–2018.

- Ringed, bearded and spotted seals can live for more than 40 years.
- The oldest ribbon seal was 25 years old.
- The age distribution of ribbon seals is different from the other seals; this could be a result of the small sample size.

Age Class Distribution

- Changes in the age distribution of harvested animals can be useful in identifying long-term population trends and short-term variability.
- Although there is annual variability in the proportion of pups in the harvest, the long-term trends indicate healthy populations.
- There were declines in the proportion of pups for ringed and spotted seals during the 2011 UME (Unknown Mortality Event) and for spotted seals in 2018 (indicated by arrows).



Annual proportions of age classes harvested.
** Sample sizes in these years were <10 seals.*

Conclusions

- Age distribution is useful for identifying changes in the population.
- Ringed, bearded, and spotted seals can live for over 40 years.
- Ribbon seals can live for up to 25 years.
- The proportion of pups in the harvest remains high.

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Hybrid fibers in the bearded seal (*Erignathus barbatus*) longissimus dorsi muscle

BALLARD, E.J.*; BARRETT, L.M.; DEAROLF, J.L.; THOMETZ, N.M.; BRYAN, A.; REICHMUTH, C.;
Hendrix College, Conway, AR, Univ. of San Francisco, CA,
Alaska Dept. of Fish and Game, Fairbanks, Univ. of California, Santa Cruz

Introduction

Bearded seals (*Erignathus barbatus*) are shallow diving pinnipeds, that mostly stay in depths of about 100 meters or less (2). Being benthic feeders, they scour the ocean floor searching for food sources like polar cod, sculpins , shrimp, spider crabs, and a variety of other invertebrate species (2). Their benthic feeding strategy makes their diving ability an interesting topic of study. Specifically, understanding the physiological aspects of the bearded seal locomotor muscle biology could provide key insights into their diving behavior.

Barrett et. al. (2018) characterized the fiber-type profile of the primary locomotor muscle of bearded seals, the longissimus dorsi (LD). Their study (2018) has revealed that this muscle is composed primarily of fast-twitch, type IIa fibers, a type of fiber often found in the muscles of long distance runners. The composition of the locomotor muscle of bearded seals is unsurprising in the context of their diving behaviors, as these seals must be able to powerfully and quickly contract their muscles to propel themselves through the water while searching for their prey.

However, Barrett and her colleagues (2018) also found that some of the IIa fibers reacted to the SC-71 antibody, which is specific for type IIa myosin heavy chain, but they also stained intermediately for myosin ATPase after alkaline pre-incubation (Fig. 1:3). In addition, some of the slow twitch fibers (type I) also reacted to the SC-71 antibody (Fig. 1:4) (1). This result demonstrates that there are hybrid fibers, or fibers that are expressing more than one type of myosin heavy chain present in the muscle, which would give these fibers contraction properties intermediate between slow- and IIa fast-twitch fibers (6). Thus, it becomes important to determine the proportion of these hybrid fibers in order to gain a more complete understanding of the role of the LD muscle in the diving abilities of bearded seals.

To quantify the I/IIa hybrid fibers in the bearded seal LD, we will identify and count the number of type I, slow twitch fibers that stain lightly after basic myosin ATPase, react to the slow twitch antibody A4.951, and react to SC-71. Quantifying these hybrid fibers will allow us to make stronger conclusions about the function of the longissimus dorsi muscle of bearded seals.

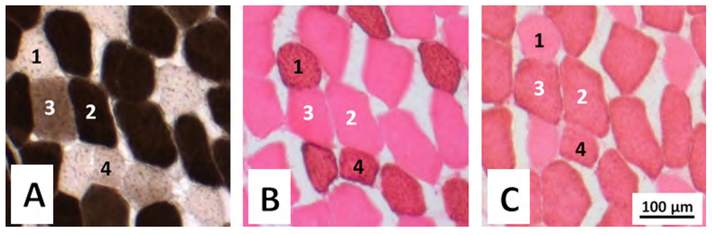

Figure 1. Representative cross-sections of bearded seal (*Erignathus barbatus*) longissimus dorsi (LD) muscle after histochemical and immunocytochemical staining. The LD was stained for myosin ATPase after basic pre-incubation (A) and for its reaction to anti-slow (A4.951) (B) and anti-fast IIa (SC-71) myosin antibodies (C). The labels 1 (type I, slow twitch), 2 (type IIa-dark, fast twitch), 3 (type IIa-intermediate on 10.3, fast twitch), 4 (type I, SC-71+, slow twitch), and 5 (type IIa – intermediate on 10.4, fast-twitch) indicate the same fibers on each of the images (Barrett et al. 2018).

Methods

Freezing, cutting, and staining tissue

Samples from six longissimus (LD) muscles of adult bearded seals (*Erignathus barbatus)* (Table 1) were collected by the Alaska Department of Fish and Wildlife with help from the local community of Point Hope, AK. These specimens were then shipped to Hendrix College in Conway, AR, and kept at -20°C until used in the study. The methods of Thometz et al. (2018) were used to prepare these samples for cutting.

To begin cutting the prepared frozen samples, we placed them in a cryostat to allow the samples to warm to -26°C. Then, we used Tissue Freezing Medium to mount the frozen samples onto the cryostats chucks and quick freeze them with Fisherbrand Super Friendly Freeze It ® spray. For each specimen, we cut eight 10 µm and eight 12 µm thick sections and placed each pair of sections on Fisherbrand Superfrost Plus ® slides. Five of the slides were used for myosin ATPase staining using the methods of Hermanson and Hurley (1990), while the remaining three slides were saved for antibody staining (3). The antibody staining was followed by hematoxylin and eosin (H&E) counterstaining (7).

Methods Continued

Data Collection and Analysis

We imaged the histochemically and immunocytochemically stained sections of the LD using a Zciss Axio Imager AI microscope and AxioVision v. 4.7 software. We took images from identical regions of the tissue to compare each staining treatment (basic ATPase and myosin heavy-chain antibodies). For each specimen, we took a total of four sets of images in different regions of the LD muscle.

In order to determine the proportion of slow-twitch fibers reacting to the SC-71 antibody (hybrid fibers) within the LD muscle, we first identified and counted all the slow-twitch fibers in each alkaline image. We then confirmed that all of these fibers reacted positively to the A4.951 antibody. Finally, we determined how many of the slow-twitch fibers reacted to the SC-71 antibody. The proportions of these I/IIa hybrid fibers relative to the number of slow-twitch fibers and to the total number of fibers were calculated for each image. Then, we calculated the overall average proportions (± S.D) of I/IIa hybrid fibers for each specimen and these values were used to determine the average proportions (± S.D) in the LD of bearded seals.

Table 1. Bearded seal (*Erignathus barbatus*) specimens analyzed in this study

Specimen I.D.	Sex	Age (years)	Standard length (cm)
EB17PH030	Male	0+	135
EB16PH022	Female	< 1	144.7
EB16PH014	Male	1	171
EB16PH024	Female	10	213.5
EB17PH033	Male	5+	225
EB16PH020	Male	5	226

Results

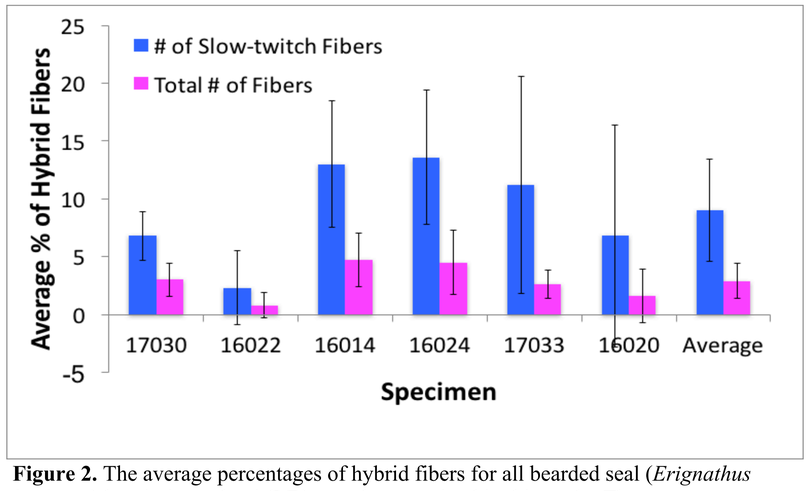

Figure 2. The average percentages of hybrid fibers for all bearded seal (*Erignathus barbatus*) longissimus dorsi (LD) muscles examined in this study. The average percentage of hybrid fibers is shown when compared to the total number of type I, slow-twitch fibers (blue), and the total number of fibers counted (pink). The specimens are presented in order of increasing body length (cm) from left to right. Error bars are ± 1 S.D.

The percentage of hybrid (I/IIA) fibers in the bearded seal longissimus dorsi (LD) muscle was found to be extremely variable both within and between specimens. EB17PH033 demonstrated 3.9% (relative to the number of type I, slow-twitch fibers) in one area of its cross-section and 25% in another. This variability exceeded the differences in the percentages of these fibers between the specimens, which ranged from 13.6% (± 5.8) in EB16PH024 to 2.3% (± 3.2) in EB16PH022 (Fig. 2). The variability in the percentages of the hybrid (I/IIA) fibers deceased when they were compared to the total number of fibers. In this case, the greatest proportion of the hybrid fibers was in the LD of EB16PH014 (4.7 ± 2.3%), while the lowest average percentage was in the muscle of EB16PH022 (0.8 ± 1.1%) (Fig. 2).

The overall average percentage of hybrid fibers in comparison to total number of type I, slow-twitch fibers in the bearded seal LD was 9.0 ± 4.4%. In comparison to the total number of fibers counted, the proportion of hybrid fibers in this muscle was 2.9 ± 1.5% (Fig. 2).

Discussion

The reason for conducting this experiment was to determine the proportion of hybrid fibers (I/IIA) found within the longissimus dorsi (LD) of bearded seals (*Erignathus barbatus*) in order to gain a more complete understanding of the role of this muscle in the diving abilities of bearded seals. We found that the overall average percentage of I/IIa hybrid fibers in the LD was 9.0% relative to the type I, slow-twitch fibers and 2.9% of the total fibers counted. These percentages are very small when compared to percentages of type I (30.3 ± 8.9%) and type IIa-dark (49.7 ± 9.2%) present in the bearded seal LD (1). Interestingly, the average percentage of hybrid fibers in this muscle are minute even when compared to the type IIa-intermediate fibers (19.9 ± 1.1%) (1), which are also a less prevalent fiber-type (Fig. 3).

In this study, we also found the presence of the I/IIa hybrid fibers to be extremely variable within and between specimens. And, the variability in the percentage of hybrid fibers between the specimens did not seem to correlate with the age, sex, or size of the animals examined. Because these fibers do not make up a consistent proportion of the cross-section of the LD, they are likely fibers that are transitioning between type I and type IIa fiber types (6). These hybrid fibers make up a small proportion (< 10%) of the slow-twitch fiber population, which are only 30% of the fibers in the bearded seal LD. Therefore, they likely do not play a major role in the swimming of these animals.

Although the I/IIa hybrid fibers in the bearded seal LD probably do not play a role in their locomotion, the reason for their presence in the muscle is unclear. Why are ~10% of the slow-twitch fibers transitioning? As we think about this question, we will learn more about the muscle physiology of bearded seals, which may allow us to gain greater insight into how to protect these animals from future threats like habitat loss and increased predator abundance due to the sea temperatures rising (5).

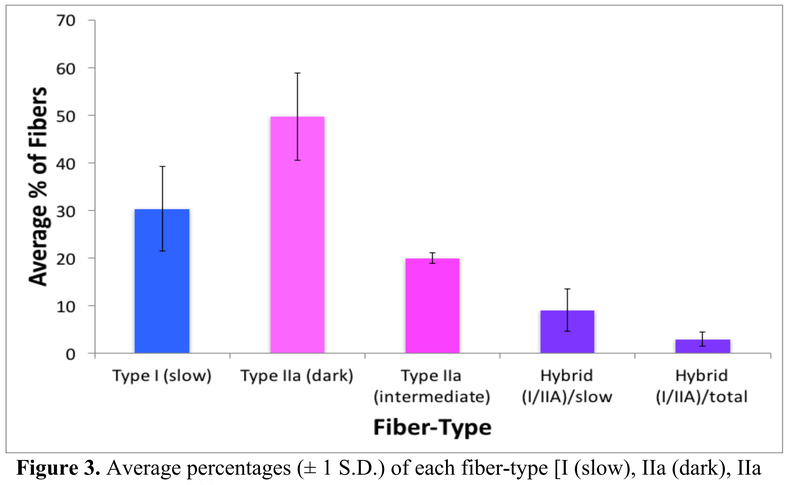

Figure 3. Average percentages (± 1 S.D.) of each fiber-type [I (slow), IIa (dark), IIa (intermediate), and I/IIa hybrid fibers (relative to slow-twitch fibers and total number of fibers)] in the bearded seal (*Erignathus barbatus*) longissimus dorsi muscle. Types I, IIa (dark), and IIa (intermediate) data from Barrett et al. (2018) and I/IIa hybrid data from this study.

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Acknowledgements

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Occurrence of Arctic and saffron cod in the diet of ringed seals at Shishmaref, 1975–2018

Louise Biderman, Anna Bryan, Justin Crawford, John Citta, and Lori Quakenbush

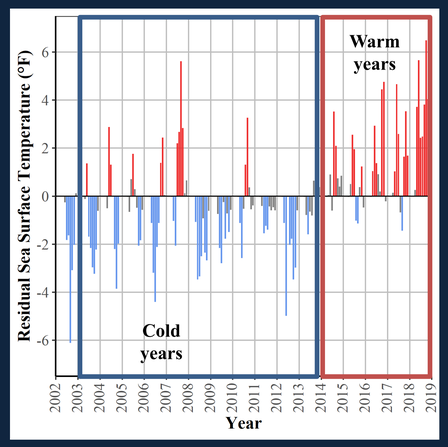
Alaska Department of Fish and Game, 1300 College Road, Fairbanks, Alaska 99701, USA louise.biderman@alaska.gov

Overview

A warming climate is expected to alter the marine food web by favoring species of fish that thrive in warmer water to the detriment of those that thrive in cooler water. As part of a long-term study of ringed seals (*Pusa hispida*) harvested near Shishmaref, Alaska, we investigated trends in the occurrence and size of the two most common fish found in their stomachs, Arctic cod (*Boreogadus saida*) and saffron cod (*Eleginus gracilis*). Arctic cod are strongly associated with sea ice and cooler waters, whereas saffron cod prefer warmer water and therefore may become more prominent in ringed seal diet as temperatures increase.

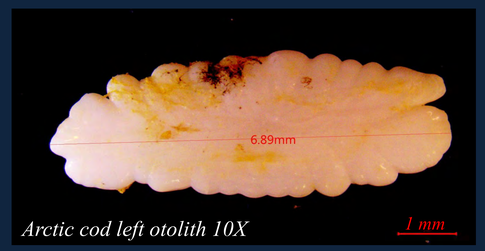
Methods

- We examined diet from stomach contents of 757 ringed seals collected by subsistence hunters in Shishmaref, Alaska between 2003–2018. Canine teeth were collected and aged. Seals were separated by age class as pups (<1 yr. old) and non-pups (>1 yr. old).
- Stomach contents were rinsed, and prey items were identified to the lowest taxonomic level.
- Fish were identified by their species-unique otolith shape.
- Each fish has two otoliths, one on the right and one of the left side of its head. Otoliths were separated by side.
- For each fish species, the side with the greatest number of otoliths was counted, photographed, and measured (from rostrum to postrostrum) using a Leica M125 stereo microscope and MU1000 AmScope.

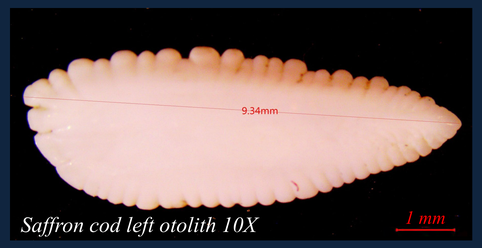


- Sea surface temperature collected near Shishmaref was used to determine warm and cold years in the 2000s and 2010s. We considered 2003–2013 to be cold years and 2014–2018 to be warm years.

Arctic cod

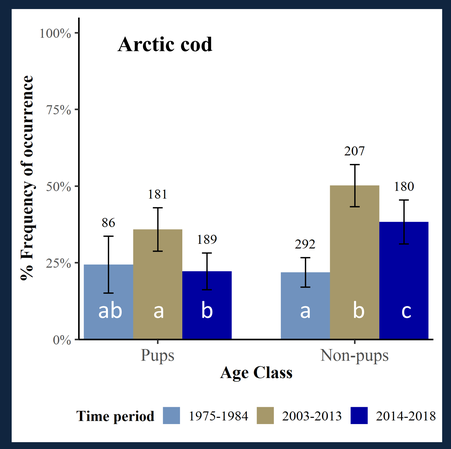


Saffron cod

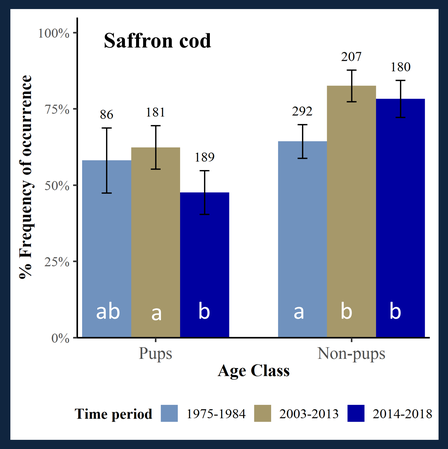


Frequency of occurrence

Frequency of occurrence (FO) was calculated by dividing the number of stomachs containing each cod species, by the number of stomachs containing any prey item. Frequencies during 2003–2013 and 2014–2018 were compared to frequencies during 1975–1984.



The FO of Arctic cod was lowest in 1975–1984 and highest in 2003–2013. Significant differences are indicated on graph with different letter labels ($p<0.05$).

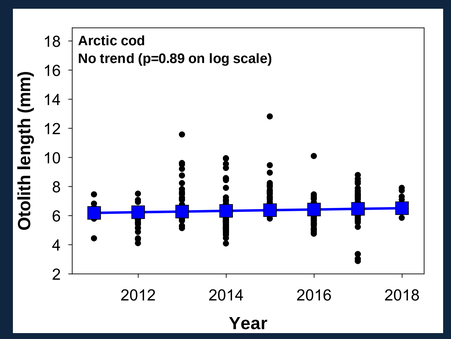


The FO of saffron cod was lowest in 2014–2018 for pups and 1975–1984 for non-pups. It was highest in 2003–2013 for both age classes. Significant differences are indicated on graph with different letter labels ($p<0.05$).

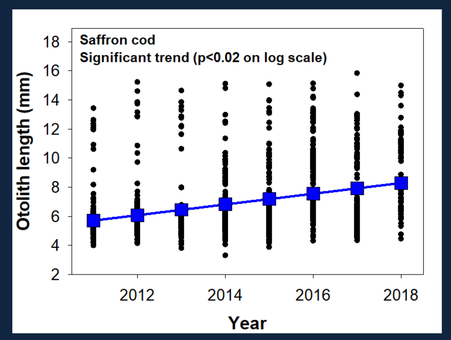
Acknowledgements: This project would not be possible without the willingness of hunters to contribute samples from their harvest, and the support of their communities, local governments, and Tribal Councils. We appreciate the support from the Ice Seal Committee. John Burns, Kathy Frost, Lloyd Lowry, and others (ADF&G) were instrumental in the early development of this monitoring program. William Walker assisted with fish identification, Gay Sheffield and our college interns assisted with field collection and sample processing. Research was funded primarily by NOAA, but has also been supported by NPRB, and the North Slope Borough. Research was conducted under NMFS Permits 358-1585, 358-1787, and 15324.

Otolith length

Otolith lengths were compared by age of seal and year of harvest. On average, otoliths in pup stomachs were approximately 1mm shorter than non-pups. However, there was no evidence that trends through time differed by age class.



Average length of Arctic cod otoliths did not change over time.



Average length of saffron cod otoliths increased significantly over time.

Conclusion

- As of 2018 Arctic cod are still present in the diet of ringed seals for both age classes.
- The trend toward larger saffron cod otoliths may indicate recent environmental conditions support the growth of larger saffron cod.
- Continued monitoring is needed to detect possible changes in the occurrence and size of prey species as the environment changes.

Next steps

❖ Examine ringed seal stomachs from 2019 for the presence of Arctic cod.

❖ Collect ocean-surveyed fish of size classes that are consumed by seals. Use these to develop length regressions that more accurately represent the size of fish consumed by seals.

Final results from hunter-assisted sampling of Pacific walruses near Saint Lawrence Island, Alaska, 2012-2014 and 2016

Anna L. Bryan[1], Lori Quakenbush[1], Jonathan A. Snyder[2], Harold Kiyuklook[3], Sheena Anningayou[4], and Mark Nelson[1]

[1]Alaska Department of Fish and Game, Fairbanks, Alaska, anna.bryan@alaska.gov, [2]U.S. Fish and Wildlife Service, Anchorage, Alaska

[3]Resident of Savoonga, Alaska; [4]Resident of Gambell, Alaska,

Introduction

Pacific walruses (*Odobenus rosmarus*) are an important subsistence species to coastal Alaska Natives and important to the Bering and Chukchi marine ecosystems. As summer sea ice declines in the Chukchi Sea an increase in energy needed by walruses to travel from terrestrial haulouts to feeding areas is expected. Increased energetic costs can cause decreased body condition and reproductive capacity, and increase disease prevalence. Data provided by sampling the harvest allows us to examine parameters that affect population health; such as ***body condition, disease exposure,*** and ***contaminant concentrations***.

Body condition

Hunters classified 98% of 208 walruses as average to very healthy based on blubber and overall body condition.

Disease exposure

Disease Agent	Antibody Prevalence # Pos./# Tested (%)
Canine and phocine distemper	0/151 (0%)
Leptospira spp.	6/151 (4%)
Toxoplasma spp.	1/151 (1%)
Brucella spp.	7/147 (5%)

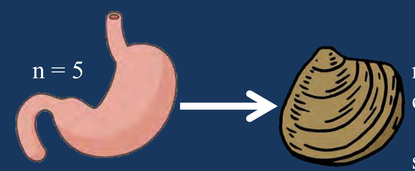
Toxic algae (HABs)

Domoic acid and saxitoxin are produced by Harmful Algae Blooms (HABs) and are predicted to occur farther north as sea surface temperatures increase.

- For domoic acid (n = 116), 49% of the walruses had detectable levels (3 to 6,457 ppb) in gastrointestinal (G.I.) tracts (ADF&G unpubl. data and Lefebvre, et al. 2016[1]).
- For saxitoxin (n = 66), 52% of the walruses had detectable levels (4 to 1,162 ppb) in G.I. tracts (ADF&G unpubl. data and Lefebvre, et al. 2016[1]).

How do digested stomach contents compare to intact clam feet and siphons from the walrus stomach?

(Undigested clams from walrus stomachs are regularly eaten by Alaska Native subsistence users.)



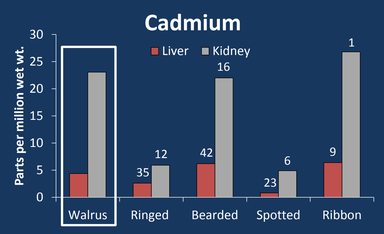
Domoic acid	0-10 ng/g	3-29 ng/g
Saxitoxin	27-81 ng/g	12-60 ng/g

*No walruses appeared to be symptomatic; however, similar concentrations of HABs have negative effects on California sea lions (*Zalophus californianus*).*

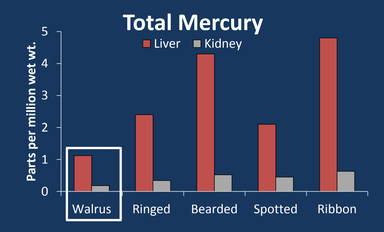
Pacific walruses in the Bering Sea near Saint Lawrence Island, Alaska.

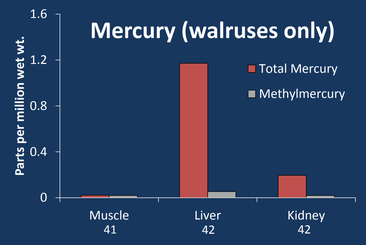
Trace elements

Trace elements were analyzed from 42 walruses, including essential elements such as iron and magnesium. Average concentrations of total mercury and cadmium in walruses compared to Alaskan seals (ADF&G unpubl. data) are shown below. Also below, concentrations of total mercury are compared to methylmercury for muscle, liver, and kidney in these walruses. ***Lead*** was near or below detection limits in all species.



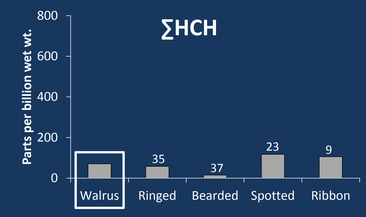
- Both cadmium and mercury occur naturally in the Arctic marine environment, although mercury has increased through industrial inputs and atmospheric transport.

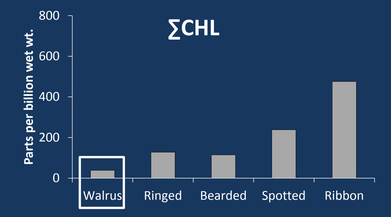




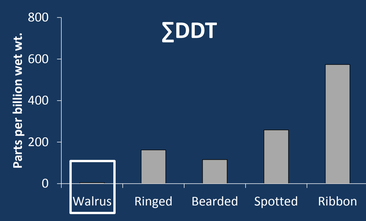
Contaminants

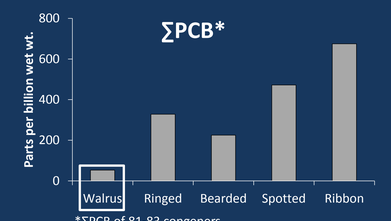
Pesticides and polychlorinated biphenyls (PCBs) are man-made chemicals that concentrate in blubber. Below are average concentrations in ***blubber*** from 42 walruses compared to Alaskan seals (ADF&G unpubl. data). Concentrations were lower in liver, kidney, and muscle (not shown) than blubber.





- HCH (Hexachlorocyclohexane) - mostly banned in 1970, except Lindane.
- CHL (Chlordane) - production in the U.S. stopped in 1997.





*∑PCB of 81-83 congeners.

- DDT (Dichlorodiphenyltrichloroethane) – banned in most countries but still used in Africa to prevent malaria. May be stockpiles in Russia.
- PCB (Polychlorinated biphenyl) – banned in the U.S. but were widely used for electrical applications.

Conclusions

- Overall walruses appear to be in good body condition.
- Exposure to diseases was low (e.g., 0% for distemper and 5% for *Brucella*).
- Walruses have similar or lower trace element and contaminant concentrations than ice seals in Alaska and walruses in Canada[2,3], where consumption of traditional foods is encouraged[4].
- Walruses are ingesting toxins produced by HABs, but no walruses appeared symptomatic. We recommend continued monitoring of domoic acid and saxitoxin in walruses. We also recommend human health organizations monitor clams from walrus stomachs to determine if they are below regulatory limits for human consumption.

Acknowledgements

This project would not have been possible without the support of the Eskimo Walrus Commission, the Native Villages of Gambell and Savoonga, and the expertise of the walrus hunters. Primary funding provided by ADF&G and the U.S. Fish and Wildlife Service (USFWS) under Section 6 of the ESA. The North Slope Borough provided funding for contaminants and vitamin analysis and NOAA's Northwest Fisheries Science Center and U.C. Santa Cruz provided funding for toxic algae analysis. Samples were provided to ADF&G by the USFWS. Seal contaminant data was funded by NOAA, National Marine Fisheries Service. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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Spotted seal productivity in Alaska using harvest-based monitoring, 1960s, 1970s, and 2000s

Anna Bryan, Lori Quakenbush, Justin Crawford, Louise Biderman, and Ryan Adam

Alaska Department of Fish and Game, Arctic Marine Mammals Program, Fairbanks, Alaska, anna.bryan@alaska.gov

Introduction

Spotted seals (*Phoca largha*) are an ice associated pinniped that forage on pelagic fish and use sea ice to rest, give birth, nurse, and molt. Arctic sea ice is declining in extent, thickness, and duration. These declines are predicted to continue and may affect the distribution, availability, or nutritional quality of fish important to spotted seals. If prey availability or quality decline, spotted seal productivity could be affected, thus monitoring productivity is important. Currently there are no estimates of spotted seal abundance or trend in Alaska that can be used to track productivity. However, the Alaska Department of Fish and Game has worked with Alaska Native hunters since the 1960s to collect data from subsistence harvested spotted seals that can be used as an index to population health and status.

We have previously reported the age at maturity and pregnancy rates for spotted seals collected between the 1960s and 2008 (Quakenbush et al. 2009). Here we update results for 2000–2016 with reproductive tracts from more than 400 spotted seals, including more than 80 mature females.

Methods

We sampled spotted seals from subsistence harvests at 9 villages in Alaska along the Bering, Chukchi, and Beaufort sea coasts from 2000–2016. Female reproductive tracts and canine teeth were collected. These data were compared to data previously collected from seals in the same region during 1964–1979. Data are broken down by decade 1960s (1964–1969), 1970s (1970–1979), 2000s (2000–2009) and 2010s (2010–2016). We examined reproductive tracts for sexual maturity and reproductive condition. Age of seals was determined by counting annuli in the dentine and cementum layers of sectioned teeth.

Age of Maturity

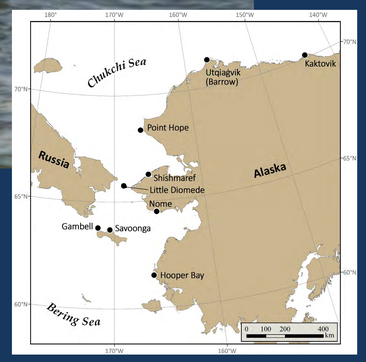
- Seals that had ovulated at least once were classified as mature.
- We estimated average age of maturity as the age at which 50% of females were mature (DeMaster 1978) using a probit regression in SAS (PROC PROBIT).

Pregnancy Rate

- We defined pregnancy rate as the proportion of mature females that were pregnant when harvested.
- We estimated average pregnancy rate and evaluated differences among periods using a logistic regression model in SAS (PROC LOGISTIC).

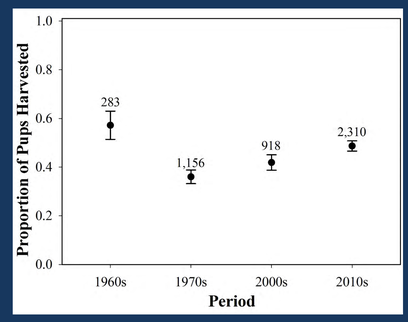
Proportion of Pups Harvested

- The proportion of pups (<1 year of age) in the sampled harvest is representative of their presence in the population. If pups were not surviving past weaning, their presence in the harvest would decrease.
- We evaluated differences in the proportion of pups harvested during each period using SAS (PROC FREQ).

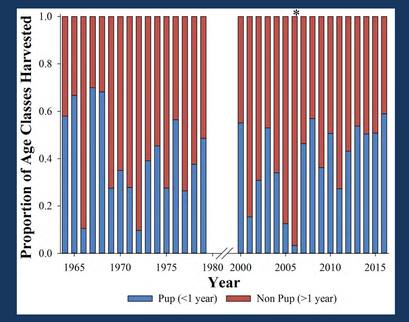


Villages in the Bering and Chukchi seas where harvested spotted seals were sampled (2000–2016).

Proportion of Pups Harvested



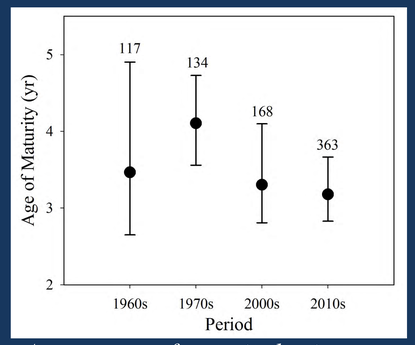
Proportion of pups harvested by time period. The number of seals harvested is listed above the error bars for each time period.



*Annual proportions of age classes harvested. *In 2006, one of 30 seals was a pup.*

- The proportion of spotted seal pups harvested was lowest in the 1970s. The proportion has increased in the 2000s and 2010s.

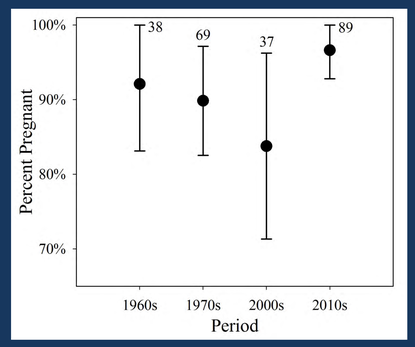
Age of Maturity



Average age of maturity by time period.

- Average age of maturity has been significantly younger since 2000 than in the 1970s, and more similar to the 1960s.

Pregnancy Rate



Average pregnancy rate by time period.

- Pregnancy rate did not change among periods, except for the 2000s which was significantly lower that the 2010s.
- Reproductive tracts from 31 females harvested in Oct.-Dec. (when a fetus should be present), had a corpora luteum, but no fetus. These may indicate unsuccessful pregnancy's and were not included.

Conclusions

- **Indices of productivity and weaning success have not declined in recent years.**
  - Age of maturity remains younger since 2000 than during the 1970s.
  - Pregnancy rates remain high, despite a dip in the 2000s.
  - Proportion of pups harvested was lowest in the 1970s.
- **Continued monitoring is important as conditions continue to change.**

Acknowledgements

This project would not be possible without the willingness of hunters to contribute samples from their harvest, the support of their communities, local governments, and Tribal Councils. We appreciate the support from the North Slope Borough and the Ice Seal Committee. John Burns, Kathy Frost, Lloyd Lowry, and others (ADF&G) were instrumental in the early development of this monitoring. We thank Mark Nelson, Heidi Isernhagen, Letty Hughes, Gay Sheffield, and our college interns for assistance in field collection and sample processing. Research was funded primarily by NOAA, but has also been supported by NSF, NPRB, and NSB. Research was conducted under NMFS Permits 358-1585, 358-1787, and 15324.

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Ringed, bearded, and spotted seal productivity in Alaska using harvest-based monitoring, 1960s–1980s and 2000s–2010s

Anna Bryan, Lori Quakenbush, Justin Crawford, Louise Biderman, and Ryan Adam

Alaska Department of Fish and Game, Arctic Marine Mammals Program, Fairbanks, Alaska, anna.bryan@alaska.gov

ID #1037

Introduction

Declines in sea ice are predicted to negatively affect ice associated seals (ringed, *Pusa hispida*, bearded, *Erignathus barbatus*, and spotted, *Phoca largha*), important to Alaska Natives for food and materials, by reducing their time to rest, pup, nurse, and molt on sea ice. Concurrent with declines in sea ice are predicted reductions in snow depth used by ringed seals to construct pupping lairs. This is expected to lower productivity and pup survival by providing less protection from weather and predators. Estimates of ice seal abundance cannot be used to detect population trends in Alaska; however, data from the subsistence harvest can be used as an index of population health and status. We compared seal productivity during the 2000s to the 1960s and 1970s, before sea ice decline.

Methods

Subsistence harvested seals were sampled at 12 villages in Alaska along the Bering, Chukchi, and Beaufort sea coasts from 2000–2018. Female reproductive tracts and canine teeth were collected. These data were compared to data previously collected from the same region during 1963–1984. Data are grouped by decade:

Ringed: 1960s (7 yrs), 1970s (9 yrs), 1980s (3 yrs), 2000s (8 yrs), and 2010s (9 yrs)
Bearded: 1960s (6 yrs), 1970s (9 yrs), 2000s (8 yrs), and 2010s (9 yrs)
Spotted: 1960s (4 yrs), 1970s (5 yrs), 2000s (9 yrs), and 2010s (9 yrs)

Age of maturity

- Seals that ovulated at least once were classified as mature.
- Average age of maturity was estimated as the age at which 50% of females were mature (DeMaster 1978) using a probit regression (PROC PROBIT).

Villages where harvested seals were sampled (2000–2018).

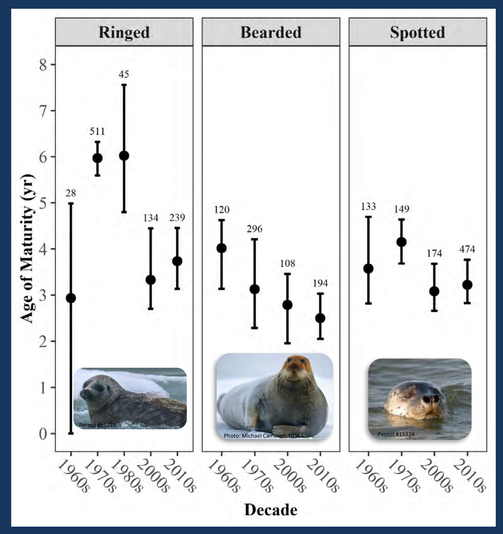
Pregnancy rate

- Pregnancy rate was defined as the proportion of mature females that were pregnant in the year of harvest. If a corpora lutea was present but no fetus was evident by November 1st, the seal was considered not pregnant.
- Differences in average pregnancy rate among time periods were evaluated using a logistic regression model (PROC LOGISTIC).

Proportion of pups harvested

- Proportion of pups (<1 year of age) in the sampled harvest is representative of their presence in the population. If pups do not survive weaning, their presence in the harvest would decrease.
- Age of seals was determined by counting annuli in the dentine or cementum layers of sectioned teeth.
- We evaluated differences in the proportion of pups harvested during each period (PROC FREQ).

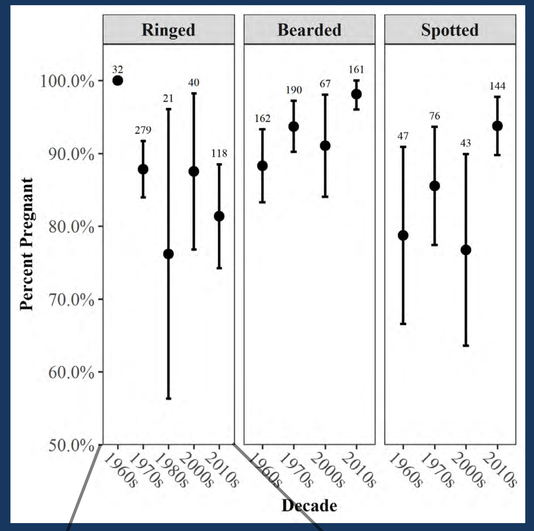
Age of maturity



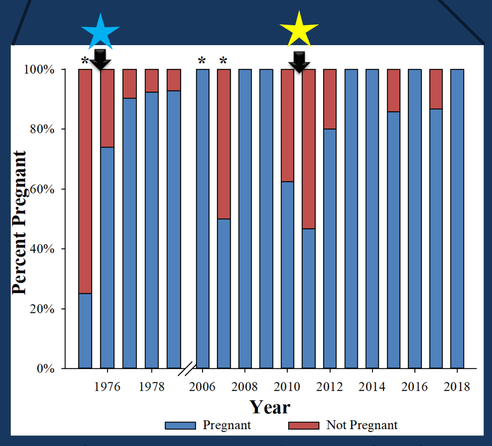
Average age of maturity by decade. Number of seals analyzed by decade is listed above the 95% confidence limits.

- Seals matured at younger ages since the 2000s than in the 1970s.

Pregnancy rate



Average pregnancy rate by decade. Number of seals analyzed by decade is listed above the 95% confidence limits.

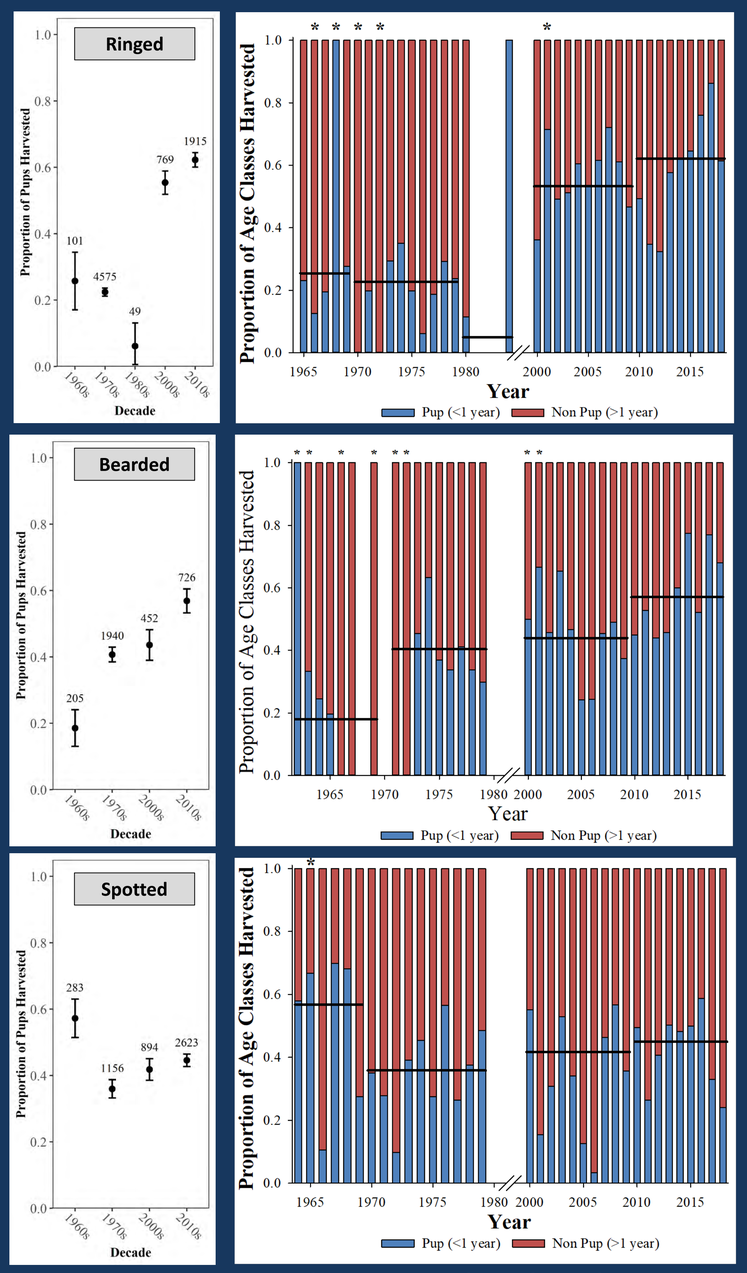


*Annual percent pregnant for ringed seals. *Only 4 mature seals were analyzed in these years. All other years had at least 7 mature seals.*

- ★ Ringed seal pregnancy rate was low prior to the 1977 regime shift.
- ★ It was also low in 2010 and 2011 during the Unusual Mortality Event (UME). During these years, reproductive tracts from six mature (13–30 yrs) females were senescent. The thickness of their uterine horns indicated previous reproductive activity, but no corpora lutea or albicans were present.

- Pregnancy rate in the 2010s was similar to other decades for ringed seals and was higher for bearded and spotted seals than all other decades.

Proportion of pups harvested



Proportion of pups harvested by decade. Number of seals analyzed by decade is listed above the 95% confidence limits.

*Annual proportions of age classes harvested. *Sample size in these years were <10 seals. All other years had >40 seals harvested. Bold black lines represent the average proportion of pups by decade.*

- The proportion of pups in the sampled harvest remains high for all three seal species in the 2010s.

Conclusions

- **Productivity and pup survival remain high in the 2010s.**
  - Ringed, bearded, and spotted seals are currently maturing at younger ages than in the 1970s.
  - Pregnancy rates remain high at 81% for ringed, 98% for bearded, and 94% for spotted seals.
  - Proportion of pups in the sampled harvest is high.
- **Ringed seals had low reproductive success during the UME (2010 and 2011) but have recovered since then.**
- **Monitoring in future years will be important as environmental conditions continue to change.**

Acknowledgements

This project would not be possible without the willingness of hunters to contribute samples from their harvest, the support of their communities, local governments, and Tribal Councils. We appreciate the support from the North Slope Borough and the Ice Seal Committee. John Burns, Kathy Frost, Lloyd Lowry were instrumental in the early development of this monitoring. We thank Mark Nelson, Gay Sheffield, and our college interns who assisted in field collection and sample processing. Research was funded primarily by NOAA, but has also been supported by NSF, NPRB, and NSB. Research was conducted under NMFS Permits 358-1585, 358-1787, 15324, and 20466.

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Helmint fauna of ice seals in the Alaskan Bering and Chukchi Seas, 2006–2015

Anna L. Bryan[1]*, Heather S. Walden[2], Antoinette McIntosh[2], Pam Tuomi[3], Anne Hoover-Miller[3], Raphaela Stimmelmayr [4,5], and Lori T. Quakenbush[1]

[1]Alaska Department of Fish and Game, Arctic Marine Mammals Program, Fairbanks, Alaska, anna.bryan@alaska.gov; [2]University of Florida College of Veterinary Medicine, Department of Comparative Diagnostic and Population Medicine; [3]Alaska SeaLife Center; [4]North Slope Borough, Department of Wildlife Management; [5]Institute of Arctic Biology, University of Fairbanks

Introduction

With warming ocean temperatures, novel parasites are predicted to spread farther north and endemic parasites may increase in prevalence. During 2006–2015, ice-associated seals of four species (ringed (*Pusa hispida*), bearded (*Erignathus barbatus*), spotted (*Phoca largha*), and ribbon *(Phoca fasciata*)) were harvested for subsistence purposes in Alaska from the Bering and Chukchi Seas and sampled for internal helminth parasites.

Methods

Samples were collected from 141 ice seals (137 harvested and four stranded) in Alaska. Most were collected during spring and fall from subsistence harvested seals at coastal Alaskan communities in the Bering and Chukchi Seas (Fig. 1). Of the four stranded seals, two were ringed seals collected during the Unusual Mortality Event (UME) in 2011 near Utqiaġvik, one was a ringed seal collected near Mekoryuk, and one was a ribbon seal collected near Adak Island (Fig. 1).

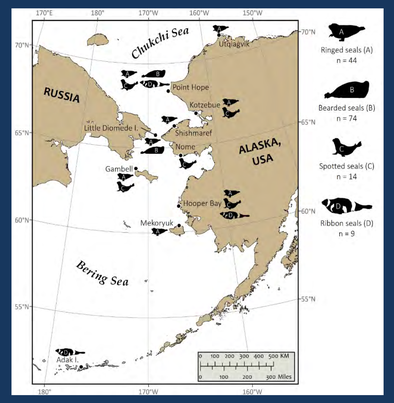


Figure 1. Locations where seals were sampled (2006–2015).

- Heart, gall bladder, and stomach were collected whole.
- Intestine and lungs (including the tracheal bifurcation) were subsampled.
- Stomachs were sorted for prey analysis and helminths were removed at that time.
- The other tissues were examined individually and rinsed into a #50 standard sieve. Remaining material was back flushed in a glass container and the helminths were removed.
- Helminths were identified to the lowest possible taxonomic level using morphological characteristics.

Number of seals with helminths

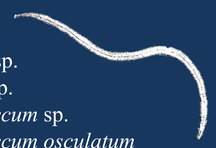
38 of 44 (86%)

73 of 74 (99%)

13 of 14 (93%)

9 of 9 (100%)

Nematoda (roundworms)



	Ringed seal	Bearded seal	Spotted seal	Ribbon seal
Nematodes	47%	97%	93%	78%
Anisakidae	21%	96%	71%	67%
Anisakid sp.	7%	1%	14%	
Anisakis sp.	2%	4%	21%	44%
Contracaecum sp.		22%		22%
Contracaecum osculatum complex	2%	28%		22%
Pseudoterranova decipiens complex	9%	91%	36%	33%
Phocascaris sp.			7%	22%
Phocascaris netski				11%
Onchocercidae		1%	7%	
Acanthocheilonema (Dipetalonema) spirocauda		1%	7%	
Filaroididae	25%	18%	57%	11%
Parafilaroides (Filaroides) sp.	7%	10%		
Parafilaroides (Filaroides) gymnurus	18%	8%	57%	11%
Crenosomatidae	11%		7%	22%
Otostrongylus sp.				11%
Otostrongylus circumlitus	11%		7%	11%
Unidentified nematode	7%	18%		11%

- **This is the first host record of the lungworm *Parafilaroides (Filaroides) gymnurus* in a ribbon seal.**
- **This is also the first report of the lungworm *Otostongylus circumlitus* in a ribbon seal and *P. (F.) gymnurus* in bearded seals from the Bering-Chukchi region (previously identified in the Sea of Okhotsk; Popov 1975 and Shults and Frost 1988).**

Trematoda (flukes)

	Ringed seal	Bearded seal	Spotted seal	Ribbon seal
Trematodes	5%	64%		
Campulidae	2%	64%		
Orthosplanchnus sp.		11%		
Orthosplanchnus arcticus	2%	51%		
Heterophyidae	2%			
Phocitrema fusiforme	2%			
Unidentified trematode		3%		

- **No trematodes were found in spotted or ribbon seal tissues.**

Cestoda (tapeworms)

	Ringed seal	Bearded seal	Spotted seal	Ribbon seal
Cestodes	9%	82%	29%	
Tetrabothriidae	2%		29%	
Anophryocephalus sp.	2%		29%	
Diphyllobothriidae	4%	78%	7%	
Diphyllobothrium sp.	4%	64%	7%	
Diphyllobothrium cordatum		8%		
Diphyllobothrium lanceolatum		19%		
Pyramicocephalus sp.		3%		
Unidentified cestode	2%	14%		

- **We found a lower prevalence of the cestode genus *Pyramicocephalus* in bearded seals (2.7%) than reported previously for the species *Pyramicocephalus phocarum* (44–100%) in the Bering-Chukchi region (Delyamure et al. 1976, Fay et al. 1978, and Fay et al. 1979).**

Acanthocephala (thorny-headed worms)

	Ringed seal	Bearded seal	Spotted seal	Ribbon seal
Acanthocephalans	61%	15%	64%	33%
Polymorphidae	61%	15%	64%	33%
Corynosoma sp.	2%	4%		22%
Corynosoma hadweni (syn. *C. wegeneri**)	39%	3%	36%	
Corynosoma reductum	9%		7%	
Corynosoma semerme	27%	4%	29%	22%
Corynosoma strumosum	32%	1%	50%	11%
Corynosoma validum	5%	4%		
Unidentified parasite	23%	3%	14%	22%

- **The acanthocephalan genus *Bolbosoma* was not found, but was found previously in ringed, spotted, and ribbon seals (Adams 1988, Shults 1982, and Shults and Frost 1988).**

Conclusions

- **None of the helminths found in this study are new to the Bering-Chukchi region.**
- **As of 2015, no new parasite species were identified, and the prevalence of endemic parasites does not appear to have increased, although some may have decreased.**
- **New lungworm reports:**
  - **This is the first host record of *P. (F.) gymnurus* in a ribbon seal.**
  - **First report of *P. (F.) gymnurus* in bearded seals from the Bering-Chukchi region.**
  - **First report of *O. circumlitus* in a ribbon seal from the Bering-Chukchi region.**

Acknowledgements

This project would not be possible without the willingness of hunters to contribute samples from their harvest, the support of their communities, local governments, and Tribal Councils. We appreciate the support from the North Slope Borough and the Ice Seal Committee. We thank Mark Nelson, Louise Biderman, and our college interns who assisted in field collection and sample processing and John Burns who helped translate Russian. Research was funded primarily by NOAA, but has also been supported by NSF, NPRB, and NSB. Research was conducted under NMFS Permits 358-1585, 358-1787, and 15324.

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Use of Nobuto™ filter papers for analyses of contaminants, nutrients, carbon (C) and nitrogen (N) stable isotopes, and serology in blood

J. Margaret Castellini[1], Todd O'Hara[1], Frances Gulland[2], Randall Wells[3], Lorrie Rea[1], George Aguiar[1], Lori Quakenbush[4], James Berner[5]

[1]Univerisity of Alaska Fairbanks, [2]The Marine Mammal Center, [3]Chicago Zoological Society's Sarasota Dolphin Research Program, [4]Alaska Department of Fish and Game, [5]Alaska Native Tribal Health Consortium

Introduction

Constraints on blood collection in the field:

- Collection:
  - specialized skills and equipment
  - usually not compatible with subsistence harvest or necropsy
- Handling:
  - specialized equipment and supplies (expensive)
  - (e.g. tubes with anticoagulant, centrifuge, cryovials, refrigeration/ice)
- Storage and shipping:
  - freezer, freezer paks, dry ice, liquid nitrogen
  - expense and logistics of shipping from remote sites
  - risk of compromised samples

Any or all may constrain the ability to use blood as an experimental or biomonitoring tissue, especially from remote locations or projects with limited funding and team members.

Solution:

Blood collection using Nobuto™ or similar filter paper strips (Figure 1)

- Can be used with live or dead (e.g. subsistence, necropsy) animals
- Minimal training and inexpensive supplies (filter papers, labels, envelopes and plastic bags)
- Air dried samples stabilize many analytes
- Ambient temperature storage and shipping
- Consistency of composition
- Utility has been demonstrated in human and wildlife studies (*Curry et al. 2014*)

Methods: Analyte Validation

Filter paper kits (Figure 1) distributed to collaborators for collection during wildlife health or rehabilitation health assessments. Agency and rehabilitation collaborators provided an aliquot of whole blood (WB) and blood soaked filter papers (FP) for comparison.

Figure 1:
Typical blood collection kit. Blood soaked, air dried cellulose filter papers (FP) were placed in envelopes and returned to the Wildlife Toxicology Laboratory, UAF for analysis

Total Mercury Concentration ([THg]):

200µl WB, 2-3 FP	Direct measurement (combustion) by DMA80

Total Selenium Concentration ([TSe]):

400µl WB, 3-4 FP	Acid digestion, mass spectrometry (ICP-MS)

Stable Isotopes Ratios of Carbon ($\delta^{13}C$) and Nitrogen ($\delta^{15}N$):

100µl WB, 1-2 FP	FP blood eluted into PBS Eluate freeze-dried Analyzed by isotope ratio mass spectrometry (UAF Stable Isotope Lab)

Serology (e.g. *Toxoplasma gondii*):

500µl serum, 5 FP WB, serum	FP WB or serum eluted into PBS Eluate concentrated using 30K centrifugal filter *Toxoplasma* analyzed by Modified Agglutination Test (MAT); Colorado State University Veterinary Diagnostic Lab

Connections between THg, TSe and stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$)

- Se and Hg have high binding affinity: may contribute to protective effects of TSe
- Se is an antioxidant
  - may be protective against oxidative damage from THg;
  - TSe antioxidant activity may be compromised by THg binding
- Stable isotope ratios of C and N provide measures of feeding ecology ($\delta^{13}C$, region/migration; $\delta^{15}N$, trophic level)

Total mercury (THg) validation

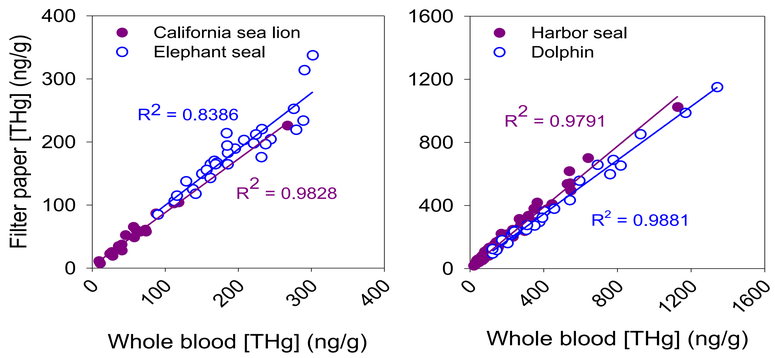

Figure 2: Comparison of WB and FP [THg] in pinnipeds sampled by the Marine Mammal Center, Sausalito, CA and Sarasota Bay bottlenose dolphins sampled by the Chicago Zoological Society's Sarasota Dolphin Research Program (from *Hansen et al. 2014*; adapted from *O'Hara et al. In Press a*)

Total selenium (TSe) validation

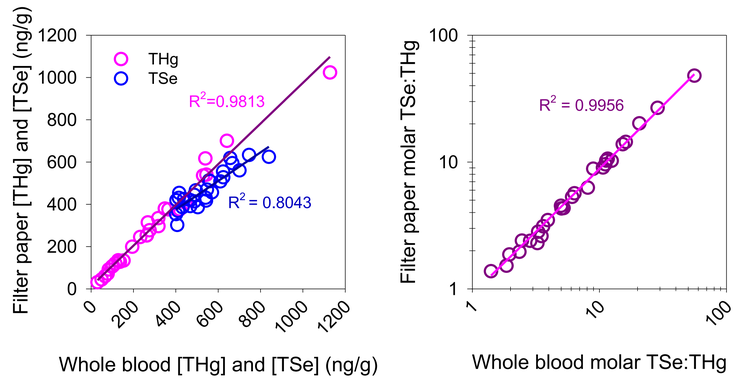

Figure 3: Comparison of WB and FP [TSe] in harbor seal pups sampled by the Marine Mammal Center, Sausalito, CA

$\delta^{13}C$ and $\delta^{15}N$ validation

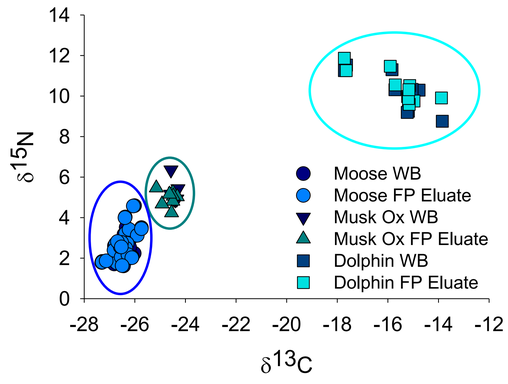

Figure 4: Comparison of WB and FP stable isotope ratios of carbon and nitrogen in Alaskan moose, musk ox and Sarasota Bay bottlenose dolphins. Species were chosen to represent a wide range of foraging ecology. (from *O'Hara et al. In Press b*)

Serology (e.g. *Toxoplasma gondii*)

Sarasota Bay bottlenose dolphin archived serum samples tested for *Toxoplasma gon*dii (n = 33) (from *O'Hara et al. In Press a*)

18 negative; 15 positive (titer ≥ 400)
Matching WB and serum FP eluates and concentrated eluates were tested;
all –ve serum samples were –ve in FP eluates and concentrated eluates (WB and serum)

Results from FP eluates and concentrated eluates for individuals that tested +ve in serum (n = 15)			
	Total –ve*	Total +ve	Total +ve within 1 titer dilution
FP Serum eluate	7/15	8/15	3/15
FP Serum eluate concentrated	6/15	9/15	5/15
FP WB eluate	10/15	5/15	2/15
FP WB eluate concentrated	8/15	7/15	5/15
* all eluates that tested –ve had the lowest serum titers (400)			

Prediction criteria for R^2 values (from *O'Hara et al. 2008*)

Weak: R^2 0.36-0.55 Moderate: R^2 0.56-0.75 **Strong: $R^2 > 0.75$**

- FP [THg] **strongly** predictive of WB [THg] (Fig. 2)
- FP [TSe] **strongly** predictive of WB [TSe] (Fig.3)
  - Despite some variability between FP and WB [TSe],
  - FP molar ratio of TSe:THg **strongly** predictive of WB molar TSe:THg
- FP $\delta^{13}C$ and $\delta^{15}N$ **moderately/strongly** predictive of WB $\delta^{13}C$ and $\delta^{15}N$ (Fig. 4)
  - FP $\delta^{13}C$ and $\delta^{15}N$ signatures were able to distinguish between species with different foraging ecologies

The ability to reliably measure [THg], [TSe], $\delta^{13}C$ and $\delta^{15}N$ using blood soaked filter papers, broadens the scope of this sampling tool to address questions of ecotoxicology, including potential protective/adverse effects (TSe) and possible pathways of exposure ($\delta^{13}C$ and $\delta^{15}N$).

Biomonitoring and One Health

RAMP: Rural Alaska Monitoring Program, Alaska Native Tribal Health Consortium (ANTHC)

- Ongoing collections of FP whole blood from Alaskan ice seals (n > 100) and northern fur seals (n > 100) provided by ADF&G (subsistence harvest and live captures), ANTHC community partners (subsistence), wildlife biologists (pinniped health assessments; NOAA)
- To date: analysis of THg in 4 ice seal species
  - Ringed seal (n = 12), Bearded seal (n = 43),
  - Spotted seal (n = 24), Ribbon seal (n = 14)

One Health: Connections between environmental, wildlife, and human health

Use of blood soaked filter papers facilitates studies of marine mammal health and marine mammals as sentinels in a One Health context, especially for subsistence and remote collections

Acknowledgements

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Samples from animals in rehabilitation were collected under MMPA permit No: 18786. Dolphin samples were collected under NMFS Scientific Research Permit No. 15543 and IACUC approvals renewed annually by Mote Marine Laboratory. ADF&G samples were collected under NMFS Permit No: 15324 and ADF&G IACUCs 2016-23 and 0027-2017-27.

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Bearded seal foraging related to benthic communities and environmental characteristics of the Chukchi Sea.

Michael Cameron, Marine Mammal Lab / NOAA
Brett McClintock
Arny Blanchard, Institute of Marine Science, University of Alaska Fairbanks
Peter Boveng
Justin Crawford, Alaska Department of Fish and Game
John Goodwin, Native Village of Kotzebue
Jacqueline Grebmeier, Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science
Stephen Jewett, Institute of Marine Science, University of Alaska Fairbanks
Bob Lauth, NOAA, NMFS, Alaska Fisheries Science Center
Josh London, NOAA, NMFS, Alaska Fisheries Science Center
James Lovvorn, Department of Zoology and Center for Ecology, Southern Illinois University
Brenda Norcross, Institute of Marine Science, University of Alaska Fairbanks
Lori Quakenbush, Alaska Dept. Fish and Game
Alex Whiting, Native Village of Kotzebue

*Presenter: Michael.cameron@noaa.gov
Society of Marine Mammalogy, 22–27 October 2017, Halifax, Nova Scotia, Canada

Bearded seals are large phocids that inhabit circumpolar Arctic and Subarctic waters. They are benthic feeders, consuming epifaunal and infaunal invertebrates and demersal fishes, primarily at depths < 200 m. Our goals were to locate ecologically important areas and to identify specific factors driving bearded seal habitat selection in the northeastern Chukchi Sea from late-June to late-November. Instead of merely examining space use, we modeled the foraging movements of bearded seals as a function of specific biological and environmental features (e.g., benthic prey and sediment type), using a two-stage analysis. In the first stage, we used a multistate movement model to identify benthic foraging activity based on biotelemetry data collected from seven adult and subadult bearded seals. In the second stage, we fit point process models for resource selection using benthic prey and other environmental covariates as predictors of the foraging locations identified in the first stage. Bearded seals exhibited positive selection (i.e., preference) for a diverse array of invertebrates and fishes. For many fish, only the smaller age-classes were selected for, supporting previous observations from stomach samples. In addition, areas of mud or finer sand were selected against (i.e., avoided). Many of the taxa that were positively selected for have spatial distributions that are concentrated within 50-90 km of the Alaska coastline, a region identified as an ecological hotspot for bearded seal foraging. Indeed, this area appears to constitute a summer “smorgasbord” for these benthic foragers. In contrast, one location farther offshore (71° 20’N 163° 00’W) contained a suite of prey and sediment types avoided by bearded seals. This location is also coincident with high densities of walrus, strong currents and significant industrial activity related to oil and gas exploration. Further research will determine the extent to which these conditions caused bearded seals to avoid the area.

Aerobic and Anaerobic Properties of Bearded Seal Locomotor Muscle

Holly Hermann-Sorensen[1], Brandi Ruscher-Hill[2], Mariah Tengler[2,3], Anna Bryan[4], Colleen Reichmuth[1,2], Nicole Thometz[2,3]

[1]Department of Ocean Sciences, University of California Santa Cruz, [2]Institute of Marine Sciences, University of California Santa Cruz, [3]Department of Biology, University of San Francisco, [4]Alaska Department of Fish and Game

Background

While climate change is expected to have pervasive effects on all Arctic marine organisms, long-lived, highly derived species such as ice-associated seals will be disproportionately affected. Bearded seals (*Erignathus barbatus*) have a circumpolar distribution in the Arctic and are commonly associated with the transition zone between land-fast and pack ice. As predominantly benthic foragers, bearded seals dive to the seafloor to search for prey. Given their reliance on sea ice as a platform for foraging, sea ice loss may result in increased travel time to foraging grounds and/or increased dive times. Examining the physiological adaptations of bearded seals may help to determine the potential consequences of rapid sea ice loss.

Question: Will the ability of bearded seals to respond to climate change be constrained by their physiology?

Aim: Measure the aerobic and anaerobic capacities of bearded seal locomotor muscle to gain a more comprehensive understanding of their diving abilities and limitations.

Methods

Skeletal muscle samples of a major locomotor muscle (longissimus dorsi) were obtained from bearded seals (n=7) harvested by subsistence hunters in Point Hope, Alaska, in partnership with local community members and the Alaska Department of Fish and Game. Samples were analyzed for both myoglobin content ([Mb]) and non-bicarbonate buffering capacity following the methods of Reynafarje (1963) and Castellini and Somero (1981), respectively.

To determine myoglobin content:

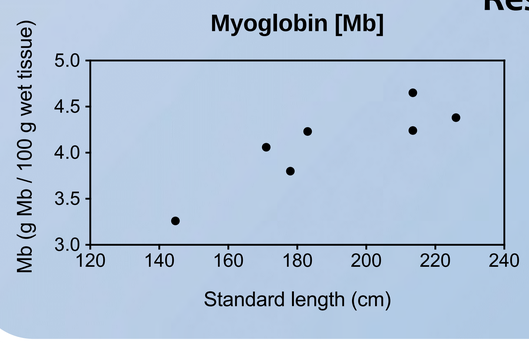
- subsamples were minced in a phosphate buffer (pH=6.6) and sonicated on ice
- homogenates were spun at 28,000G for 50min at 0°C
- supernatant of each sample was bubbled with CO
- absorbance of each sample was determined at 538 nm and 568 nm
- differential absorbance was used to calculate [Mb]

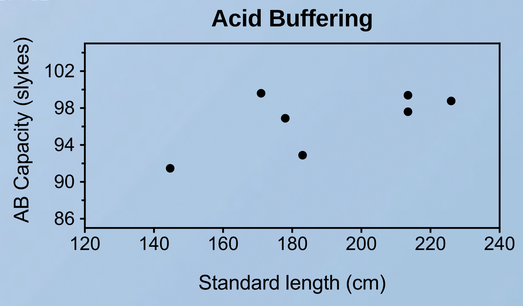
To determine acid buffering capacity:

- subsamples were minced in saline and homogenized via sonication on ice
- homogenates were equilibrated in a 37°C water bath
- samples were titrated over a pH range of approximately 6 to 7 using NaOH
- the µmoles needed to cause this change were used to determine buffering capacity in unit of slykes

Photo Credit: M. Cameron/NOAA Polar Ecosystems Program

Results





Summary

Compared to other Arctic marine mammals , the locomotor muscle of bearded seals has comparatively low myoglobin content, but high buffering capacity. This unique muscle physiology may be an adaptation to benthic foraging under sea ice.


Photo Credit: M. Cameron/NOAA Polar Ecosystems Program

The physiological properties of marine mammal skeletal muscle play important roles in defining species-specific limits to diving and foraging. In combination with environmental projections, these types of physiological data can be used to inform predictions as to how climate change will impact individual species.

Table 1. Known myoglobin content and acid buffering capacity of Arctic and Antarctic marine mammals.

Species	Myoglobin (g 100g^{-1})	Buffering Capacity (slykes)
Arctic Phocids		
Bearded seal	4.1[A]	97[A]
Harp seal	8.6[C]	~85[C]
Hooded seal	9.5[C]	~85[D]
Ringed seal	4.1[E]	
Ribbon seal	8.1[F]	
Arctic Odobenid		
Walrus	3.8[B]	42[B]
Arctic Cetaceans		
Beluga	6.9[G]	84[G]
Narwhal	7.9[H]	
Antarctic Phocids		
Leopard seal	5.1[J]	
Weddell seal	5.4[I]	

[B]Noren et al. 2015; [C]Lestyk et al. 2009; [D]Burns et al. 2007; [E]Lydersen et al. 1991; [F]Lenfant et al. 1970; [G]Noren et al. 2016; [H]Williams et al. 2011; [I]Ponganis et al. 1993; [J]Kuhn et al. 2006

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Acknowledgements:

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Detecting population structure in bearded and ringed seals: current understanding and future challenges

A. R. Lang[1], L. Quakenbush[2], H. Ziel[3], K. Robertson[1], M. Lauf[1], B. L. Taylor[1], and P. Boveng[3]

[1] Southwest Fisheries Science Center, La Jolla, CA; [2] Alaska Department of Fish & Game, Fairbanks, AK; [3]Alaska Fisheries Science Center, Seattle, WA

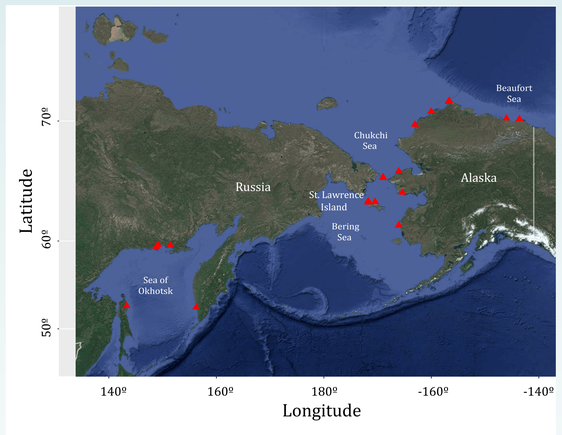
Rationale

- Bearded seals (*Erignathus barbatus*) and ringed seals (*Pusa hispida*) depend on sea ice for hauling out, whelping, and molting.
- Projections of diminishing ice and snow cover have raised concern for both species
- A better understanding of stock structure in both species is needed to evaluate the risk of localized depletions.

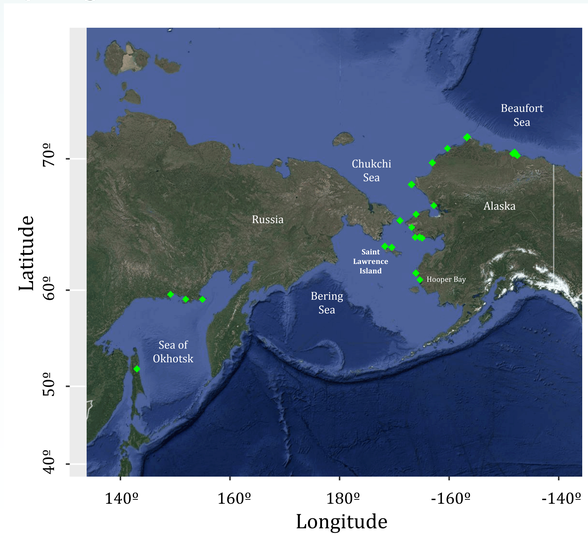
Objective: To use existing tissue samples and available published data to assess whether population structure can be detected among breeding sites used by bearded and ringed seals during spring (March – May) in the Pacific Arctic and Okhotsk Sea using mitochondrial DNA (mtDNA) control region sequences

Figure 1. Distribution of available samples:

a) Bearded seals



b) Ringed seals



Results

Table 1. Summary of the number of samples, number of haplotypes, and haplotype diversity within each stratum. Measures of haplotype diversity range from 0 to 1, with higher values indicating increased levels of diversity.

Region	# of samples	# of haplotypes	Haplotype diversity
Bearded seals:			
DPS level:			
Okhotsk Sea	20	18	0.989
Pacific Arctic	176	86	0.979
Within Pacific Arctic:			
Saint Lawrence Island, AK	33	23	0.973
Bering Strait	88	58	0.984
Chukchi Sea	49	28	0.969
Ringed seals:			
Subspecies level:			
Lake Saimaa	10[1]	5	0.667
Baltic Sea	11[1]	11	1.000
Okhotsk Sea	6	6	1.000
Pacific Arctic	232[1]	227	1.000
Within the Pacific Arctic:			
Hooper Bay, AK	39	38	0.999
Saint Lawrence Island, AK	25	25	1.000
Bering Strait	87	87	1.000
Beaufort Sea	74[1]	73	1.000

Table 2. Measures of mtDNA genetic differentiation between strata. PHI_{ST} measures the amount of sequence variation between strata and generally ranges from 0 to 1, with higher values indicating greater degree of structure.

Comparison	PHI_{ST}	p-value
Bearded seals:		
DPS level:		
Okhotsk Sea (20) v. Pacific Arctic (176)	0.018	0.1317
Within Pacific Arctic:		
Saint Lawrence Island, AK (33) v. Bering Strait (88)	-0.004	0.5289
Saint Lawrence Island, AK (33) v. Chukchi Sea (49)	0.010	0.1856
Bering Strait (88) v. Chukchi Sea (49)	0.000	0.4152
Ringed seals:		
Subspecies level:		
Lake Saimaa (10) v. Okhotsk Sea (6)	0.689	0.0020
Lake Saimaa (10) v. Pacific Arctic (232)	0.337	0.0020
Lake Saimaa (10) v. Baltic Sea (11)	0.514	0.0020
Baltic Sea (11) v. Okhostk Sea (6)	0.320	0.0040
Baltic Sea (11) v. Pacific Arctic (232)	0.050	0.0240
Okhotsk Sea (6) v. Pacific Arctic (232)	0.155	0.0060
Within Pacific Arctic:		
Hooper Bay, AK (39) v. Saint Lawrence Island, AK (25)	0.005	0.2754
Hooper Bay, AK (39) v. Bering Strait (87)	0.006	0.1836
Hooper Bay, AK (39) v. Beaufort Sea (74)	0.015	0.0559
Saint Lawrence Island, AK (25) v. Bering Strait (87)	-0.005	0.6208
Saint Lawrence Island, AK (25) v. Beaufort Sea (74)	0.006	0.2275
Bering Strait (87) v. Beaufort Sea (74)	0.008	0.0858

Findings

- At the taxonomic level:
  - No significant differences were identified between the Okhotsk Sea DPS and the Beringia DPS of bearded seals.
  - Significant differences were identified between all four of the ringed seal subspecies represented here, consistent with previous analyses[1].
  - Although represented by few samples, the magnitude of mtDNA differentiation seen between the Okhotsk Sea ringed seal subspecies and the Pacific Arctic subspecies is greater than that seen between the Arctic and Baltic ringed seal subspecies.
- Within the Pacific Arctic:
  - No population structure was detected within bearded seals
  - Although no significant differences were identified between the ringed seal strata, a couple of the comparisons approached significance, suggesting that some low level of differentiation might exist.
- However, existing sample sets represent only a small proportion of animals and contain many sampling gaps, limiting the ability to draw any firm conclusions.

Challenges and future plans:

1) Difficulty in identifying biologically meaningful stratifications for existing sample sets given high variation in the environment
2) Detecting low but potentially biologically significant levels of genetic differentiation is difficult in highly diverse species
   - Power to detect genetic differences can be increased by:
     - Adding more samples
       - Highlights the need for additional sampling efforts, particularly during the reproductive season
     - Increasing the number of loci analyzed

- Ongoing work includes the use of genotyping-by-sequencing (GBS) to generate data from thousands of loci in each species. This work will:
  - Markedly increase the power to detect genetic differences
  - Provide a resource that can be used to design assays to genotype additional samples, such as those currently archived at the University of Alaska's Museum of the North

[1] Includes published data from:

Martinez-Bakker M.E., Sell S.K., Swanson B.J., Kelly B.P., Tallmon D.A. 2013. Combined genetic and telemetry data reveal high rates of gene flow, migration, and long-distance dispersal potential in Arctic ringed seals (*Pusa hispida*). *PLOS One* 8:e77125.

Arctic - Mammals

Exposure Risks and Health Effects of Algal Toxins in Marine Mammals Using both Environmental Surveillance and Biomedical Laboratory Models

Kathi Lefebvre
NOAA Northwest Fisheries Science Center, Kathi.Lefebvre@noaa.gov
David Marcinek
University of Washigton, dmarc@uw.edu
Kathy Burek Huntington
Alaska Veterinary and Pathology Services, avps.kbh@gmail.com
Lori Quakenbush
Alaska Department of Fish & Game, lori.quakenbush@alaska.gov
Anna Bryan
Alaska Department of Fish & Game, anna.bryan@alaska.gov
Raphaela Stimmelmayr
North Slope Borough, Raphaela.Stimmelmayr@north-slope.org
Gay Sheffield
University of Alaska Fairbanks, ggsheffield@alaska.edu
Heather Ziel
NOAA Alaska Fisheries Science Center, heather.ziel@noaa.gov
Tracey Goldstein
University of California, Davis, tgoldstein@ucdavis.edu
Jonathon Snyder
U.S. Fish and Wildlfie Service, jonathan_snyder@fws.gov
Tom Gelatt
NOAA Alaska Fisheries Science Center, tom.gelatt@noaa.gov
Frances Gulland
The Marine Mammal Center, Gullandf@TMMC.org
Bobette Dickerson
NOAA Alaska Fisheries Science Center, bobette.dickerson@noaa.gov
Verena Gill
Bureau of Ocean Energy Management, verena.gill@boem.gov

The Wildlife Algal-Toxin Research and Response Network (WARRN-West) provides environmental surveillance for the presence of algal toxins in marine wildlife from the Arctic Ocean to Southern California. Over the last decade the program has analyzed several thousand samples from stranded and harvested animals from more than a dozen species. Additionally, the biomedical diagnostics part of this program has performed controlled laboratory studies using mammalian models to identify health effects of exposure to the algal toxin domoic acid. Data on the prevalence of algal toxins in marine mammals as well as results from controlled laboratory studies will be presented. The effects of acute high level exposure and chronic low-level exposure to domoic acid will be compared. A new paradigm of chronic low-level toxicity has been identified in which a reversible impairment of spatial memory, learning, and activity occurs in the absence of gross morphological lesions in the brain of mammals.

ALGAL TOXINS IN ALASKAN ARCTIC FOOD WEBS: SEAWATER, ZOOPLANKTON, BIVALVES, FISH, ICE SEALS, WALRUSES AND WHALES!

Kathi Lefebvre[1], Raphaela Stimmelmayr[2], Lori Quakenbush[3], Gay Sheffield[4], Alicia Hendrix[5], Anna Bryan[4], Anna Mounsey[1], Maryjean Willis[1], Emily Iversen[1], Dave Kimmel[6], Janet Duffy-Anderson[6], Jim Murphy[7], Kristin Cieciel[7], Elizabeth Siddon[7], Lisa Eisner[7], Emily Fergusson[7], Spencer Showalter[1], Ellen Yasumiishi[7], Jeanette Gann[7], Jackie Grebmeier[8], Jonathan Snyder[9], Donald M. Anderson[10], Evangeline Fachon[10]

[1]Environmental and Fisheries Science Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Seattle, WA
[2]North Slope Borough Department of Wildlife Management, PO Box 69, Barrow, AK
[3]Alaska Department of Fish and Game, Arctic Marine Mammal Program, Fairbanks, AK
[4]University of Alaska Fairbanks, Alaska Sea Grant, Marine Advisory Program, PO Box 400, Nome, AK
[5]Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, USA
[6]EcoFOCI, Alaska Fisheries Science Center, NOAA, Seattle, WA
[7]Auke Bay Laboratory, Alaska Fisheries Science Center, NOAA, Juneau, AK
[8]Chesapeake Biological Laboratory, University of Maryland, Solomons, MD
[9]US Fish and Wildlife Service, Marine Mammals Management, Anchorage, AK
[10]Woods Hole Oceanographic Institution, Woods Hole, MA 02543

Changing ocean conditions threaten to increase harmful algal bloom (HAB) frequency, severity and geographic extent in northern seas raising concerns regarding the trophic transfer of algal toxins in marine food webs and potential exposure risks to marine wildlife and humans. Coastal Alaskan communities in Arctic and subarctic regions rely heavily on non-commercial acquisition of marine wildlife for nutritional, economic, and cultural well-being. Thus, the health of marine wildlife is not solely a wildlife conservation issue, but includes public health and food security issues. Here we present data on the prevalence of two algal toxins of concern, domoic acid (DA) and saxitoxin (STX) in multiple levels of Alaskan Arctic food webs. DA and STX are neurotoxic and are responsible for the shellfish poisoning syndromes known as Amnesic Shellfish Poisoning (ASP) and Paralytic Shellfish Poisoning (PSP), respectively. We will present algal toxin prevalence results from long-term data sets (up to 15 years) of subsistence-harvested marine mammals including bowhead whales, walruses, and four species of ice seals (bearded, ringed, spotted and ribbon) representing well over 1,000 animals. We will also present results on the presence of algal toxins quantified in seawater, zooplankton, bivalve, and fish samples collected during 2019 research cruises in the Beaufort Sea, Chukchi Sea, Bering Strait Region, Bering Sea, and Gulf of Alaska. Both DA and STX were detected in all regions examined and the potential for increased algal toxin prevalence and food web transfer in Arctic waters will be discussed.

BRUCELLA INFECTIONS IN PHOCIDS VERSUS OTARIIDS IN ALASKA

Nymo Ingebjørg Helena [1,5], Larsen Anett Kristin [1], Rødven Rolf [1,2], Tryland Morten [1], Foster Geoff [3], Beckmen Kimberlee [4], Quakenbush Lori [4], Godfroid Jacques [1]

[1] UiT-The arctic university of Norway, Department of arctic and marine biology, Arctic infection biology, Box 6050 Langnes, Tromsø, Norway;
[2] Department of natural resources and rural development, Norwegian institute for bioeconomy research, Box 115, 1431 Ås, Norway;
[3] SAC Consulting veterinary services, Drummondhill, Stratherrick Road, Inverness IV10 8SP, Scotland;
[4] Alaska dept. of fish and game, Division of wildlife conservation, 1300 College Road, Fairbanks, 99701 Alaska, USA;
[5] Corresponding author: ingebjorg.h.nymo@uit.no / +47 918 38 421

Alaskan pinnipeds

Alaskan waters accommodate five phocid (Picture 1-5) and two otariid species (Picture 6-7) [1]. The Arctic ringed seals, the bearded seals in the Bering Sea distinct population segment, and the Steller sea lions in the western distinct population segment are listed as threatened by the Endangered Species Act and the Northern fur seal Pribilof Island stock (Picture 8) is listed as depleted by the Marine Mammal Protection Act [2].

Brucella pinnipedialis

The bacteria *Brucella pinnipedialis* was included in genus *Brucella* in 2007 [3], and has since been isolated from several phocid species, however, the number of isolates from otariids are sparce. Few pathological findings have been associated with infection in phocids, while reproductive pathology and abortions, and isolation of the zoonotic strain type 27, have been documented in otariids [4].

Picture 1. Eastern North Pacific harbor seal (*Phoca vitulina richardii*). Source: Dave Withrow, NOAA.

Picture 2. Spotted seal (*Phoca larga*). Source: Jay Verhoef, NOAA.

Picture 3. Ribbon seal (*Histriophoca fasciata*). Source: Josh London, NOAA.

Picture 4. Ringed seal (*Phoca hispida*). Source: Michael Cameron, NOAA.

Picture 5. Bearded seal (*Erignathus barbatus*). Source: Michael Cameron, NOAA.

Picture 6. Steller sea lions (*Eumetopias jubatus*). Source: Vladimir Burkanov, NOAA.

Picture 7. Northern fur seals (*Callorhinus ursinus*). Source: Beth Sinclair, NOAA.

Picture 8. Northern fur seal pups on St. Paul Island, one of the Pribilof Islands. Source: Beth Sinclair, NOAA.

Materials and methods

Serum samples were collected (1975-2011) from Eastern North Pacific harbor seals (n=1122), spotted seals (n=85), ribbon seals (n=55), ringed seals (n=150), bearded seals (n=124), Steller sea lions from the Western (n=76) and Eastern (n=48) distinct population segments, and Northern fur seals (n=107) in Alaskan waters (Figure 1) and Argo Bay, Canada (n=7 ringed seals). Sex (n=795 females and 790 males) and age category (n=394 pups: <1 yr, 390 juveniles: 1-3 yrs and 636 adults: <3 yrs) were known for most animals. Sera were analyzed for anti-*Brucella* antibodies with a Protein A/G indirect enzyme-linked immunosorbent assay (ELISA) [5].

Results

The total seroprevalences were much higher in phocids than in otariids (harbor seals; 25 %, spotted seals; 19 %, ribbon seals; 16 %, ringed seals; 14 %, bearded seals; 13 %, Steller sea lions from the Western; 1 %, and Eastern; 2 % distinct population segments, and Northern fur seals; 0 % (Figure 1).

In phocids the pups in general had the lowest seroprevalence, juveniles had the highest, and adults were in between (Figure 2).

Seropositive seals were detected in all locations investigated (Figure 1) and throughout the sampling period.

There was no difference between sexes.

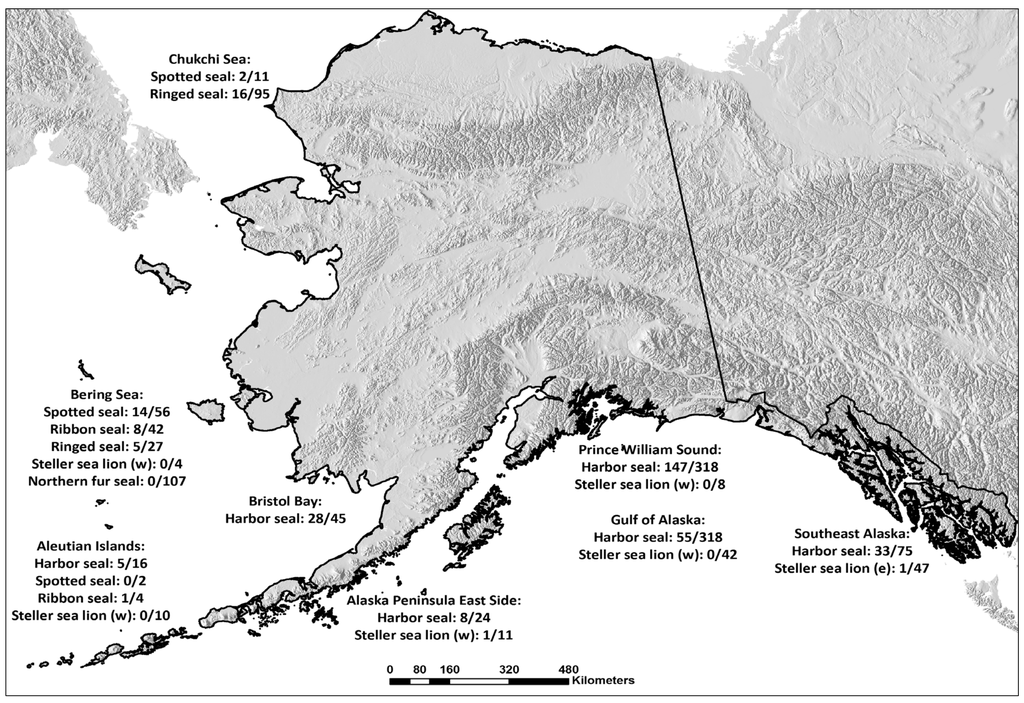


Figure 1. Number of *Brucella*-seropositives per species and sampling area.

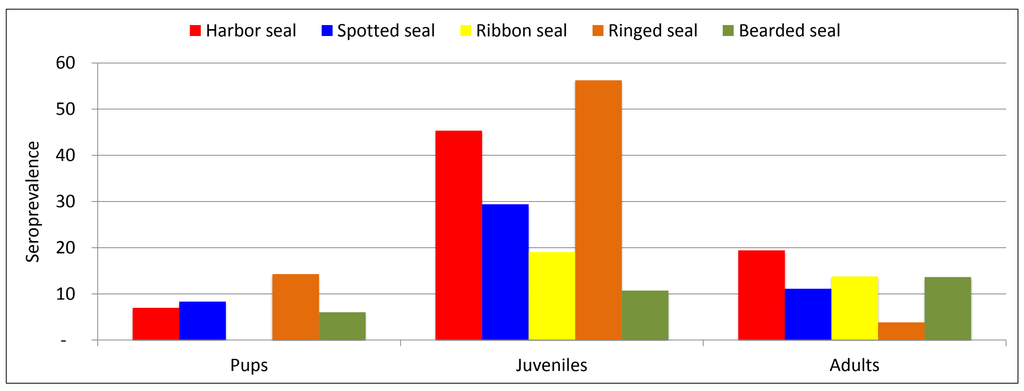


Figure 2. Seroprevalence (anti-*Brucella* antibodies) in phocid pups, juveniles and adults.

Conclusions

The age-dependent serological patterns in **phocids** suggests environmental exposure to *B. pinnipedialis* at the juvenile stage, with a later clearance of infection. This is in line with the lack of pathology in phocids [4], our work on *B. pinnipedialis* in phocid cell models [6, 7] and experimental infection in Atlantic Cod (*Gadus morhua*) [8]. **The infection in phocids could hence be transient and not causing pathology.**

The low seroprevalences in **otariids** could suggest a low level of exposure or innate resistance to the infection. **However, morbidity/mortality due to the infection is plausible and in line with previous findings in otariids [4].**

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

Technical Report to:

Alaska Department of Fish and Game, State Wildlife Grant
and
U.S. Fish and Wildlife Service

Lori Quakenbush
Anna Bryan
Mark Nelson
Arctic Marine Mammal Program
Alaska Department of Fish and Game
1300 College Road, Fairbanks AK 99701

And

Jonathan Snyder
Marine Mammals Management
U.S. Fish and Wildlife Service
1100 E. Tudor Road, Anchorage AK 99501

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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.

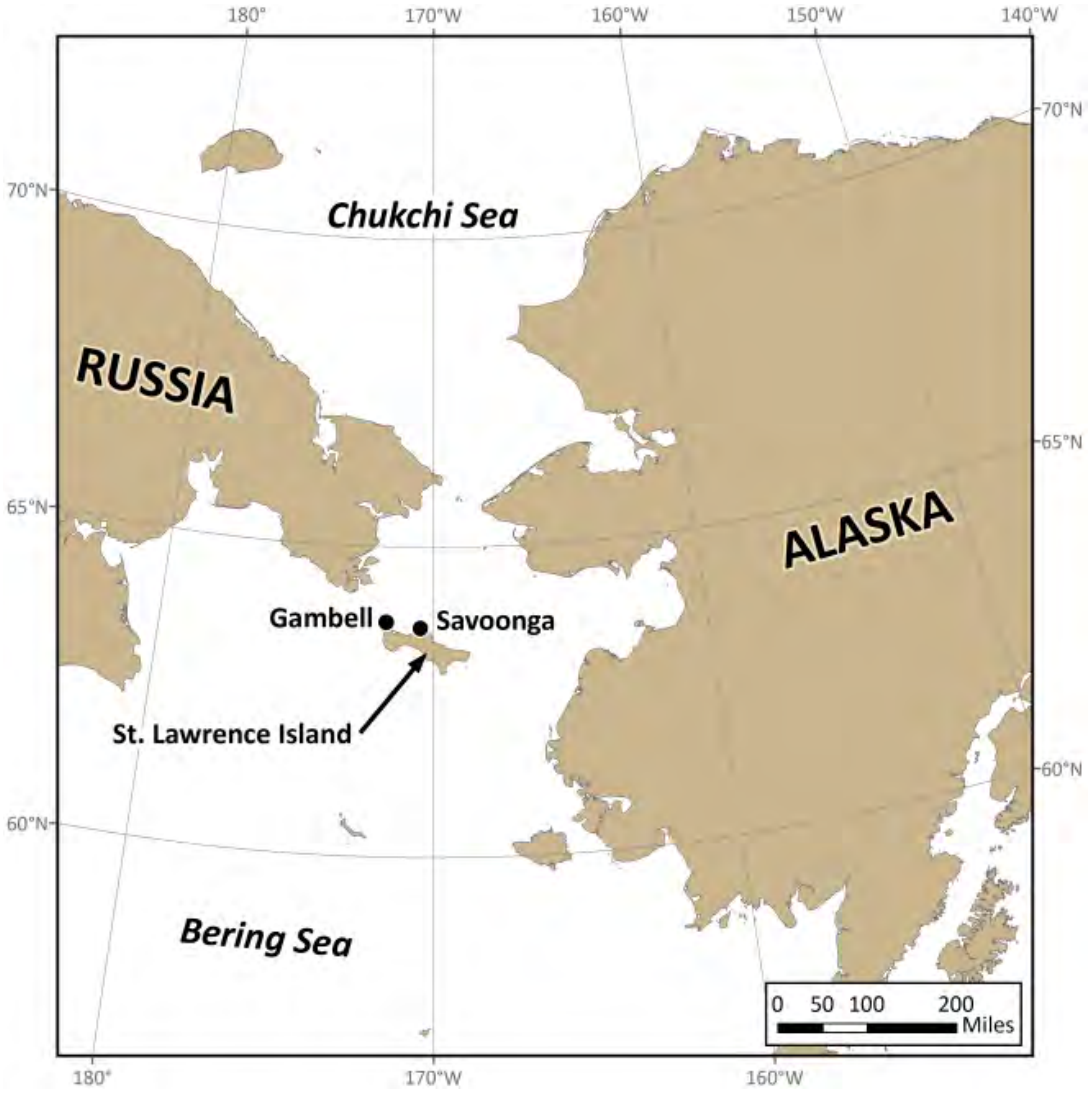

Figure 1. Walrus sample collection locations.

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.

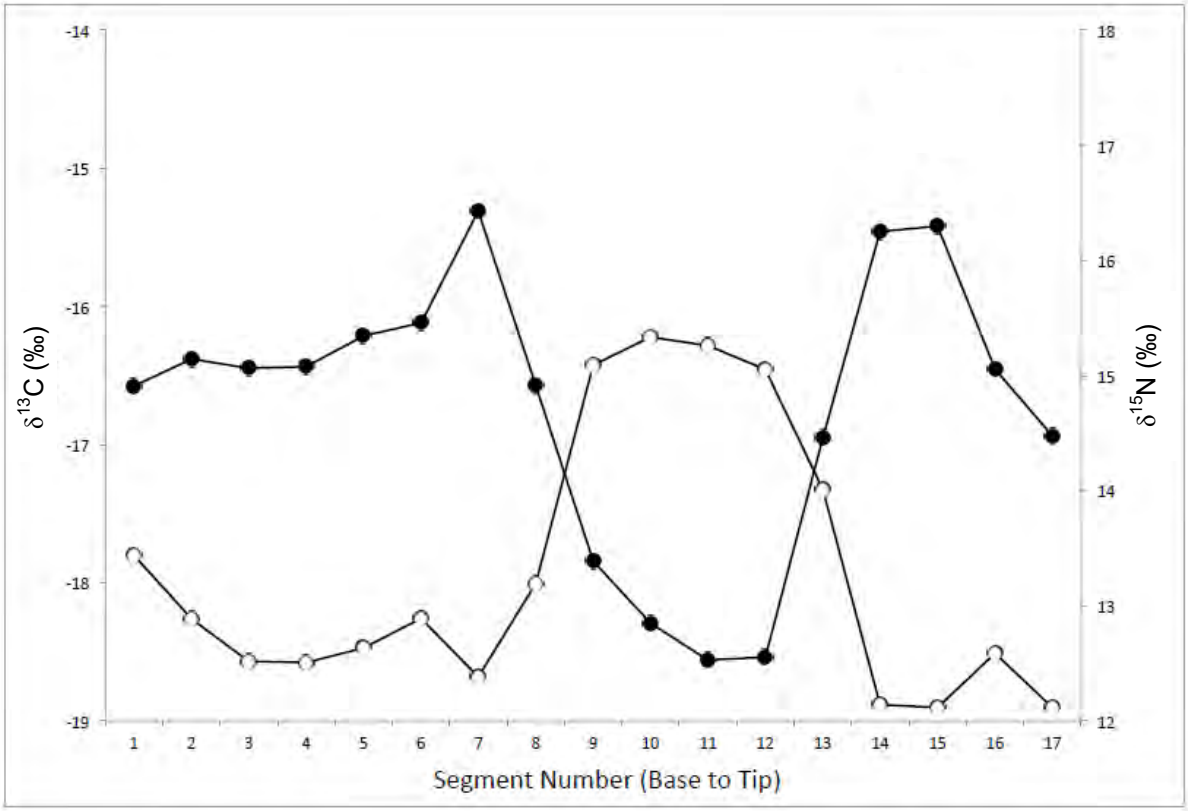

Figure 2. Stable carbon ($\delta^{13}C$, closed circles) and nitrogen ($\delta^{15}N$, open circles) values of a whisker from an 11-yr-old female walrus (G12-0086) harvested near Gambell in May 2012. Figure prepared by Seth Newsome.

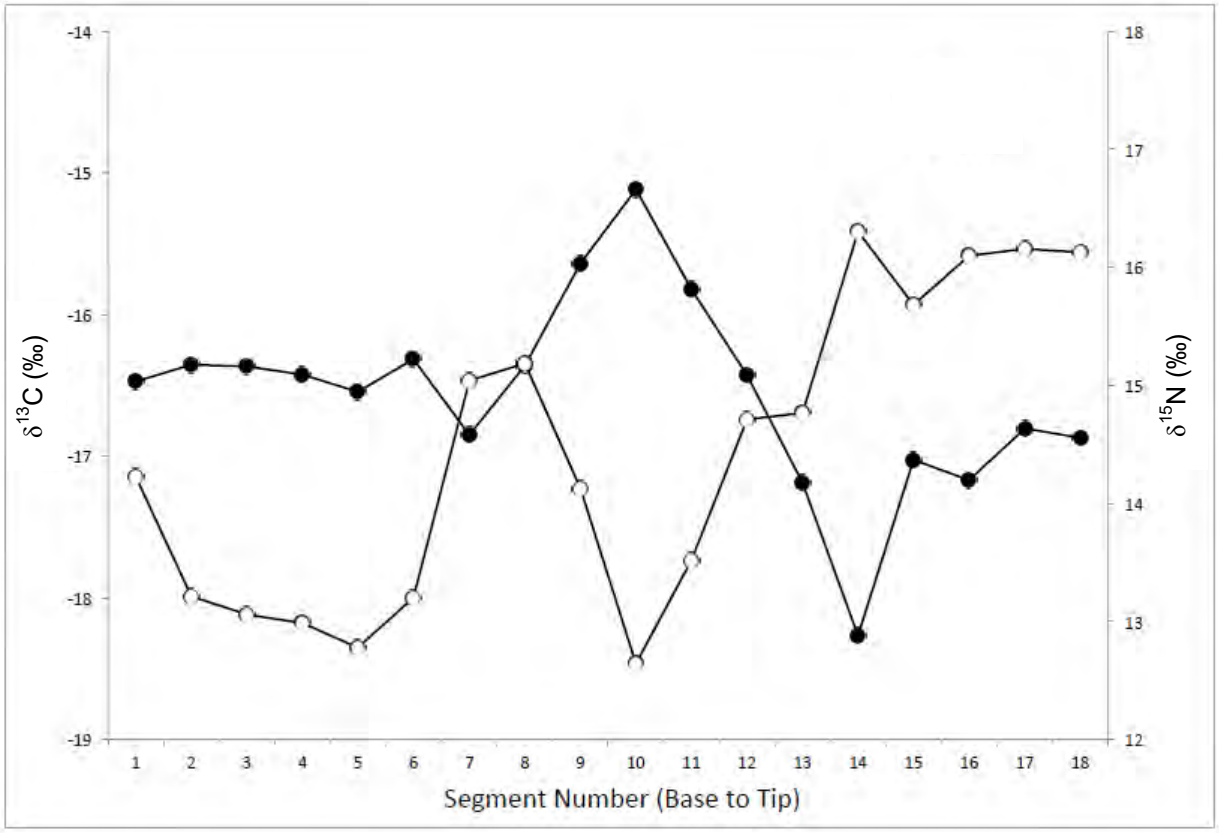

Figure 3. Stable carbon ($\delta^{13}C$, closed circles) and nitrogen ($\delta^{15}N$, open circles) values of a whisker from a 10-yr-old female walrus (G12-0088) harvested near Gambell in May 2012. Figure prepared by Seth Newsome.

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.

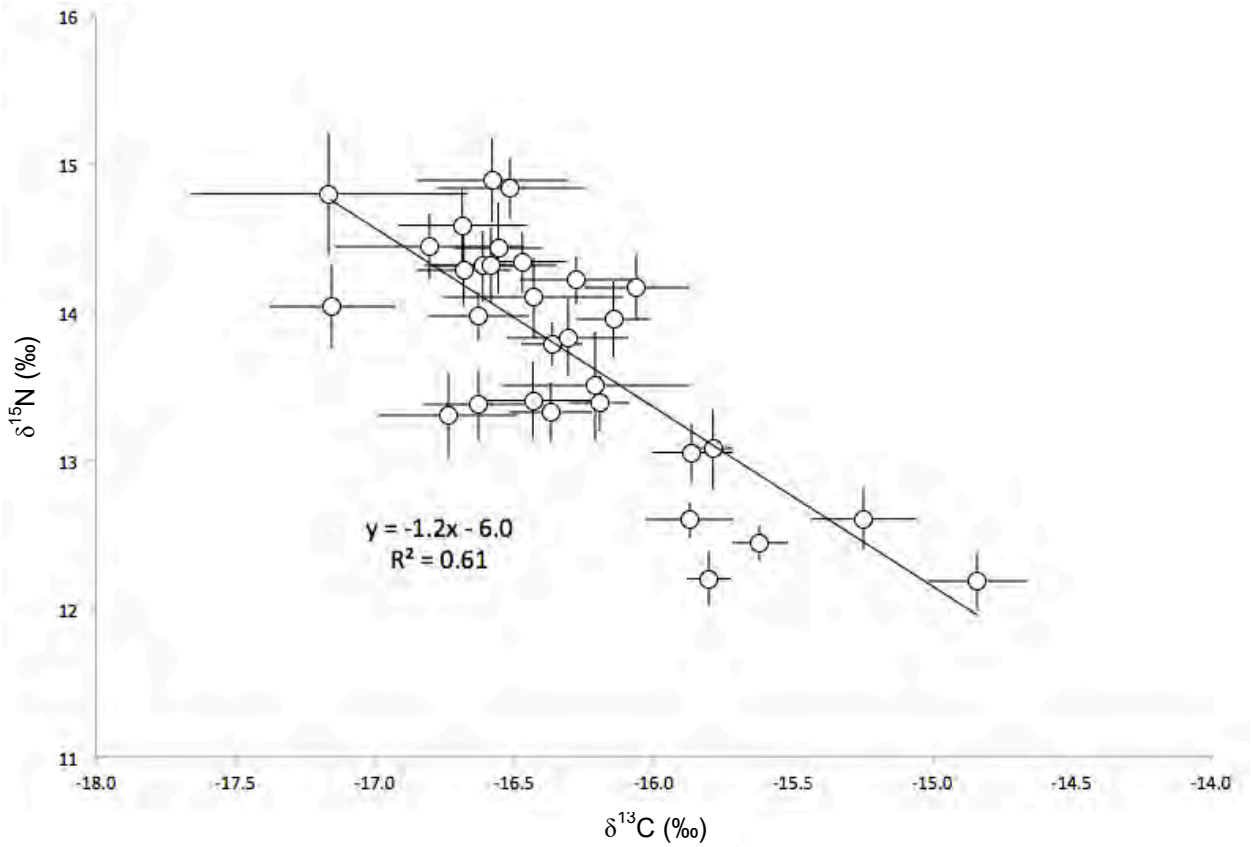

Figure 4. Mean stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) values of whiskers of 28 walruses harvested in May 2012 near Saint Lawrence Island and three near Hooper Bay. Error bars are standard error. Figure prepared by Seth Newsome.

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)
∑ 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
∑ 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).

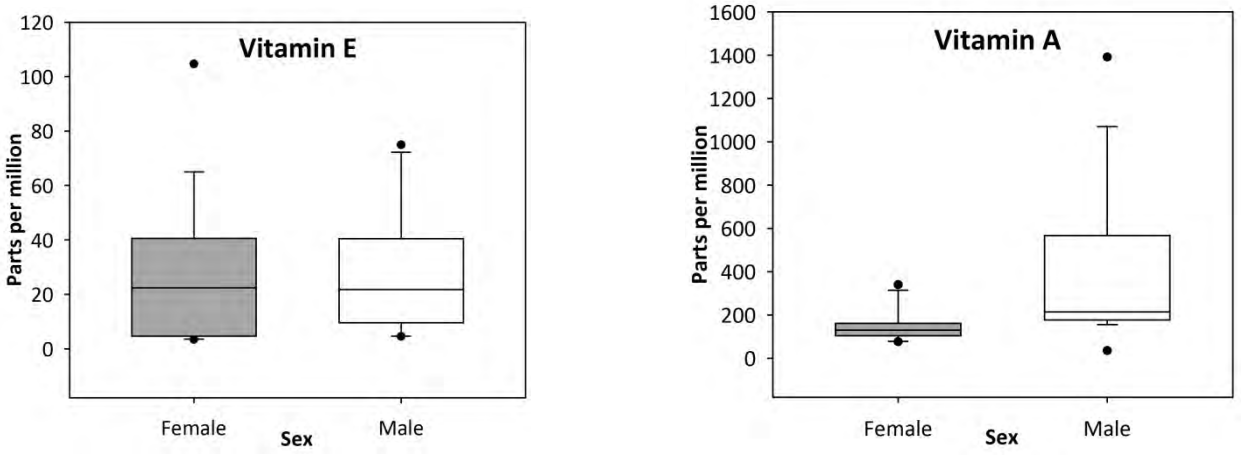

Figure 5. Vitamin E and A concentrations in liver from 37 walruses harvested at Gambell and Savoonga during 2012–2014.

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).

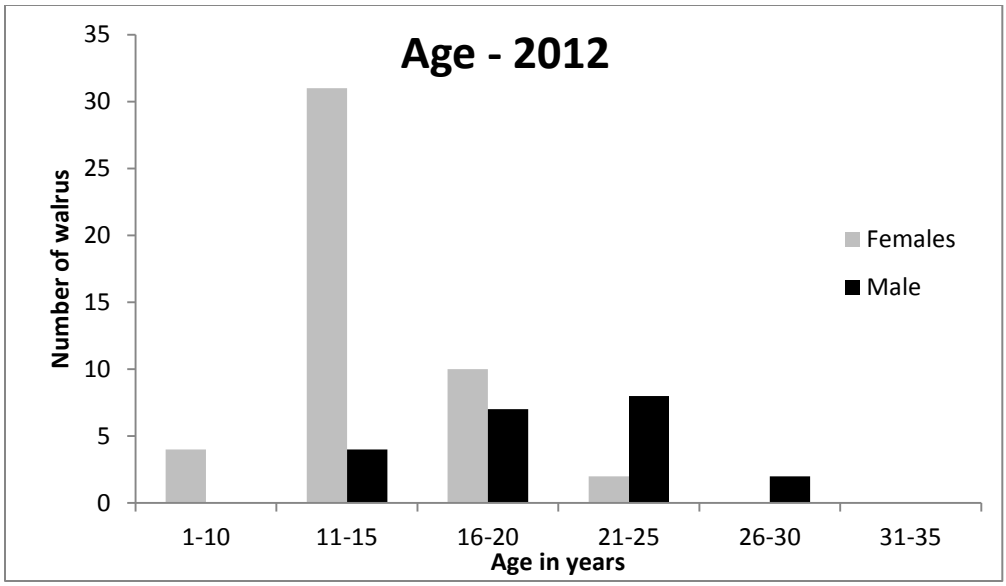

Figure 6. Harvested walruses (n=68) by sex and age category sampled at Gambell and Savoonga in 2012.

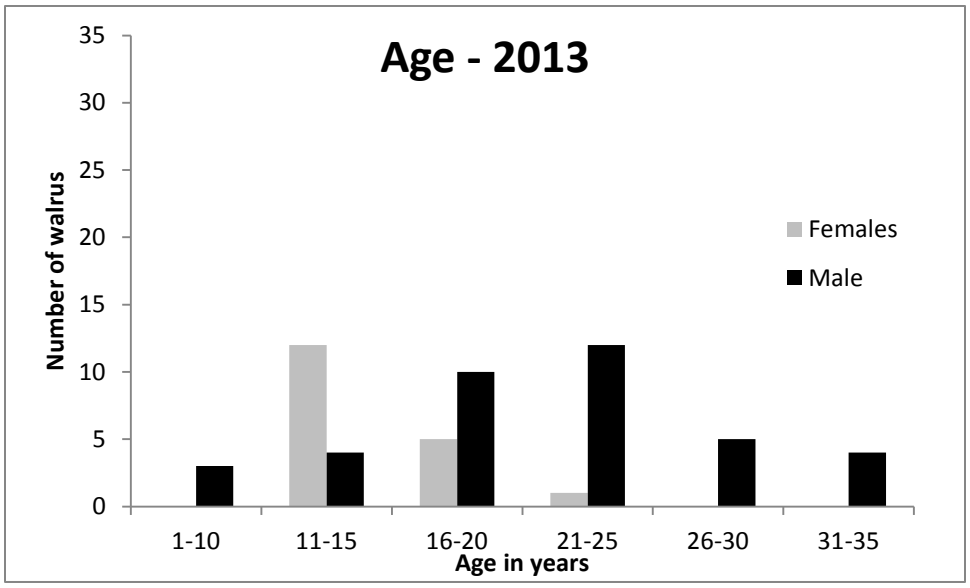

Figure 7. Harvested walruses (n=56) by sex and age category sampled at Gambell and Savoonga in 2013.

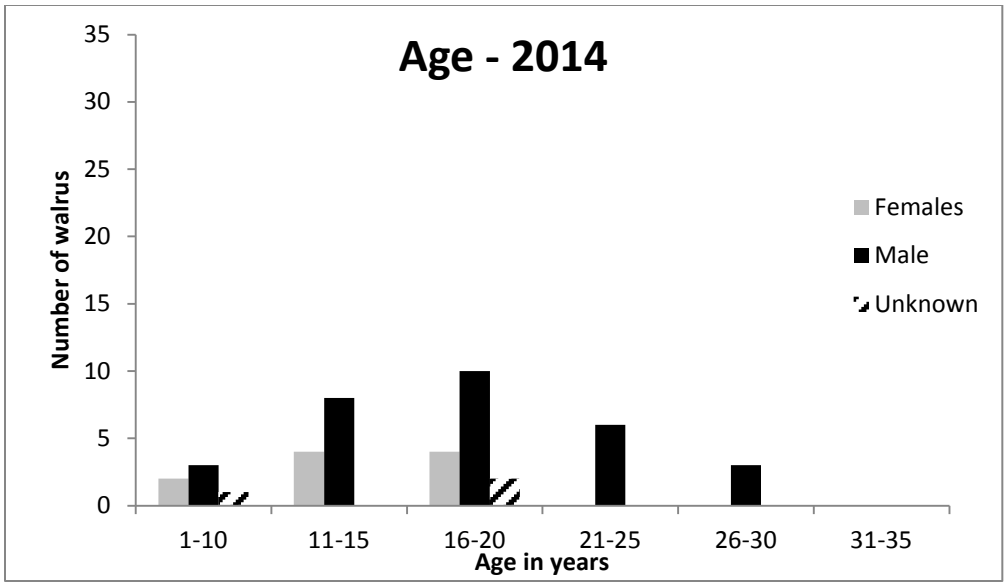

Figure 8. Harvested walruses (n=43) by sex and age category sampled at Gambell and Savoonga in 2014.

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).

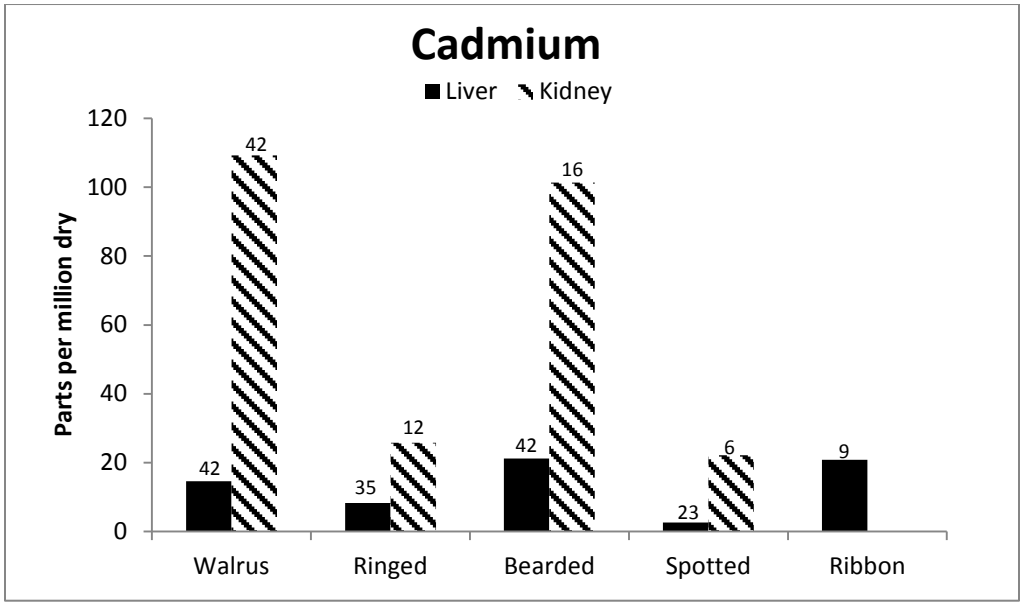

Figure 9. Comparison of cadmium concentrations (µg/g = ppm dry weight) in liver and kidney of walruses and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar. Only one ribbon seal kidney was analyzed and is not presented.

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).

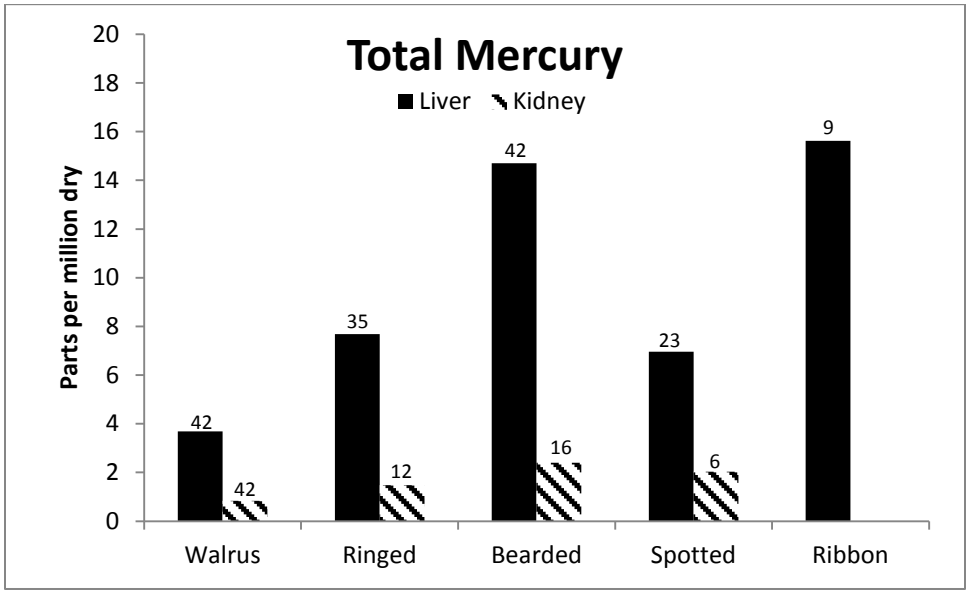

Figure 10. Comparison of total mercury concentrations (µg/g = ppm dry weight) in liver and kidney of walruses and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar.

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).

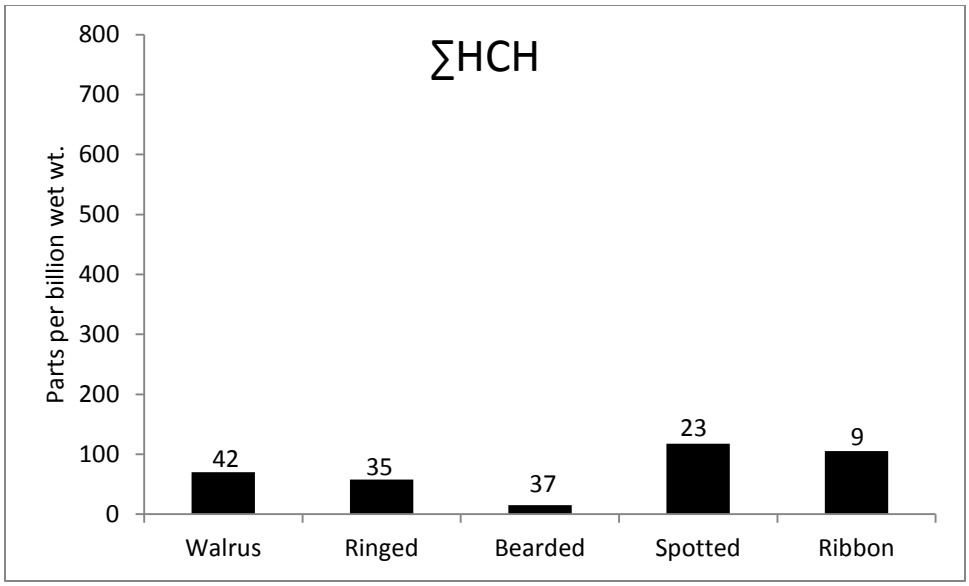

Figure 11. Comparison of the sum of alpha, beta, delta, and gamma hexachlorocyclohexane (ΣHCH) concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003-2007. Sample size appears above each bar.

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.

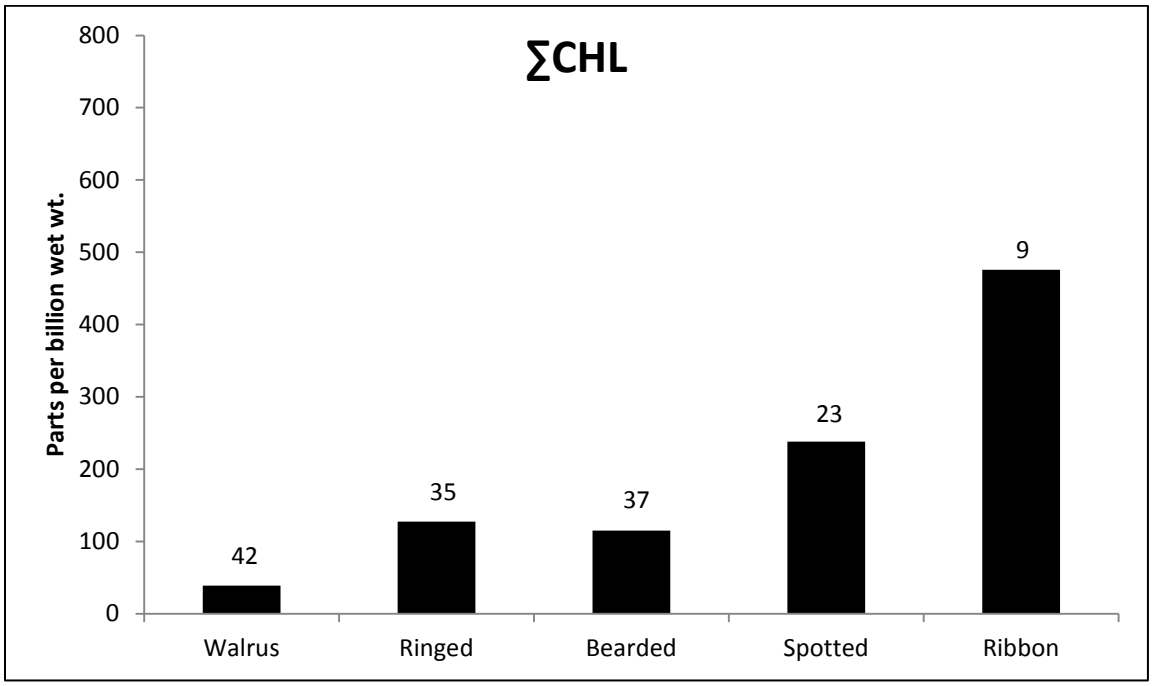

Figure 12. Comparison of the sum of six chlordane compounds concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003-2007. The number of samples analyzed appears above each bar.

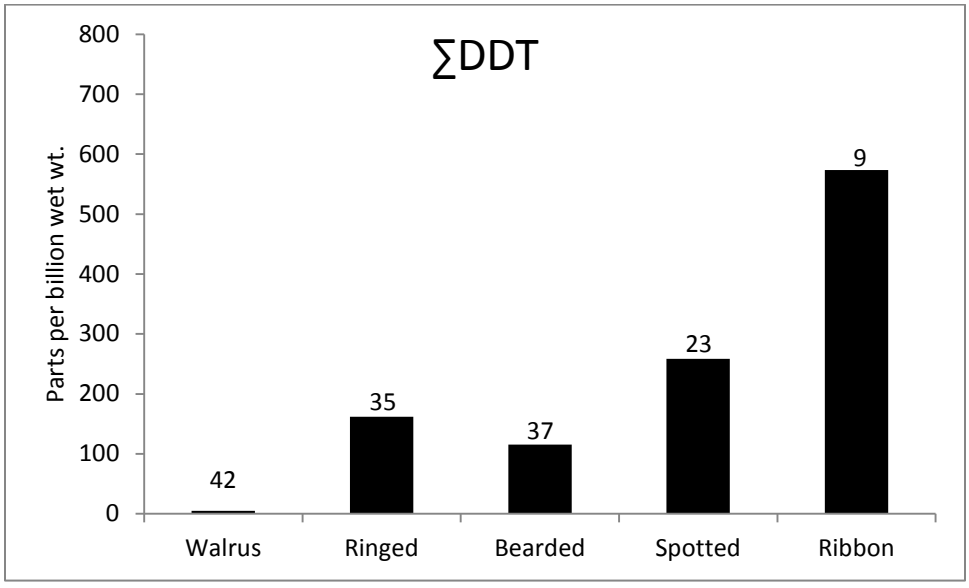

Figure 13. Comparison of the sum of seven dichlorodiphenyltrichloroethane (ΣDDT) concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar.

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.

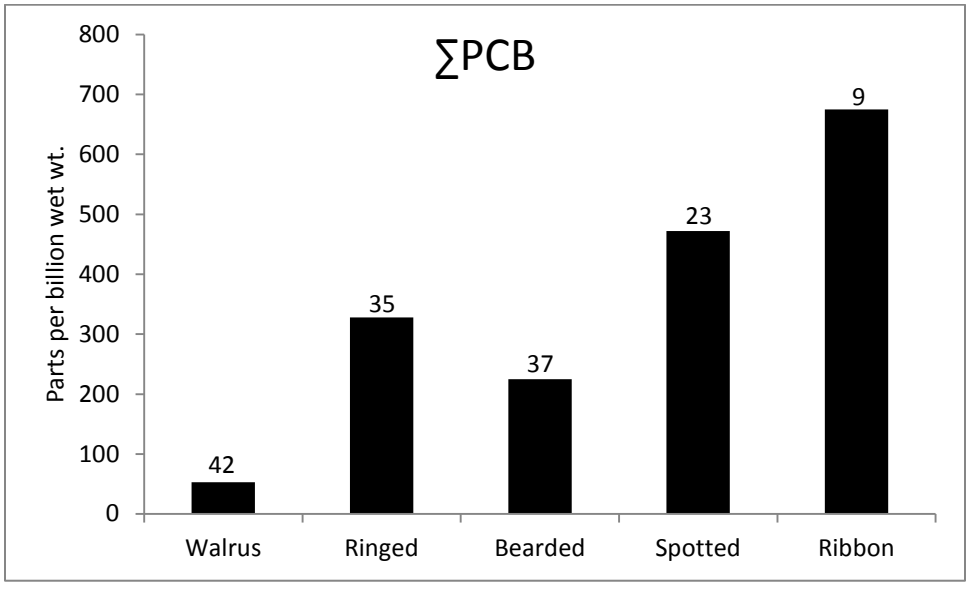

Figure 14. Comparison of the sum of all (81–83) polychlorinated biphenyls (PCB) concentrations (parts per billion wet weight) in blubber of walruses (this study) and seals sampled in the Bering and Chukchi seas in 2003–2007. Sample size appears above each bar.

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).

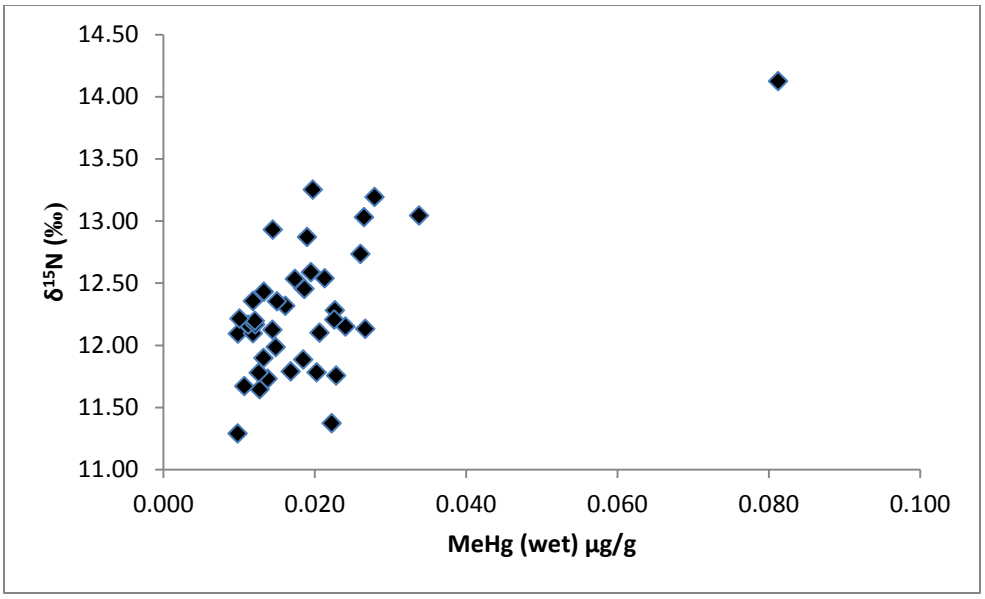

Figure 15. Stable nitrogen isotope values ($\delta^{15}N$) and MeHg in walrus muscle from 40 walruses collected at Gambell and Savoonga between 2012 and 2014.

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.

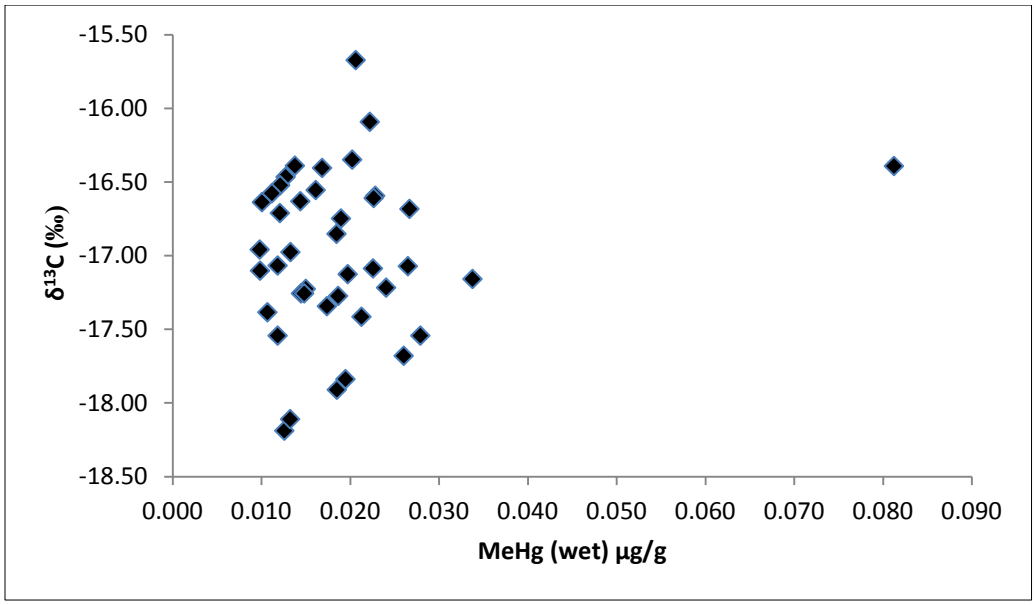

Figure 16. Stable carbon isotope values ($\delta^{13}C$) and MeHg in walrus muscle from 40 walruses collected at Gambell and Savoonga between 2012 and 2014.

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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Ringed, bearded, and spotted seal productivity in Alaska using harvest-based monitoring, 1960s–1980s and 2000s–2010s

Lori Quakenbush, Anna Bryan, Justin Crawford, Louise Biderman, and Ryan Adam

Alaska Department of Fish and Game, Arctic Marine Mammals Program, Fairbanks, Alaska, anna.bryan@alaska.gov

Introduction

Declines in sea ice are predicted to negatively affect ice associated seals (ringed, *Pusa hispida*, bearded, *Erignathus barbatus*, and spotted, *Phoca largha*), important to Alaska Natives for food and materials, by reducing their time to rest, pup, nurse, and molt on sea ice. Concurrent with declines in sea ice are predicted reductions in snow depth used by ringed seals to construct pupping lairs. This is expected to lower productivity and pup survival by providing less protection from weather and predators. Estimates of ice seal abundance cannot be used to detect population trends in Alaska; however, data from the subsistence harvest can be used as an index of population health and status. We compared seal productivity during the 2000s to the 1960s and 1970s, before sea ice decline.

Methods

Subsistence harvested seals were sampled at 12 villages in Alaska along the Bering, Chukchi, and Beaufort sea coasts from 2000–2018. Female reproductive tracts and canine teeth were collected. These data were compared to data previously collected from the same region during 1963–1984. Data are grouped by decade:

Ringed: 1960s (7 yrs), 1970s (9 yrs), 1980s (3 yrs), 2000s (8 yrs), and 2010s (9 yrs)
Bearded: 1960s (6 yrs), 1970s (9 yrs), 2000s (8 yrs), and 2010s (9 yrs)
Spotted: 1960s (4 yrs), 1970s (5 yrs), 2000s (9 yrs), and 2010s (9 yrs)

Age of maturity

- Seals that ovulated at least once were classified as mature.
- Average age of maturity was estimated as the age at which 50% of females were mature (DeMaster 1978) using a probit regression (PROC PROBIT).

Villages where harvested seals were sampled (2000–2018).

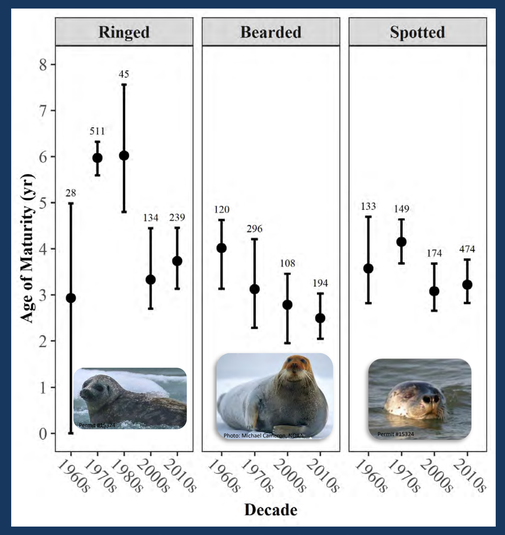
Pregnancy rate

- Pregnancy rate was defined as the proportion of mature females that were pregnant in the year of harvest. If a corpora lutea was present but no fetus was evident by November 1st, the seal was considered not pregnant.
- Differences in average pregnancy rate among time periods were evaluated using a logistic regression model (PROC LOGISTIC).

Proportion of pups harvested

- Proportion of pups (<1 year of age) in the sampled harvest is representative of their presence in the population. If pups do not survive weaning, their presence in the harvest would decrease.
- Age of seals was determined by counting annuli in the dentine or cementum layers of sectioned teeth.
- We evaluated differences in the proportion of pups harvested during each period (PROC FREQ).

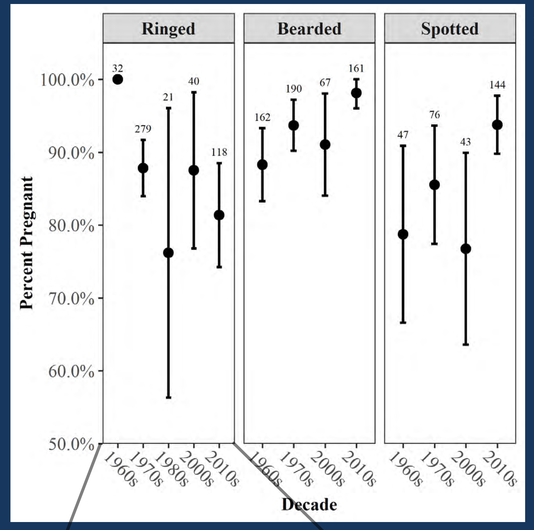
Age of maturity



Average age of maturity by decade. Number of seals analyzed by decade is listed above the 95% confidence limits.

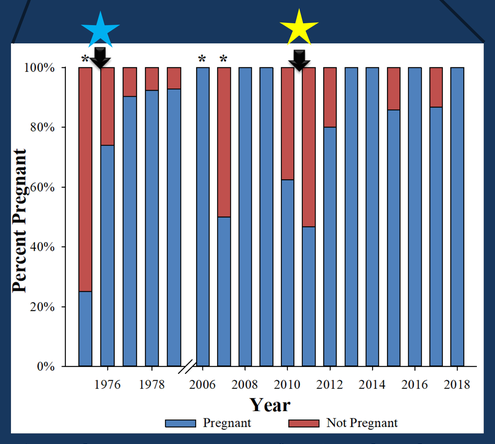
- Seals matured at younger ages since the 2000s than in the 1970s.

Pregnancy rate



Average pregnancy rate by decade. Number of seals analyzed by decade is listed above the 95% confidence limits.

- Pregnancy rate in the 2010s was similar to other decades for ringed seals and was higher for bearded and spotted seals than all other decades.

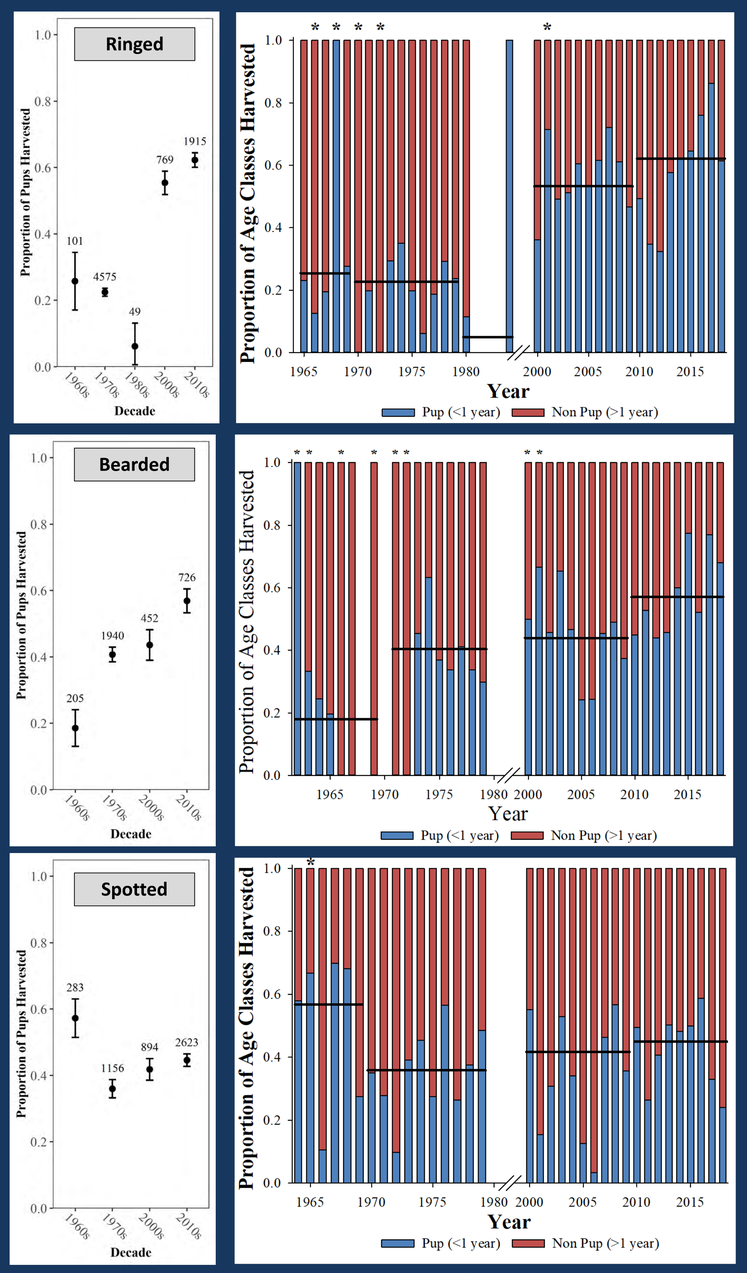


*Annual percent pregnant for ringed seals. *Only 4 mature seals were analyzed in these years. All other years had at least 7 mature seals.*

★ Ringed seal pregnancy rate was low prior to the 1977 regime shift.

★ It was also low in 2010 and 2011 during the Unusual Mortality Event (UME). During these years, reproductive tracts from six mature (13–30 yrs) females were senescent. The thickness of their uterine horns indicated previous reproductive activity, but no corpora lutea or albicans were present.

Proportion of pups harvested



Proportion of pups harvested by decade. Number of seals analyzed by decade is listed above the 95% confidence limits.

*Annual proportions of age classes harvested. *Sample size in these years were <10 seals. All other years had >40 seals harvested. Bold black lines represent the average proportion of pups by decade.*

- The proportion of pups in the sampled harvest remains high for all three seal species in the 2010s.

Conclusions

- **Productivity and pup survival remain high in the 2010s.**
  - Ringed, bearded, and spotted seals are currently maturing at younger ages than in the 1970s.
  - Pregnancy rates remain high at 81% for ringed, 98% for bearded, and 94% for spotted seals.
  - Proportion of pups in the sampled harvest is high.
- **Ringed seals had low reproductive success during the UME (2010 and 2011) but have recovered since then.**
- **Monitoring in future years will be important as environmental conditions continue to change.**

Acknowledgements

This project would not be possible without the willingness of hunters to contribute samples from their harvest, the support of their communities, local governments, and Tribal Councils. We appreciate the support from the North Slope Borough and the Ice Seal Committee. John Burns, Kathy Frost, Lloyd Lowry were instrumental in the early development of this monitoring. We thank Mark Nelson, Gay Sheffield, and our college interns who assisted in field collection and sample processing. Research was funded primarily by NOAA, but has also been supported by NSF, NPRB, and NSB. Research was conducted under NMFS Permits 358-1585, 358-1787, 15324, and 20466.

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Physiological development of locomotor muscles influence diving capacities in free-ranging bearded seals

Tengler, M.[1], Bryan, A.[2], Reichmuth, C.[3], Thometz, N.M.[1]

[1]University of San Francisco
[2]Alaska Department of Fish and Game
[3]University of California Santa Cruz

Seals must store and efficiently use oxygen while diving and foraging at depth. Like all mammals, they store oxygen in their lungs, blood, and muscle, but the physiological properties of skeletal muscles play a disproportionately large role in defining diving capacities. Further, pups are not born with the same physiological abilities as adults, with muscle oxygen stores typically beginning to develop at the onset of independent foraging. Bearded seals (*Erignathus barbatus*) are large ice-dependent Arctic seals. They dive to the seafloor to search for and consume benthic fish and invertebrates, and use sea ice as a platform to rest between foraging bouts. In this study, we examined the physiological development of bearded seal locomotor muscle (longissumus dorsi). Samples were obtained from subsistence harvested bearded seals (n = 37) of different ages collected at Point Hope, Alaska. All muscle samples were analyzed for both myoglobin content and non-bicarbonate buffering capacity. We found clear and progressive ontogenetic trends in skeletal muscle physiology, which indicate that young bearded seals are at a physiological disadvantage in diving and foraging ability when compared to adults. These data provide insight into potentially sensitive life-stages, during which individuals are likely constrained in their behavior. Ultimately, defining age-specific diving capacities and physiological limitations can inform understanding of bearded seal habitat use and aid in predicting behavioral responses to environmental change.

Physiology of locomotor muscle in ringed, bearded, and spotted seals

Tengler, M.[1], Bryan, A.[2], Reichmuth, C.[3,4], Thometz, N.M.[1]

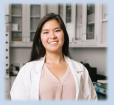
[1]Department of Biology, University of San Francisco
[2]Alaska Department of Fish and Game
[3]Institute of Marine Sciences, University of California Santa Cruz
[4]Alaska SeaLife Center

Marine mammals must store and efficiently use oxygen while diving. In general, locomotor muscles have large oxygen reserves to fuel aerobic metabolism, but they must also be capable of managing the buildup of anaerobic byproducts. Thus, the physiological properties of locomotor skeletal muscles play a large role in defining species-specific limits to diving and foraging. Given that pups are not born with the same physiological capacities as adults, defining both species- and age-specific muscle physiology can provide a more comprehensive understanding of diving constraints and behavioral flexibility. We examined the physiological development of myoglobin content [Mb] and non-bicarbonate buffering capacity (β) in the longissimus dorsi of three Arctic seal species. Samples were obtained from subsistence harvested ringed (*Pusa hispida*; n=11), bearded (*Erignathus barbatus*; n=37), and spotted (*Phoca largha*; n=12) seals. We found adult ringed and spotted seal muscle [Mb] to be similar to other seal species at 6.7±0.3 g Mb · 100 g wet tissue^{-1} and 5.4±0.6 g Mb · 100 g wet tissue^{-1}, respectively. In contrast, adult bearded seals had comparably low muscle [Mb] (4.7±0.4 g Mb · 100 g wet tissue^{-1}), exhibiting muscle physiology more similar to walruses than to other seals. We also documented increasing ontogenetic trends in [Mb] and β for all three species, with bearded seals exhibiting the most subtle developmental pattern. Our data suggest strong links between muscle physiology, ontogeny, and life-history strategies and provide insight into the diving capacities and limitations of data-deficient species.

Muscle Physiology of Ice-Associated Arctic Seals

Mariah Tengler[1], Anna Bryan[2], Colleen Reichmuth[3,4], Jennifer Dearolf[5], Nicole Thometz[1]

[1]University of San Francisco, [2]Alaska Department of Fish and Game, [3]University of California, Santa Cruz, [4]Alaska SeaLife Center, [5]Hendrix College



Corresponding Author:
mtengler@usfca.edu

Background

Arctic ecosystems are rapidly changing, affecting the availability of sea ice habitats as well as the distribution of ice-associated seals and their prey. Here we examine key physiological properties of ringed, spotted, and bearded seals that define their diving and foraging abilities. This information is essential to understanding potential behavioral responses of ice-associated seals to changing environmental conditions.

We examined the development of muscle physiology in three Arctic seal species to gain a more comprehensive understanding of their diving abilities and limitations.

Species of Interest:

Ringed seal

small size (50 – 70kg)
pelagic forager (20 – 100 m)
use land fast-ice

Spotted seal

medium size (60 – 110 kg)
pelagic forager (10 – 50 m)
use pack-ice edge

Bearded seal

large size (250 – 360 kg)
benthic forager (10 – 60 m)
use transition zone

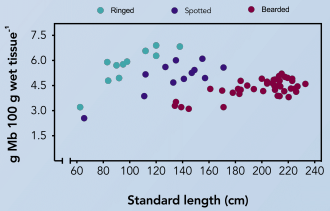
Objectives:

- Measure the aerobic and anaerobic capacities of locomotor muscle
- Assess developmental trends across species
- Evaluate species-specific patterns in muscle physiology

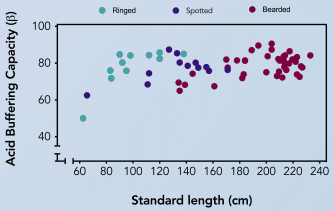
Methods

- Skeletal muscle samples of the longissimus dorsi, a major locomotor muscle, were obtained from ringed (n=11), spotted (n=12), and bearded (n=41) seals harvested for subsistence purposes.
- Samples were analyzed for myoglobin content ([Mb]), non-bicarbonate buffering capacity (β), and fiber-type profiles following the methods of Reynafarje (1963), Castellini and Somero (1981) and Hermanson and Hurley (1990).

Results: Myoglobin content

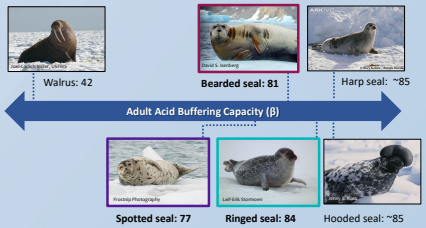


Developmental changes in [Mb] with increasing body size. Factorial ANOVA: Significant differences by species ($F_{(2,52)} = 43.33$, $p < 0.0001$), age-class ($F_{(2,52)} = 28.28$, $p < 0.0001$), and species*sex ($F_{(2,52)} = 4.42$, $p = 0.02$).

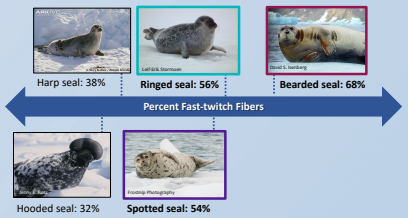


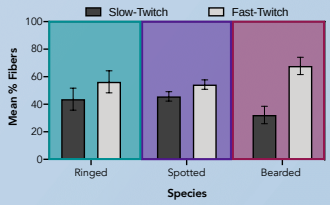
Developmental changes in β (slykes) as a function of body size. Factorial ANOVA: Significant effect of age-class ($F_{(2,52)} = 10.58$, $p = 0.0001$).

Results: Acid buffering capacity



Preliminary Results: Fiber-Typing





Average percentages of slow- and fast-twitch muscle fibers in the longissimus dorsi of sub-adult and adult ringed, spotted, and bearded seals. Error bars are ± 1 s.d.

Summary

- [Mb] and β increase across development for all three species with bearded seals exhibiting the most subtle developmental trend
- Differences in the development of muscle physiology may be driven by timing of key life-history events, such as timing of first entry into water
- Variability in adult [Mb] and fiber-type profiles across species are likely related to species-specific diving strategies (e.g. benthic vs mid-water foraging)

Samples were obtained under NMFS Permits 15324 and 20466 with corresponding LOAs from the NMFS West Coast Region.

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Arctic - Fishes and Fish Habitats

Sizes of Fishes Consumed by Ice Seals in the Alaskan Arctic

Kelly Walker
University of Alaska Fairbanks, klwalker2@alaska.edu
Brenda Norcross
University of Alaska Fairbanks, bnorcross@alaska.edu

Climate change effects such as changing sea ice cover in the Arctic will affect the distributions of Arctic fishes and ice seals. A large component of bearded (*Erignathus barbatus*), spotted (*Phoca largha*) and ringed (*Pusa hispida*) seal diets is fish, making it important to fully understand diets of these top-level predators. Otoliths are often the only remains of fish that can be used to identify species of fishes consumed, making it impossible to physically measure and weigh individual consumed fish. Otolith length – fish length and fish length – fish weight relationships were used in this project to estimate sizes of fish consumed by bearded, spotted and ringed seals. Stomachs contents of seals harvested by northern Alaskan communities were processed by the Alaska Department of Fish and Game. Otoliths from these stomachs were used in this project to estimate lengths of fishes consumed by each seal using otolith length – fish length relationships previously developed in this project. Sizes of fish consumed were compared by seal species, age class (pup, subadult and adult), harvest location, and sex. For bearded seals, seal harvest location was the primary factor influencing sizes of consumed fishes. Bearded seals harvested near Little Diomede consumed larger Arctic Cod, Arctic Staghorn Sculpin and Shorthorn Sculpin than those harvested near Barrow. Seal age class and seal harvest location were the primary drivers influencing spotted seal fish consumption. Adult spotted seals consumed larger Saffron Cod than pups, and spotted seals harvested near Shishmaref consumed larger Arctic Cod than those harvested near Little Diomede. Seal age class was the only factor influencing fish size in ringed seal diets. Adult ringed seals consumed larger Saffron Cod than pups. With this new information, fish weight can be calculated from fish length, which will make it possible to investigate energetic requirements of ice seals. A thorough understanding of ice seal diets is needed to better understand how flexible their nutritional requirements will be in the face of increasing climate change in the Alaskan Arctic.

ESTIMATING SIZES OF FISH CONSUMED BY ICE SEALS USING OTOLITH LENGTH – FISH LENGTH RELATIONSHIPS

By Kelly Walker, B.S.

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of

Masters of Science

in

Fisheries

University of Alaska Fairbanks

December 2017

APPROVED:

Brenda L. Norcross, Committee Chair

Randy Brown, Committee Member

Andres Lopez, Committee Member

Lori Quakenbush, Committee Member

Milo Adkison, Chair

Department of Fisheries

Brad Moran, Dean

College of Fisheries and Ocean Sciences

Michael Castellini,

Dean of the Graduate School

Abstract

Arctic fishes and ice seals are key components of the Alaskan Arctic ecosystem. Bearded (*Erignathus barbatus*), spotted (*Phoca largha*) and ringed (*Pusa hispida*) seals are consumers of Arctic marine fishes. Little is known about the sizes of fish that ice seals consume because prey items are digested quickly once exposed to stomach acids. Otoliths, fish ear bones, are often the only parts of a fish that remain in a seal stomach. Otolith length relates directly to fish length, making size estimations of consumed fish possible for piscivore diet studies. Otoliths were measured from fishes collected from cruises in the Beaufort and Chukchi seas during 2009 – 2014. Otolith length – fish length and fish length – fish weight relationships were developed for 11 Arctic marine fish species that are commonly consumed by ice seals in Alaska. Otoliths from seal stomachs provided by subsistence hunters to the Alaska Department of Fish and Game were identified to species level and measured for total length. A mixed effects model was used to determine how the variables of seal species, harvest location, seal age class and sex influenced the sizes of fish consumed. Harvest location and seal age class were the primary factors that affected fish size in ice seal stomachs. Estimating length and weights of fishes consumed by ice seals will help further diet and energetics studies that have not previously been possible in the Alaskan Arctic.

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Acknowledgements

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General Introduction

Arctic fishes provide an essential connection between lower tropic levels (Craig 1984) and sea ice-associated seals, and seals provide major links between humans and the Arctic marine ecosystem (Bluhm and Gradinger 2008). With the Arctic rapidly changing, it is important to understand the current status of important trophic linkages to determine how future ecosystem changes might affect these food webs (Wassmann et al. 2011). Fishes are a major component of seal diets and are likely to be strongly affected by the changing climate. The purpose of this study was to develop otolith length – fish length relationships of fish species commonly present in ice seal diets and to apply these relationships to otoliths found in ice seal stomachs to estimate fish sizes consumed by ice seals.

Four species of seals in Alaska, bearded (*Erignathus barbatus*), spotted (*Phoca largha*), ringed (*Pusa hispida*), and ribbon (*Histriophoca fasciata*) seals are called ice seals because they depend on sea ice for some part of their life history. This study focuses on three of these species; ribbon seals were not included as they are relatively rare, samples are less available, and stomachs are mostly empty (Frost and Lowry 1980). All three seal species prey upon fishes to varying degrees in the Bering, Chukchi and Beaufort seas (Dehn et al. 2007). Bearded seals are mostly benthic feeders, including benthic fish prey such as sculpins and flatfishes, whereas both spotted and ringed seals are mostly pelagic feeders; this results in some prey species differences among these piscivores.

The fish diets of ice seals can be described using fish ear bones, called otoliths. Otoliths are calcified structures that are typically the last part of the fish to be digested and can be passed through the intestinal system minimally digested (Murie and Lavigne 1986). Otoliths have species-specific shapes that allow identification of fishes that have been consumed. Otolith length (OL) is strongly related to fish length (FL), making it possible to estimate length of the fish consumed (Campana 1990). In turn, fish length is strongly related to fish weight (FW) making it also possible to estimate weights of consumed fishes. Developing relationships

between OL and FL and FL and FW can aid in developing a better understanding of quantitative diet composition of Arctic piscivores, such as ice seals.

Fish species differ in their energetic values. Fishes with higher energy content are considered nutritionally more beneficial to an ice seal. For example, Capelin (*Mallotus villosus*), Pacific Sand Lance (*Ammodytes hexapterus*), and Arctic Cod (*Boreogadus saida*) have especially high energetic values (Van Pelt et al. 1997, Harter et al. 2013) and may thus be more valuable to an ice seal than species with lower energetic content. At the same time, energy intake by a predator is also determined by prey size, because a larger fish generally has higher total energetic value than a smaller fish. By applying OLFL relationships, the identity and size of fish can be determined; using known energy content of Arctic fishes, it is then possible to determine how much energy fish contribute to ice seal diet.

Multiple factors likely drive the composition and size structure of prey fishes in ice seal diets. On the prey side, one of these factors is that fish size can vary by region. For example, sizes of fish in the Beaufort and Chukchi seas are different from their counterparts in the Bering Sea (Helser et al. 2015). This indicates that OLFL relationships also could differ by region. This regionally specific feature makes it important to examine OLFL relationships for species of interest among study regions, to ensure proper size estimation of fish prey species. In addition, it is possible that OLFL relationships may change in the environment as energy allocation to various body functions such as growth, respiration, reproduction, etc. change with warming water temperatures (Mosegaard et al. 1988). OLFL relationships were developed for some Arctic fishes in the 1970s (Frost and Lowry 1981), but no more recent or regionally-resolved OLFL relationships are available for fish species in the Alaskan Arctic. Given the dramatic changes in the Arctic environment of decreasing sea ice cover and increasing heat budget over the past 40 years (Stroeve et al. 2007, Frey et al. 2015, Wood et al. 2015), the results of this study are especially timely. On the predator side, seal age and location of foraging area are factors that can influence the composition and size structure of an ice seal's fish diet. Seal age determines a seal's ability to dive deeper and longer (Noren et al. 2005). The longer a seal can remain underwater, the larger the opportunity there is to encounter larger numbers of fishes to capture

and consume. Foraging location could influence sizes of fishes consumed, if fish sizes or population structures indeed differ among regions.

The goal of this study was to provide a better understanding of ice seals and their fish prey under current conditions in the Arctic. The specific objectives of this study were to 1) provide OLFL and FLFW relationships for 11 fish species in the Chukchi and Beaufort seas, 2) determine sizes of fishes consumed by three common ice seal species in the Alaskan Arctic by applying these relationships to otoliths found in ice seal stomachs, and 3) determine differences in fish prey size among and within seal species by harvest location, age and sex.

Chapter 1

Otolith length – fish length and fish length – fish weight relationships for 11 Pacific Arctic marine fish species

Abstract

Arctic fishes are a key component of the Arctic ecosystem. Determining sizes of fish consumed by marine predators is difficult because otoliths are often the only part of the fish left after digestion. By developing species-specific otolith to body morphometric relationships for Arctic marine fishes, length and weight can be estimated for fish eaten by marine piscivores. Fishes were collected during ice free months in the Beaufort and Chukchi seas during 2009 – 2014, and the most prevalent species captured that were also those most often eaten by ice associated seals were chosen for analysis. Otoliths from 11 fish species from seven families were measured. Strong linear relationships between otolith length and fish total length were observed in all species examined. Coefficient of determination values over 0.80 were recorded for nine of the species examined. All 11 species had very strong fish length – fish weight relationships. The development of otolith length – fish length and fish length – fish weight relationships for key Arctic fish species is an important advance allowing for bioenergetics studies of marine piscivores, providing insight into prey requirements of predators and predator-prey interactions in the Arctic.

Introduction

Fishes are an important part of the Arctic food web (Craig 1984, Bluhm and Gradinger 2008, Eriksen et al. 2012) representing essential connections between upper trophic level predators, such as seals, and lower trophic level species, such as benthic and pelagic invertebrates (Cooper et al. 2009, Majewski et al. 2013). Birds and marine mammals prey on a mixture of demersal and pelagic fishes. There are 45 known families of fishes present in the Pacific Arctic (Mecklenburg et al. 2011). From these 45 families, species such as Capelin (*Mallotus villosus*), Arctic Cod (*Boreogadus saida*), Saffron Cod (*Eleginus gracilis*), sculpins,

eelpouts, eelblennies, Pacific Sand Lance (*Ammodytes hexapterus*) and flatfishes are commonly consumed by marine piscivores.

Fish otoliths or ear bones have been used to reconstruct the diets of fish predators. Otoliths are calcium carbonate structures that are more resistant to digestion than soft tissues. Of the three pairs of otoliths found in fishes, the sagittal pair is the most widely used for aging and species identification purposes (Campana and Neilson 1985). Otolith shape varies by species (Harvey et al. 2000). This trait, and their resistance to digestion, makes them useful in identification of consumed prey (Harvey et al. 2000). Otoliths found in scat and stomachs can be identified to species level if they have not been too eroded during digestion. If otolith length and fish length relationships have been established they can also be used to determine sizes of fishes eaten. Otolith length and fish length are strongly correlated (Campana 1990) and this relationship is species-specific (Campana 2005, Harvey et al. 2000). Once a species-specific relationship has been determined, it is possible to estimate fish length using otolith length (Frost and Lowry 1981, Lidster et al. 1994, Ross et al. 2005). Fish weight is also positively related to fish length, making it possible to estimate fish weight when otolith length can be measured (Gamboa 1991, Harvey et al. 2000) although the weight relationship tends to be more variable.

Otolith length – fish length (OLFL) and fish length – fish weight (FLFW) relationships have been used to reconstruct diets of marine mammals (Frost and Lowry 1981), birds (Ross et al. 2005) and even other fish (Jackson et al. 2000). Otolith length – fish weight relationships have been used to estimate biomass of fishes consumed by California sea lions (*Zalophus californianus*) (Gamboa 1991). Several marine mammal – fisheries interactions were studied using OLFL relationships including grey seals (*Halichoerus grypus*) preference for commercial sizes of Silver Hake (*Merluccius bilinearis*) and Atlantic Herring (*Clupea harengus*) (Bowen et al. 1993) and fur seals, sea lions, spotted seals and Walleye Pollock (*Gadus chalcogrammus*) in the Bering Sea (Lowry et al. 1986). OLFL relationships have added to the understanding of diet of several piscivorous whale species such as Baird's beaked whales (*Berardius bairdii*) and beluga whales (*Delphinapterus leucas*) (Walker et al. 2002, Quakenbush et al. 2015). OLFL relationships were used to estimate biomass of fishes consumed by American white pelicans (*Pelecanus erythrorhynchos*) and double crested cormorants (*Phalacrocorax auritus*) on the

North Platte River and Great Lakes region (Derby and Lovvorn 1997, Ross et al. 2005). To determine sizes of forage fishes consumed by Lancetfish (*Alepisaurus ferox*), Swordfish (*Xiphias gladius*) and Yellowfin Tuna (*Thunnus albacores*), OLFL relationships were developed for several species of forage fishes, sardines and myctophid species, in the Indian Ocean (Potier et al. 2007). Salmonid diet studies in Norway used OLFL relationships to determine sizes of sticklebacks consumed by different sizes of adult Arctic Char (*Salvelinus alpinus*) and Brown Trout (*Salmo trutta*) (L'Abée-Lund et al. 1992).

OLFL relationships have been found to vary by region. For example, Red Snapper (*Etelis carbunculus*) OLFL relationships differ among populations in the Pacific Ocean (i.e., Hawaii, Vanuatu, Fiji and French Polynesia; (Smith 1992), as do Haddock (*Melanogrammus aeglefinus*) on Georges Bank and Atlantic Herring in the Atlantic Ocean (Munk et al. 1991, Begg and Brown 2000). Therefore, it is reasonable to expect that the OLFL for Pacific Arctic species may be different among the Bering, Chukchi, and Beaufort seas due to known differences in water temperature and available nutrients (Carmack and Macdonald 2002, Helser et al. 2015). These regional differences reinforce the need to develop OLFL relationships for fish species in the Pacific Arctic to best understand marine predator diet and energetics.

The Chukchi and Beaufort seas compose the Pacific portion of the Arctic Ocean. These two seas have different oceanographic and biological characteristics (Macdonald et al. 1987, Carmack and Macdonald 2002, Crawford et al. 2012). The Chukchi Sea receives high amounts of nutrients from the Bering Sea through Bering Strait and is considered to be more productive than the Beaufort Sea (Macdonald et al. 1987, Carmack and McLaughlin 2011). These differences in productivity suggest a potential for greater growth and size of fishes present in the Chukchi Sea than in the Beaufort Sea.

In this study, I determined OLFL and FLFW relationships for 11 fish important as prey to marine mammals, birds and fish in the Chukchi and Beaufort seas to better understand marine predator diet in the Pacific Arctic.

Methods

Fishes were collected during ice free months (August and September) in the Chukchi and Beaufort seas 2009 – 2014 (Figure 1.1). In the Chukchi Sea, fishes were collected from stations in water 17 – 80 m in depth during 2009–2012. Stations were sampled from the Bering Strait north to latitude 75° N. In the Beaufort Sea, fishes were collected during four research cruises during 2011 – 2014 at on-shelf and off-shelf stations in water depths from 13 to 1,000 m. In 2011, a total of 81 stations were sampled between 155.25 and 145.09° W. Cruises in 2012, 2013 and 2014 sampled a total of 53 stations between longitudes 151.50° W and 137.00° W. These cruises covered the area from Point Barrow to the Mackenzie River Delta in Canada.

Specimens were captured using four types of bottom trawls: 1) the plumb staff beam trawl had 7 mm mesh in the body and 4 mm mesh in the codend, 2) the Canadian beam trawl had a 10 mm mesh in the body and a 6 mm mesh in the codend, 3) the otter trawl had 38 mm mesh in the body and 19 mm mesh in the codend, and 4) the NOAA 83-112 eastern trawl had 102 mm mesh in the body and 32 mm in the codend. Captured fishes were euthanized with MS-222 using a protocol approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee (IACUC protocol #07-047). Fishes were identified to species level using the methods of Mecklenburg et al. (2002). After identification, fishes were frozen in seawater and shipped to the UAF Fisheries Oceanography Laboratory for further processing.

Eleven fish species were selected for OL vs. FL analysis based on their prevalence in trawl catches and their frequency of occurrence in ice seal stomachs sampled by the Alaska Department of Fish and Game, Arctic Marine Mammal Program: Capelin, Arctic Cod, Saffron Cod, Arctic Staghorn Sculpin (*Gymnocanthus tricuspis*), Shorthorn Sculpin (*Myoxocephalus scorpius*), Canadian Eelpout (*Lycodes polaris*), Stout Eelblenny (*Anisarchus medius*), Slender Eelblenny (*Lumpenus fabricii*), Pacific Sand Lance, Bering Flounder (*Hippoglossoides robustus*) and Yellowfin Sole (*Limanda aspera*). In the lab, all fish were weighed to the nearest 0.01 g and measured for total length to the nearest 1.0 mm. Total length was measured from the tip of the snout to the end of the longest lobe of the caudal fin. A total of 1,396 fish were measured. For each species, 20 fish from each 10 mm size class were randomly selected from the Beaufort Sea

samples and from the Chukchi Sea samples, separately, for otolith measurement. Both sagittal otoliths were removed, cleaned, dried and stored in vials. Otoliths were photographed using a Leica DFC295 camera mounted onto a Leica M165C dissecting scope. One otolith from each fish was measured from rostrum to postrostrum to the nearest 0.0001 mm using a Leica imaging software program. The software was calibrated before each session of measurements.

Least squares linear regressions (linear regressions) were used to describe the relationship between OL and FL (Frost and Lowry 1981). Linear regressions were calculated using SigmaPlot Version 12.5 (Systat Software, San Jose, CA). The following linear equation was used to explain the relationship between otolith length and fish length: $y = a + bx$, where y is fish length, a is intercept, b is slope and x is otolith length

To determine if OLFL relationships within a species were different between the Chukchi and Beaufort seas, a least squares linear regression was calculated for each species for both seas and slope and intercept coefficients were compared using a two sample t-test as described by Zar (1999). If the test of slope coefficients were not significant ($\alpha > 0.05$), the intercept coefficients were then tested. If both slope and intercept coefficients were similar, a single linear regression equation was used for the species. If at least one coefficient was significantly different, separate linear regression equations were used for each sea.

Several methods were used to determine if a linear regression provided sufficient estimation precision for use in fish length estimation. Confidence intervals (CI) were used to interpret how close observed data points were to the mean (Montgomery et al. 2012). Prediction intervals identified the expected range of additional (i.e., larger or smaller) observations based on the current data's relationship with the mean. Values of r^2 determined how much variation the linear regression explained. Data points that were greater than or less than three studentized residuals from the mean were considered outliers and omitted from further analysis to further strengthen the relationship. The remaining data were used in all analyses.

Weight – length relationships were also calculated for each species. A power function was used to fit the data and show the curvilinear relationship between FW and FL. Equations

were developed for all 11 species using data from all specimens with available weight information (n = 11,057) from the UAF Fisheries Oceanography Laboratory specimen collection, including, but not limited to, the specimens used for developing the OLFL relationships. These specimens were chosen from the same cruises and years to accurately reflect FLFW relationships in those years. The standard fisheries weight regression (Ricker 1975) was used: $W = aL^b$, where W is fish weight, a is intercept, L is fish total length and b is the regression coefficient.

Results

Otolith shape was unique for each species. Capelin otoliths were round with a pronounced rostrum and were always semitransparent (Figure 1.3a). Otoliths of both cod species were elongate in shape (Figures 1.3b, c). Both sculpin species had elongated otoliths with a distinct rostrum (Figures 1.3d, e). Canadian Eelpout (Figure 1.3f) and both eelblennies (Figures 1.3g, h) had smaller, round otoliths. Pacific Sand Lance otoliths were almond shaped with a small rostrum (Figure 1.3i). Flatfish species had circular otoliths that were large for their body size (Figures 1.3j, k). Capelin had the shortest otolith length range (0.562-1.241 mm) and Bering Flounder had the longest (0.292-4.829 mm).

Each species had a unique OLFL and FLFW relationship. For OLFL, nine of the 11 species had r^2 values > 0.85 (Figure 1.4). The other two species, Capelin and Pacific Sand Lance, had lower r^2 values (0.66 and 0.76), potentially due to smaller sample sizes for these species (Table 1.1). However, Bering Flounder and Yellowfin Sole, the two flatfish species, also had small sample sizes but high r^2 values (0.98, Table 1.1). Significant differences in OLFL relationships between Chukchi and Beaufort samples were found for Arctic Staghorn Sculpin ($t = 1.88$, $p = 0.000$), Canadian Eelpout ($t = -2.35$, $p = 0.009$) and Stout Eelblenny ($t = 0.407$, $p = 0.027$). All three species had different slope coefficients, indicating that the relationship between otolith length and fish length was different for these species between the Chukchi and Beaufort seas. Therefore, in Table 1.1, OLFL are displayed separately for these three species for each sea. Linear regressions for all other species were similar among seas so samples were pooled and a single relationship was calculated. FLFW relationships for all 11 species were strongly curvilinear with very high r^2 values (Table 1.2). The r^2 values for FLFW ranged from 0.77

(Capelin) to 0.99 (Arctic Staghorn Sculpin and Yellowfin Sole) (Table 1.2). Capelin and Pacific Sand Lance had the lowest r^2 values (0.77 and 0.92).

Discussion

These newly characterized OLFL relationships for Arctic marine fishes can be used to reconstruct diets of marine piscivores and increase knowledge of Arctic marine ecosystems. These relationships can also be compared to those established for past populations to detect changes over time. If changes are identified in OLFL relationships for the same species, this could indicate different environmental conditions for growth. In addition to being able to determine lengths of fishes consumed by piscivores, relationships developed in this study can be used to estimate fish weight at time of consumption. These OLFL relationships are new for the Alaskan Arctic and will enable investigations of the energetic needs of marine piscivores in the Arctic. A continuation of this study will apply these otolith lengths and body size relationships to otoliths found in ice seal stomachs.

The reliability of this method depends on how well the sizes of the fish sampled represent the fish populations. If the full length range of a species is not represented in the data set used to create the linear regression equation, then estimates of FL at the higher and lower ends of the length range are less accurate (Tarkan et al. 2007). Therefore, it is not recommended to estimate the length of a fish based on an OL that is outside of the range of the sample dataset used to make the linear regression. Most of the fish sampled in this study were less than 400 mm and probably representative of small to medium sized fish for each species. If marine piscivores prey upon fish larger than those used to develop the equations, then OL and FL measurements from larger fish need to be collected, added to the linear regression, and a new regression fit.

Although differences in OLFL relationships can exist between regions thus limiting the use of these relationships to the area of collection (Campana and Casselman 1993), only three of the eleven fish species analyzed in this study showed a difference in OLFL between the Chukchi and Beaufort seas. One possible explanation could be uneven sample sizes between the Chukchi and Beaufort seas; however, that was only true for one of these species, Canadian Eelpout.

Another possible explanation is that the Chukchi Sea is a more productive region for the growth of these two species, though that is unlikely, as more species would have had a difference in their OLFL relationships between the two seas.

Otoliths erode when exposed to digestive acids (Murie and Lavigne 1986, Lidster et al. 1994, Christiansen et al. 2005). In phocid seal stomachs, most otoliths are completely digested ~12 hours after consumption (Murie and Lavigne 1986). Smaller otoliths from Capelin and Pacific Herring (*Clupea pallasii*) will erode much faster than larger Arctic Cod otoliths (Lidster et al. 1994, Christiansen et al. 2005). The OLFL relationships developed in this study used fresh otoliths dissected from fish caught during sampling cruises, therefore the application of these relationships to otoliths that are eroded by digestive acids would underestimate the size (length and weight) of the fish at time of consumption. Any changes to otolith shape can result in underestimation of FL, therefore only relatively fresh otoliths should be used to estimate FL from stomach contents. Otoliths that show intact species-specific characteristics (such as rostrums, post-rostrums and ventral and dorsal ridges) can be used for species identification and length estimation (Harvey et al. 2000).

Environmental factors may influence OLFL relationships (Gauldie and Crampton 2002). In the Arctic, the effects of climate change are occurring at an accelerated rate (Laidre et al. 2015). Rising temperatures and decreasing sea ice cover are likely to affect distributions of fishes in the coming years (Logerwell et al. 2015). Warmer water temperature could modify OLFL relationships by affecting fish and otolith growth rates. For example, OL at a given FW was shorter for faster growing Arctic Char in warmer waters than for slower growing Arctic Char in colder waters (Mosegaard et al. 1988). Sea ice cover is closely related to food availability for all trophic levels (Springer and McRoy 1993, Grebmeier et al. 2006, Bluhm and Gradinger 2008). Decreasing sea ice cover could influence seasonal nutrient availability and the exchange of nutrients between the benthic and pelagic food webs (Grebmeier et al. 2006). In turn, this could change growth rates of fishes, changing the relationship between OLFL (Gauldie and Nelson 1990, Wright et al. 1990). Therefore, as climate change continues in the Pacific Arctic, established OLFL relationships are expected to change.

The OLFL and FLFW relationships developed in this study can be used to estimate the size, weight, and thus energetic value of fishes. Applying these relationships to fishes consumed by marine piscivores in Alaska will allow diet and energetics studies that have not previously been possible.

Figures

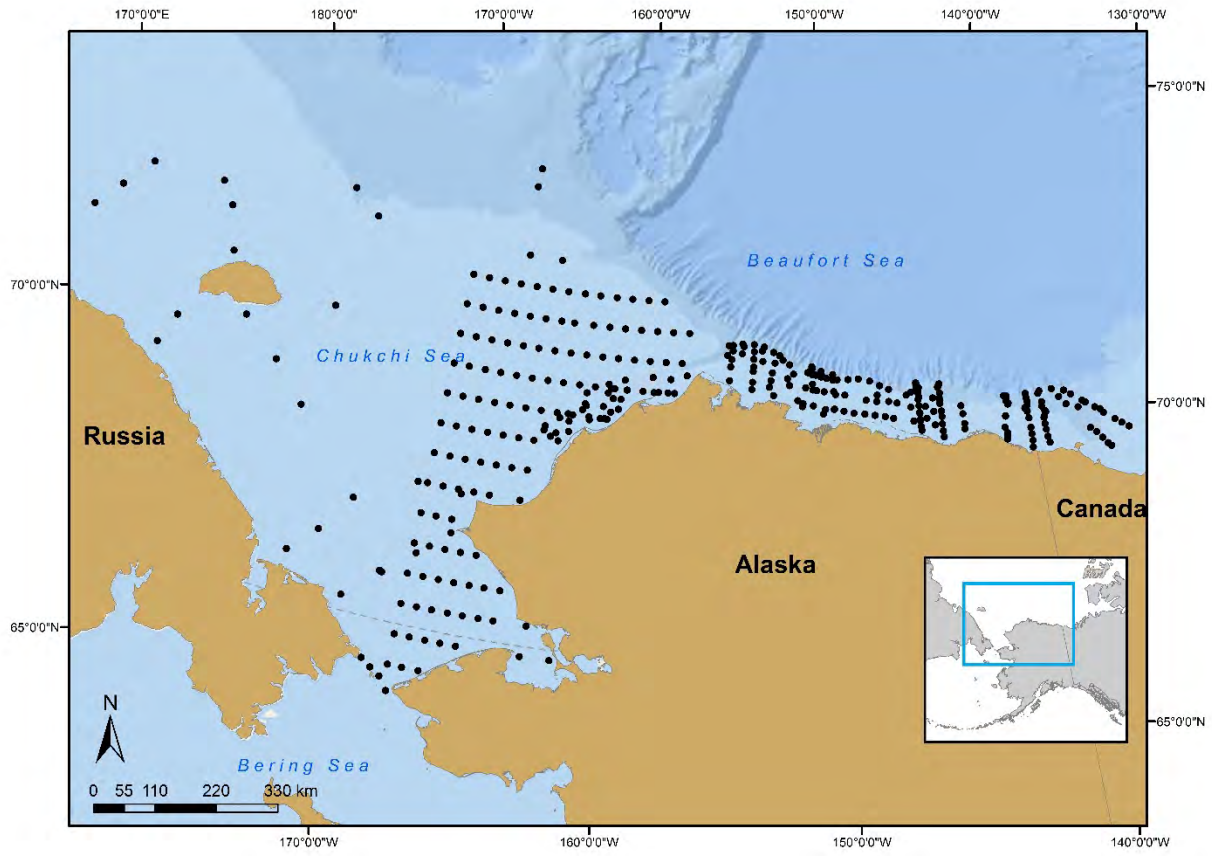

Figure 1.1. Study area with fish sample locations (black dots) during eight research cruises, 2009 – 2014.

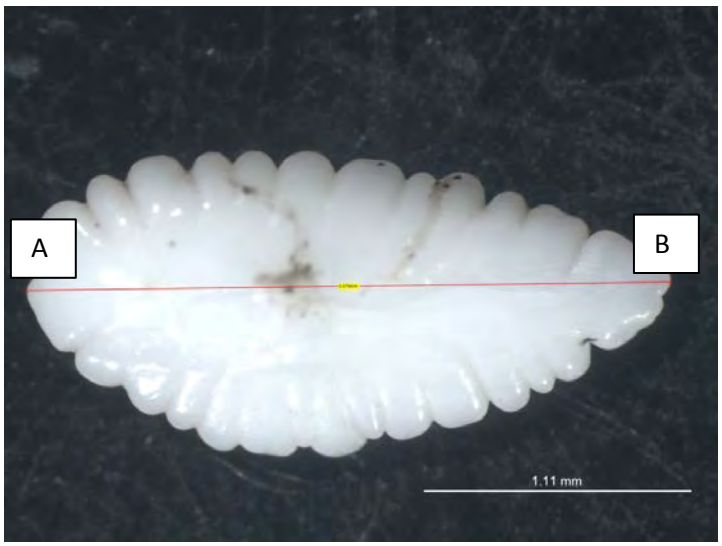

Figure 1.2. Arctic Cod otolith. Red line indicates where length was measured from the rostrum (A) to the postrostrum (B).

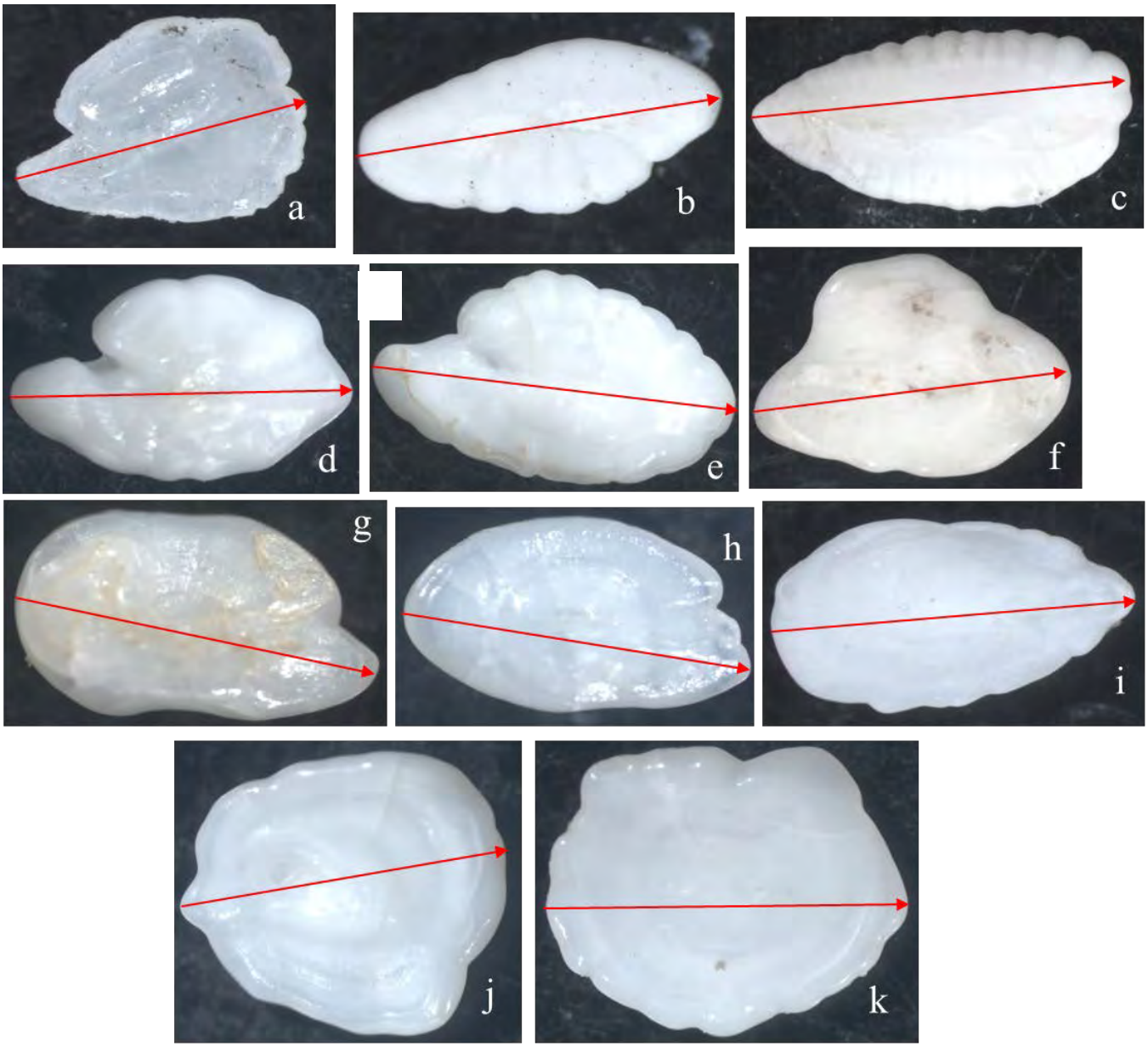

Figure 1.3. Sagittal otoliths from each species with red line designating where lengths were measured. a: Capelin, b: Arctic Cod, c: Saffron Cod, d: Arctic Staghorn Sculpin, e: Shorthorn Sculpin, f: Canadian Eelpout, g: Stout Eelblenny, h: Slender Eelblenny, i: Pacific Sand Lance, j: Bering Flounder, k: Yellowfin Sole

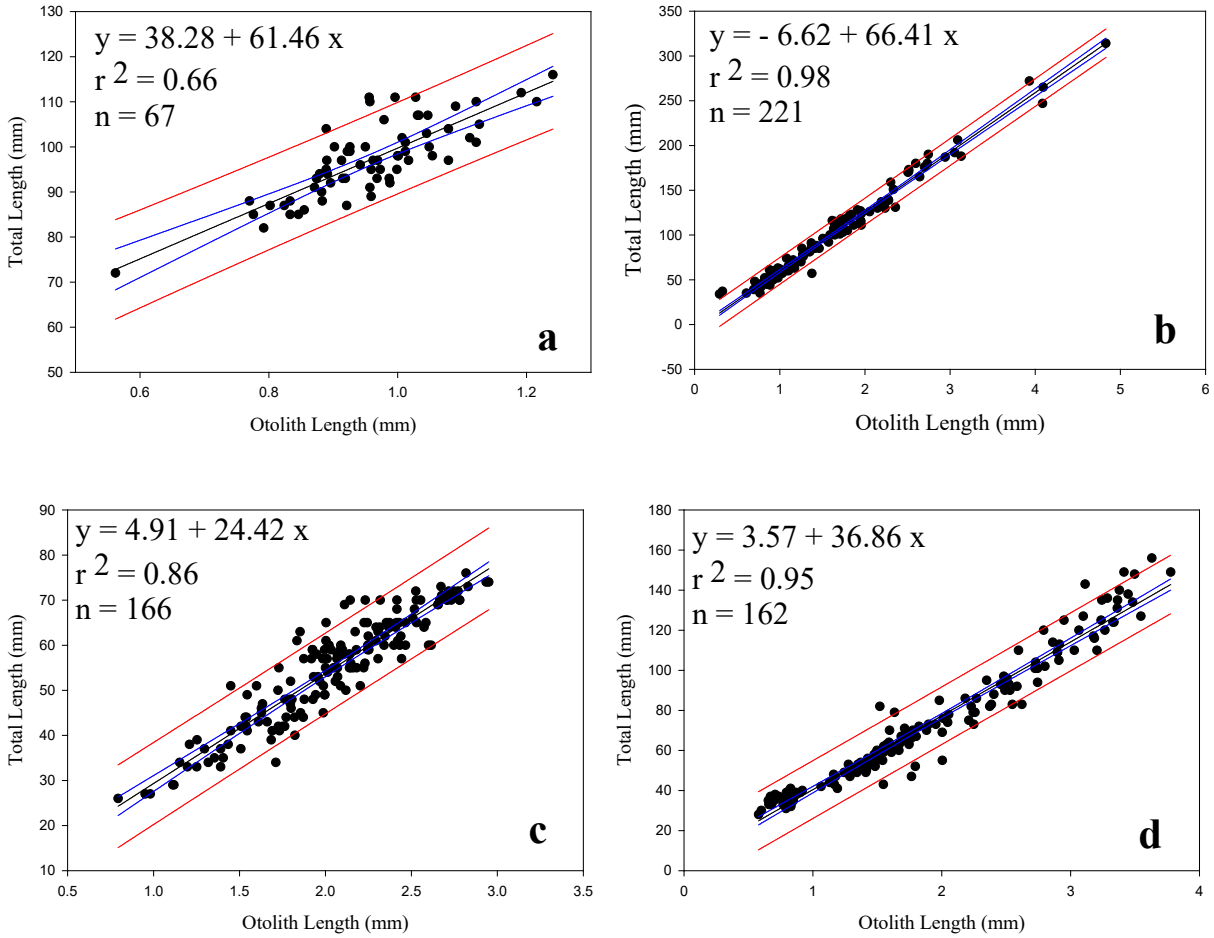

Figure 1.4. OLFL relationships for 11 fish species with linear regression equation, coefficient of determination (r^2) and sample size (n). Blue lines are confidence intervals and red lines are prediction intervals, both 95%. a: Capelin, b: Arctic Cod, c: Saffron Cod, d: Arctic Staghorn Sculpin, e: Shorthorn Sculpin, f: Canadian Eelpout, g: Stout Eelblenny, h: Slender Eelblenny, i: Pacific Sand Lance, j: Bering Flounder, k: Yellowfin Sole

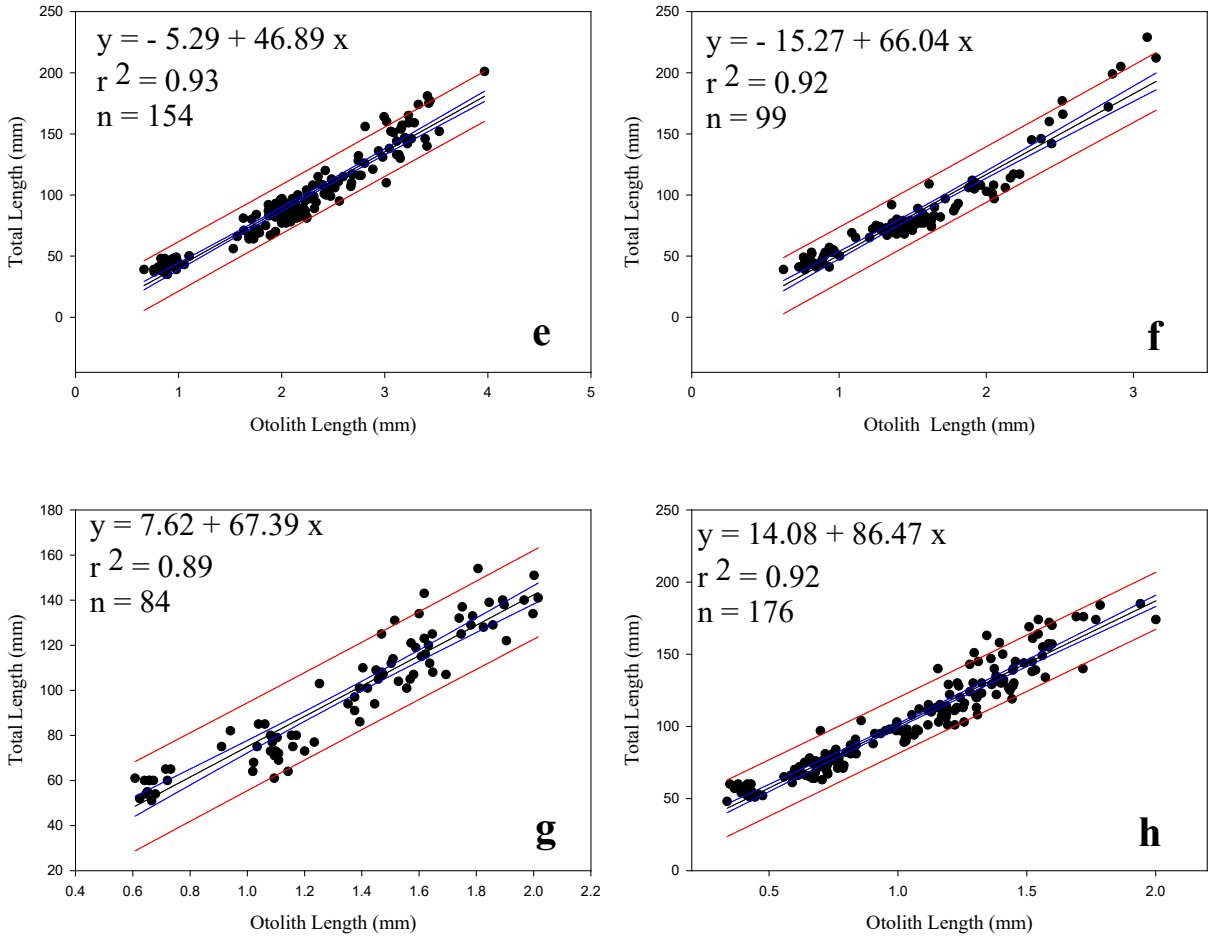

Figure 1.4 continued. OLFL relationships for 11 fish species

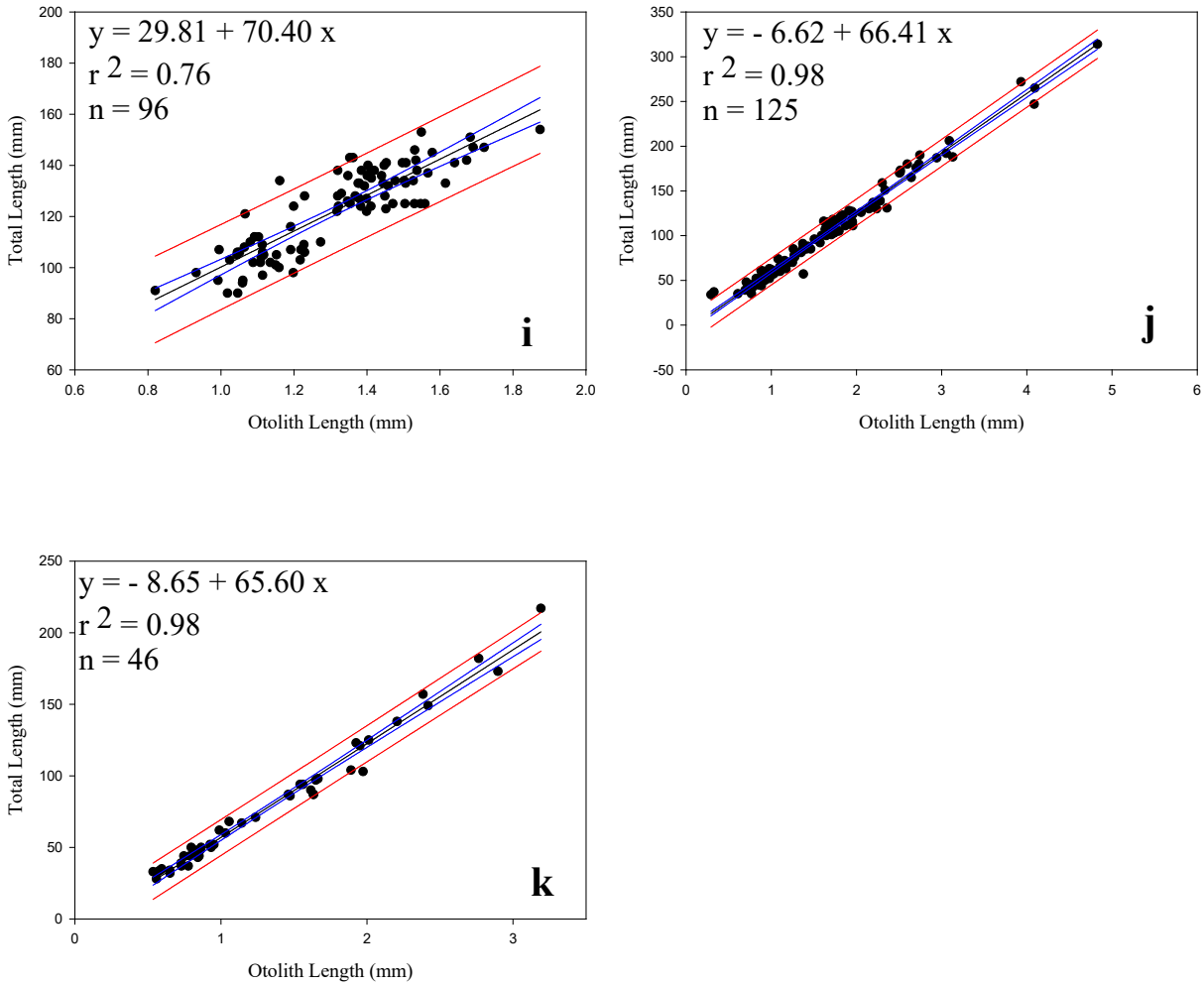

Figure 1.4 continued. OLFL relationships for 11 fish species

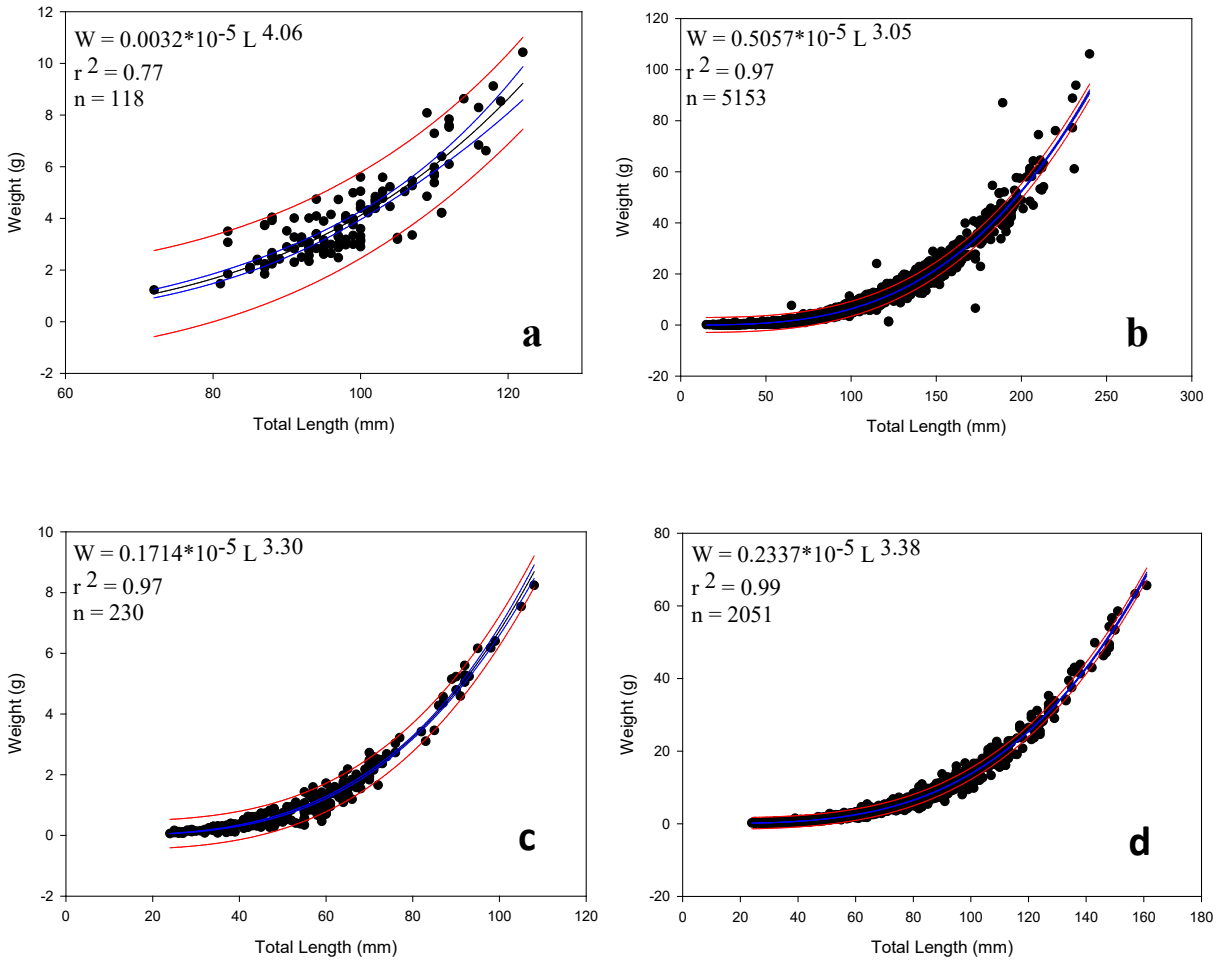

Figure 1.5. FLFW relationships for 11 fish species with linear regression equation, coefficient of determination (r^2) and sample size (n). Blue lines are confidence intervals and red lines indicate prediction intervals, both 95%. a: Capelin, b: Arctic Cod, c: Saffron Cod, d: Arctic Staghorn Sculpin, e: Shorthorn Sculpin, f: Canadian Eelpout, g: Stout Eelblenny, h: Slender Eelblenny, i: Pacific Sand Lance, j: Bering Flounder, k: Yellowfin Sole

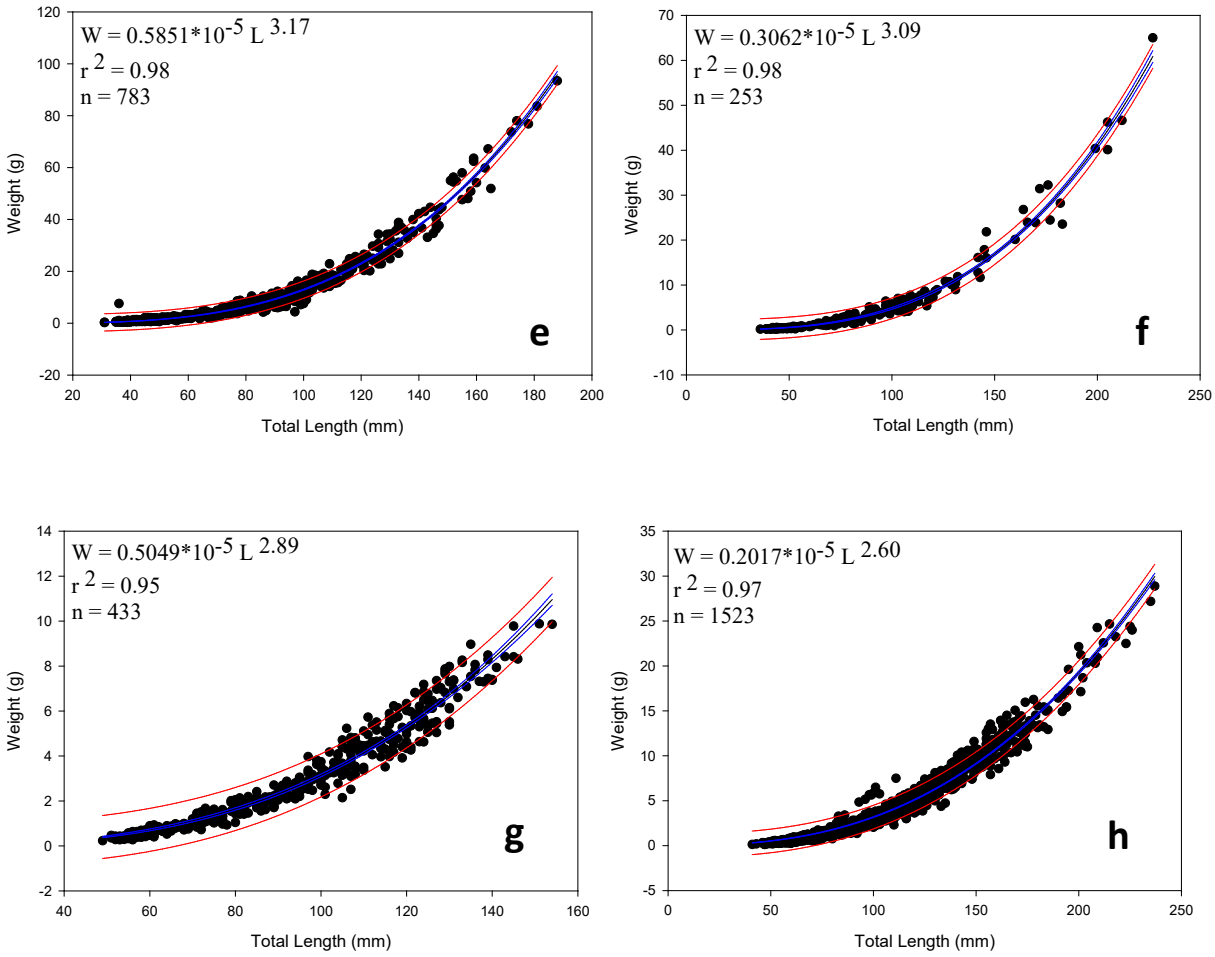

Figure 1.5 continued. FLFW relationships for 11 fish species

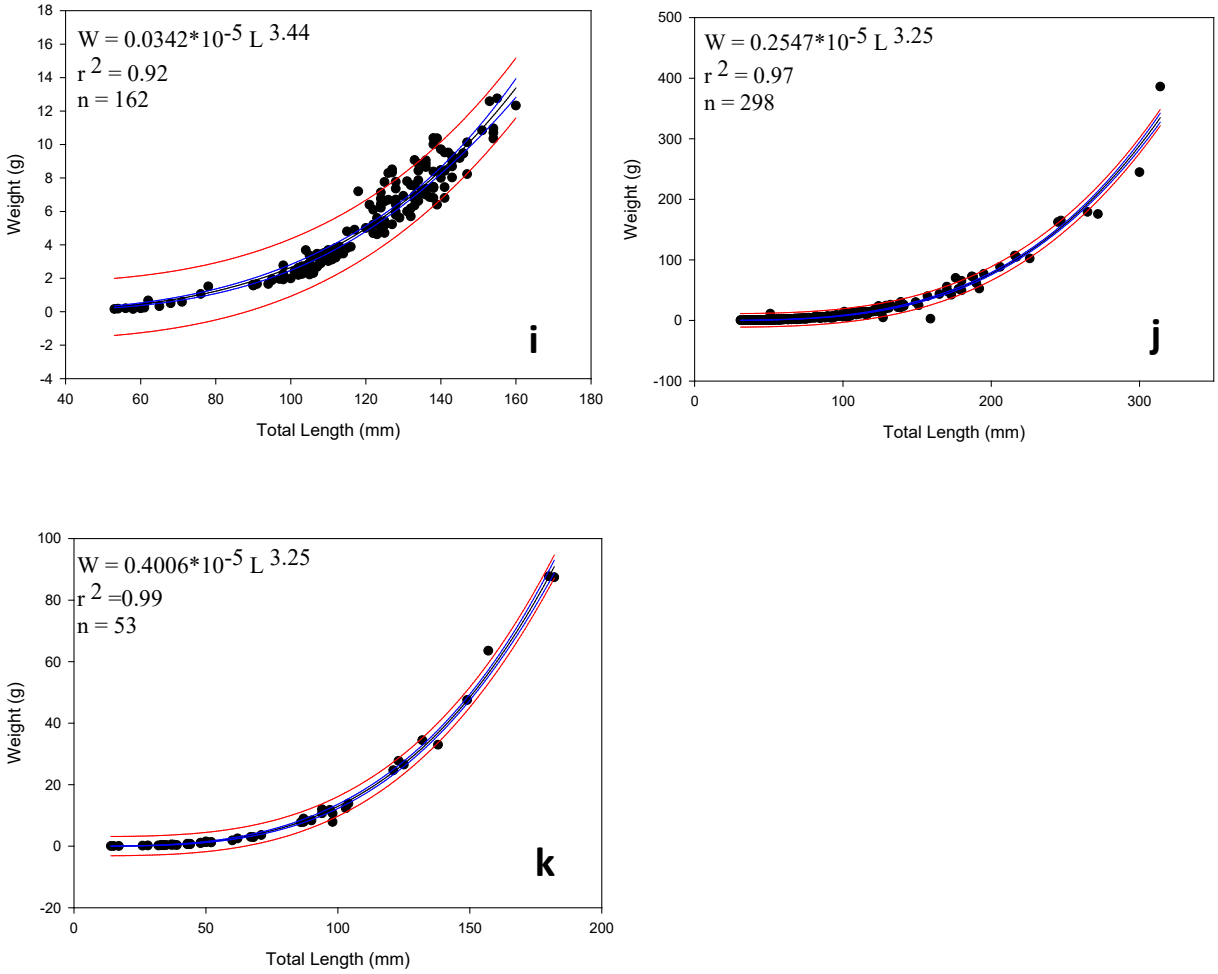

Figure 1.5 continued. FLFW relationships for 11 fish species

Tables

Table 1.1. OLFL relationships for 11 fish species from the Chukchi and Beaufort seas where n is number of fish measured for each species. Fish length is the total length for each species in mm and "min" and "max" provide the range of lengths used to form the relationship equations, "a" is slope, "b" is intercept and r^2 is the coefficient of determination.

Species	n	min	max	a	b	r^2
Osmeridae						
Mallotus villosus	67	72	116	38.28	61.46	0.66
Gadidae						
Boreogadus saida	221	26	158	-6.62	66.41	0.98
Eleginus gracilis	166	33	76	4.91	24.42	0.86
Cottidae						
Gymnocanthus tricuspis						
Chukchi Sea	111	28	156	3.23	37.35	0.96
Beaufort Sea	51	31	101	11.49	30.13	0.85
Myoxocephalus scorpius	154	35	223	-5.29	46.89	0.93
Zoarcidae						
Lycodes polaris						
Chukchi Sea	7	41	229	-28.50	78.44	0.98
Beaufort Sea	92	39	205	-7.47	2.02	0.91
Stichaeidae						
Anisarchus medius						
Chukchi Sea	41	55	154	18.30	63.62	0.94
Beaufort Sea	43	51	134	2.42	67.47	0.84
Lumpenus fabricii	176	48	204	14.08	86.47	0.92
Ammodytidae						
Ammodytes hexapterus	96	90	155	29.81	70.40	0.76
Pleuronectidae						
Hippoglossoides robustus	125	34	314	-6.62	66.41	0.98
Limanda aspera	46	28	217	-8.65	65.60	0.98

Table 1.2. FLFW relationships for 11 fish species from the Chukchi and Beaufort seas combined where n is number of specimens used for analysis. Fish weight is in g and "min" and "max" provide the range of weights used to form the relationship equations, "a" denotes intercept, "b" denotes regression coefficient, and r^2 is the coefficient of determination.

Species	n	min	max	a * 10^{-5}	b	r^2
Osmeridae						
Mallotus villosus	118	1.23	10.43	0.0032	4.06	0.77
Gadidae						
Boreogadus saida	5153	0.03	106.1	0.5057	3.05	0.97
Eleginus gracilis	230	0.06	8.24	0.1714	3.30	0.97
Cottidae						
Gymnocanthus tricuspis	2051	0.11	65.6	0.2337	3.38	0.99
Myoxocephalus scorpius	783	0.22	93.42	0.5851	3.17	0.98
Zoarcidae						
Lycodes polaris	253	0.16	65.01	0.3062	3.09	0.98
Sticheaidae						
Anisarchus medius	433	0.23	9.88	0.5049	2.89	0.95
Lumpenus fabricii	1523	0.13	28.88	0.2017	2.60	0.97
Ammodytidae						
Ammodytes hexapterus	162	0.16	12.76	0.0342	3.44	0.92
Pleuronectidae						
Hippoglossoides robustus	298	0.16	386	0.2547	3.25	0.97
Limanda aspera	53	0.03	65.01	0.4006	3.25	0.99

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Chapter 2

Sizes of fish consumed by three species of ice seals in the Alaskan Arctic

Abstract

Alaskan Arctic seals (Family Phocidae), often referred to as "ice seals", are consumers of fish and invertebrates. A large component of the diet of bearded (*Erignathus barbatus*), spotted (*Phoca largha*) and ringed (*Pusa hispida*) seals is fish. Fish otoliths are resistant to digestion and are commonly used to identify fish species during seal stomach content analysis. Although much is known about what fish species are eaten by these ice seals, less is known about the sizes of fish that are eaten. Recently described otolith length – fish length and fish length – fish weight relationships for Arctic fish species were used here to estimate sizes of fish found in stomachs of subsistence-harvested bearded, spotted, and ringed seals in Alaska based on otoliths. The influence of age class (pup, subadult, and adult), sex, harvest location (Utqiagvik (formerly Barrow), Wainwright, Point Hope, Kotzebue, Shishmaref, Little Diomede, and Nome), and seal species on the sizes of fish consumed was investigated. For bearded seals, harvest location was the primary factor influencing sizes of fish consumed. Stomachs from bearded seals harvested near Little Diomede (Bering Strait) contained larger otoliths (i.e., larger fish) of Arctic Cod, Arctic Staghorn Sculpin, and Shorthorn Sculpin than those harvested near Utqiagvik (in the northern Chukchi Sea). Spotted seals harvested near Shishmaref (southern Chukchi Sea) consumed larger Arctic Cod than those harvested near Little Diomede. Adult ringed seals consumed larger Saffron Cod than pups. Estimating the length and weight of fish consumed by ice seals will contribute to studies of diet and energetics that have not been possible previously in the Alaskan Arctic and will aide in understanding changes that may occur as climate change increases water temperatures, decreases sea ice cover, and potentially alters fish distributions.

Introduction

Seals from the family Phocidae that use sea ice as a pupping, resting and feeding platform are called "ice seals". Three ice seal species are common in the Alaskan Arctic: bearded (*Erignathus barbatus*), spotted (*Phoca largha*), and ringed (*Pusa hispida*) seals. Ice seals are ecologically important as major consumers of fish and invertebrates (Dehn et al. 2007), and are relied upon for subsistence by Native coastal communities in western and northern Alaska (Moore and Huntington 2008).

Ice seals vary by size and diet composition. Bearded seals are the largest ice-associated seal in Alaskan Arctic waters, with adults reaching lengths of up to 2.4 m and weights up to 360 kg (Lowry et al. 1980a). Spotted seals are medium-sized seals up to 1.7 m in length and weights up to 110 kg (Boveng et al. 2009). Lastly, ringed seals are the smallest ice seal with lengths of 1.5 m and weights of up to 75 kg (Lowry et al. 1980b, Kelly et al. 2010). Bearded seals are benthic feeders of fish and invertebrates (Lowry et al. 1980a, Hjelset et al. 1999). Pups typically have a diet of mostly gastropods and bivalves (Crawford et al. 2015) while adult bearded seals consume more fish, mostly sculpins, Arctic Cod (*Boreogadus saida*), flatfish, and pricklebacks (Dehn et al. 2007, Crawford et al. 2015). Spotted seals are primarily pelagic feeders though they are known to prey on some demersal fishes and invertebrates (Dehn et al. 2007). Spotted seals consume mostly Saffron Cod (*Eleginus gracilis*), Arctic Cod, Pacific Herring (*Clupea pallasii*), and Rainbow Smelt (*Osmerus mordax*) (Quakenbush et al. 2009). Ringed seals are also primarily pelagic feeders of fishes and invertebrates (Holst et al. 2001, Dehn et al. 2007), mostly Arctic Cod, Pacific Herring, Rainbow Smelt, and Walleye Pollock (*Gadus chalcogrammus*) (Crawford et al. 2015).

Otoliths, fish ear bones, are somewhat resistant to digestion and can be used to identify prey fish species by inspection of stomach contents. Otolith shape is species specific (Harvey et al. 2000), allowing for fish species identification after consumption. Otolith length and fish length show a positive relationship, allowing estimation of fish length at time of consumption from otoliths found in stomachs. Fish length can also be used to estimate fish weight (Gamboa et al. 1991, Harvey et al. 2000). Otolith length – fish body size relationships have been used to

describe fish prey in other fish species, birds, whales, and seals (Frost and Lowry 1981, Ross et al. 2005, Potier et al. 2007, Quakenbush et al. 2015). Recently, relationships for 11 fish species consumed by ice seals have been estimated using fishes collected by the UAF Fisheries Oceanography Laboratory (Chapter 1).

Regional differences in size may occur in fishes commonly consumed by ice seals. For example, Arctic Cod, a common ice seal prey item (Crawford et al. 2015), are larger in the northern Bering Sea relative to conspecifics farther north in the Chukchi Sea, likely due to the warmer temperatures nearshore waters and increased availability of nutrients (Helser et al. 2015). Sizes of fish in ice seal diets could change in the future due to physiological stresses on fishes, which could shift seal distributions as a response to fish prey size in a changing environment (Laidre et al. 2008). By using otolith length to fish length relationships to estimate sizes of fish seals are consuming, it is possible to assess regional size variability of fish in ice seal diets. Information on fish size and energy content are essential components of diet and energetics studies.

Energy content is known to vary by species and size of fish. Pacific Sand Lance (*Ammodytes hexapterus*), Arctic Cod, Saffron Cod, and Capelin (*Mallotus villosus*) are especially energy rich (Van Pelt et al. 1997, Harter et al. 2013, Hop and Gjøsæter 2013), making them potentially valuable forage species for ice seals in the Alaskan Arctic. In addition to being energy rich, Arctic Cod is the most abundant forage fish in the Arctic and is considered a key link between trophic levels (Lowry and Frost 1981, Craig 1984, Hop and Gjøsæter 2013, Helser et al. 2015).

The quantity, quality, and diversity of forage fish consumed by ice seals is not only determined by the available fish species and, in turn, sizes of available fish but also by the age of the seal. Older seals are more experienced foragers and are physiologically able to dive deeper and longer than pups, enabling them to capture more, and potentially larger, fish (Noren et al. 2005). For example, adult ringed seal consume more Arctic Cod than pups (Dehn et al. 2007), and bearded seal adults contain more fish in their diets than do pups (Crawford et al. 2015). Less

is known about the sizes of fish eaten by adult seals and pups, which affects the energy intake per foraging effort of these two age classes.

Ice seal diet is likely to be influenced by the changing climate in the Alaskan Arctic. The decrease of sea ice cover in the Arctic and subsequent increase of water temperature has the potential to affect distribution, abundance, and size of important prey for seals (Bluhm and Gradinger 2008, Grebmeier et al. 2010). If changes in prey result in less energy available to seals, seal behavior and distribution will likely change to maintain their energy needs for survival and reproduction (Kovacs et al. 2011). Such ecological consequences of changes in the Arctic increase the urgency for a better understanding of ice seal energy requirements.

The objectives of this study were to 1) determine the sizes of fish consumed by three common ice seals species (bearded, spotted, and ringed seals) in the Alaskan Arctic by applying otolith size to fish size relationships (Chapter 1) to otoliths found in ice seal stomachs, and 2) determine differences in fish prey size among and within seal species by harvest location, age, and sex. Results will support future studies on the energetic value of fishes to ice seals and detect changes in fish size in ice seal diets relative to climate change.

Methods

Ice seals in the Chukchi and Beaufort seas in the Alaskan Arctic are legally harvested by subsistence hunters. Most of the harvest occurs in spring (April, May and June) and fall (September, October, November). Native Alaskan subsistence hunters work with the Alaska Department of Fish and Game, Arctic Marine Mammal Program (ADF&G) to provide samples, including stomachs, from harvested ice seals. This long-term biomonitoring program has an extensive archive of ice seal stomach content data and hard parts (including fish otoliths) that were available for this study.

Seal stomachs were available from villages bordering the Beaufort, Chukchi and Bering seas (Utqiagvik, Wainwright, Point Hope, Kotzebue, Shishmaref, Little Diomede, and Nome; Figure 2.1). This study only included seals whose stomachs contained fish. A stomach was

chosen for analysis if it contained more than 10 individual fish from any of the 11 fish species for which otolith length – fish length (OLFL) and fish length – fish weight (FLFW) relationships have been established (Chapter 1). These fish species were Capelin, Arctic Cod, Saffron Cod, Arctic Staghorn Sculpin (*Gymnocanthus tricuspis*), Shorthorn Sculpin (*Myoxocephalus scorpius*), Canadian Eelpout (*Lycodes polaris*), Slender Eelblenny (*Lumpenus fabricii*), Stout Eelblenny (*Anisarchus medius*), Pacific Sand Lance, Bering Flounder (*Hippoglossoides robustus*), and Yellowfin Sole (*Limanda aspera*).

Otoliths were removed after stomach contents were rinsed over a 1.0 mm sieve (Crawford et al. 2015). Otoliths were identified to lowest taxonomic level, usually to species, by William Walker (private contractor with ADF&G). Otoliths from each sample were separated by species, and then right and left otoliths were separated and counted to determine the minimum number of individual fish present in that stomach. Using only right or left otoliths ensured only one measurement per fish, as OLFL relationships generally do not differ between right and left otoliths (Harvey et al. 2000). The exception to this generalization are right-eyed flatfishes, where the left otolith can be somewhat larger than the right otolith (Lychakov et al. 2008). However, when OLFL relationships were described for Bering Flounder and Yellowfin Sole in this study, no difference was detected between the right and left otolith. The otolith (right or left side) to be photographed and measured was determined by the side that was most abundant in each stomach. Otolith lengths were measured using a Leica DFC295 camera mounted on a Leica M165C dissecting scope. Before each photographing session, the camera was calibrated to 0.0001 mm using a slide micrometer. Each otolith length was then measured from the photographs to the nearest 0.0001 mm using a Leica imaging software program.

Fish lengths were estimated from otoliths found in ice seal stomachs using linear equations previously estimated for each of the 11 species listed above using fishes collected during sampling surveys in the Chukchi and Beaufort seas (Chapter 1). Due to regional variability in the OLFL and FLFW relationships between the Chukchi and Beaufort seas for three fish species, Arctic Staghorn Sculpin, Stout Eelblenny and Canadian Eelpout, fish lengths for these three fish species were estimated using the relationship from the Chukchi Sea. Otoliths of fish caught during research surveys and used to establish OLFL and FLFW relationships were

often smaller than those found in seal stomachs. Therefore, estimation of fish length of seal prey was restricted to the range of otolith lengths used to produce each linear regression for that fish species (Chapter 1). However, when otoliths found in seal stomachs were larger than those used to establish the OLFL and FLFW relationships, valid comparisons of relative fish size could still be made by using otolith length directly because larger fish within the same species have larger otoliths (Harvey et al. 2000). For this study, a general linear relationship is assumed for each species (Harvey et al. 2000). Therefore, differences in sizes of fish eaten by age class, sex, harvest location, and seal species can be determined using otolith length directly and in this way, all otoliths from seal stomachs could be included.

A linear mixed effects model was used to compare mean otolith length found in seal stomachs. This method was chosen because of the nested nature (i.e. multiple individuals of several different fish species consumed by individual seals) of the data set and to account for the variability within each seal sample. Age class, sex, harvest location and seal species were factors considered to be fixed effects, and individual seals were the random effect. An ANOVA determined if the effects of seals species, harvest location, age class (pup, subadult, adult) or sex significantly affected mean otolith length for each seal species for each fish species. A full model of:

Otolith Length ~ Age class + Sex + Location + Seal Species + (1|Seal)

was used for each fish species consumed by seals, where 1|Seal indicates the random effect of the individual seal and Otolith Length ~ indicates average otolith length. When a factor did not have a significant effect on mean otolith length of that fish species, that factor was removed from the model. Interactions between and among factors were also analyzed to determine if two factors were jointly affecting mean otolith length in a seal stomach. All analyses were completed in R version 3.2.4 using the lme4 (Bates et al. 2015) and lmerTest packages (Kuznetsova et al. 2016).

Results

Among seal species comparisons

A total of 1,867 otoliths from 128 bearded seal stomachs, 1,411 otoliths from 82 spotted seals, and 2,435 otoliths from 145 ringed seals were measured. Roughly 77% of the otoliths in seal stomachs were larger than the range of otolith lengths used to create the OLFL relationships from the results of Chapter 1 and were not used to estimate fish length in this study (Figure 2.4). Many otoliths from Capelin, Arctic Cod, Saffron Cod, Arctic Staghorn Sculpin, Shorthorn Sculpin, Slender Eelblenny, Canadian Eelpout, Pacific Sand Lance, Bering Flounder and Yellowfin Sole consumed by ice seals were larger in length than otoliths used to create the OLFL relationships. Overall, fishes eaten by seals had otoliths that averaged 0.78 mm longer. Maximum otolith lengths used to create the OLFL relationships ranged from 2.18 to 10.45 mm among these ten fish species. All Saffron Cod and Canadian Eelpout consumed by ice seals had otolith lengths larger than those used to establish the OLFL relationships (Figure 2.4) and those lengths could not be used to estimate sizes of fish found in stomachs. Saffron Cod consumed by ice seals had otoliths that averaged 4.96 mm longer than those used to create the original OLFL. For Canadian Eelpout, otolith lengths were 2.25 mm longer in fish eaten by seals. Stout Eelblenny was the only species where average otolith lengths of fish eaten by seals were similar to those used to create the OLFL relationships.

Saffron Cod, Arctic Cod, Capelin, and Pacific Sand Lance were consumed by all three ice seal species (n = 86 bearded seals, n = 81 spotted seals, and n = 145 ringed seals). All three seal species also ate Saffron Cod larger than fish specimens caught during research cruises used to develop OLFL relationships (Chapter 1). Because the Saffron Cod consumed were too large to use in the OLFL equations, a direct comparison of otolith lengths was used and showed that spotted seals consumed the largest Saffron Cod among seal species (mean = 7.96 mm, SD = ± 2.95, $p < 0.001$, n = 56 spotted seals, n = 656 Saffron Cod), followed by ringed seals (mean = 6.42 mm, SD ± 1.76, $p < 0.001$, n = 70 ringed seals, n = 870 Saffron Cod), and then bearded seals (mean = 5.27 mm, SD ± 2.19, $p < 0.001$, n = 37 bearded seals, n = 143 Saffron Cod). In contrast, no differences were found in average otolith lengths for Capelin or Pacific Sand Lance among the three seal species. Spotted seals consumed the largest Arctic Cod (242 mm and

116.18 g, Table 2.1), bearded ate the second largest (229 mm and 85.14 g), followed by ringed seals (214 mm and 73.05 g).

Within seal species comparisons

Average otolith sizes of fish consumed within each of the three seal species were compared by harvest location, seal age class and sex. For bearded seals, only harvest location was significant in the average otolith lengths of fish consumed (Table 2.2); otoliths of Arctic Cod, Arctic Staghorn Sculpin and Shorthorn Sculpin from bearded seals harvested near Little Diomede were significantly larger than those harvested near Utqiagvik, Point Hope, and Shishmaref ($p < 0.001$, Figure 2.3). Based on otoliths found in bearded seal stomachs, fish lengths could be estimated for nine of 11 fish species commonly eaten. Mean estimated fish lengths ranged from 108–163 mm (Table 2.1, Figure 2.2); with Arctic Cod being the longest. Mean fish weights ranged from 4.28–85.14 g; with Arctic Cod weighing the most (Table 2.1). Bering Flounder (mean weight: 36.12 g) and Yellowfin Sole (mean weight: 54.77 g), both flatfishes, were next and the smallest species of fish consumed was Stout Eelblenny at an average 4.28 g.

For spotted seals, harvest location was also the only significant factor influencing fish size (Table 2.2). Arctic Cod found in spotted seals harvested near Shishmaref were larger than those harvested near Little Diomede ($p < 0.001$). Mean fish lengths for seven of eight species consumed by spotted seals ranged from 113–220 mm (Table 2.1, Figure 2.2). Again, otoliths of Saffron Cod were larger than the size range used to develop the relationships. Mean fish weights ranged from 7.08–116.24 g with Shorthorn Sculpin being the heaviest fish species consumed by spotted seals (Table 2.1).

For ringed seals, harvest location and seal age class were significant factors that influenced sizes of fish consumed, but only for Arctic Cod (Table 2.2). Ringed seals harvested near Shishmaref consumed larger fishes than ringed seals harvest near Little Diomede ($p = 0.05$). Adult ringed seals consumed Arctic Cod with larger otoliths than pups ($p = 0.05$). For ringed seals, fish lengths could be estimated for three of seven fish species consumed (mean lengths included Arctic Cod: 214 mm, Shorthorn Sculpin: 158 mm, and Yellowfin Sole: 176 mm, Table

2.1, Figure 2.2). The other four fish species consumed by ringed seals (Capelin, Saffron Cod, Arctic Staghorn Sculpin, and Pacific Sand Lance) had otoliths that were larger than those used to develop the OLFL relationships. Mean fish weights ranged from 54.24 – 79.28 g. Yellowfin Sole weighed the most (79.28 g, Table 2.1) followed by Arctic Cod at 73.05 g.

Discussion

The relationships used to estimate fish length from otolith length did not cover the full range of otoliths found in seal stomachs. Most otolith length – fish size relationships, however, are known to be linear (e.g., Harvey et al. 2000) although they may have inflection points where the relationship changes (Frost and Lowry 1981, Francis 1990). Although an inflection may change the slope of the relationship, it does not change the general relationship that larger otoliths equate to larger fish and smaller otoliths to smaller fish. Therefore, while it was not possible to accurately estimate fish length for large otoliths found in seal stomachs, the assumption that relative otolith length relates to relative fish size is likely valid and can be used for general fish size comparisons using otolith lengths.

To make OLFL relationships more applicable to ice seal diet studies, larger fish need to be added to the dataset used to calculate the relationships for all fish species consumed by ice seals. Identifying fish species and estimating fish length from otolith length are useful tools for studying piscivore diets from stomach contents. Species identification is dependent upon the shape and integrity of species-specific features found on the otolith, such as ridges, scallops and various other identifying features (Campana 1990). Estimating fish lengths accurately is dependent on using otoliths that are not too eroded by digestion (Murie and Lavigne 1986). By using otoliths minimally affected by digestion, this method can be used to study fish prey species composition and size of fish prey in ice seals or other predators (Frost and Lowry 1981).

Fish size (analyzed as otolith size) was significantly related to harvest location for all three seal species. Spotted and bearded seals harvested near Shishmaref and Little Diomede consumed larger fishes (i.e., Arctic Cod, Arctic Staghorn Sculpin, and Shorthorn Sculpin) than those harvested near Utqiagvik or Point Hope (Fig. 2.3). These results suggest either that some

fish species are larger in the southern than northern Chukchi Sea or that seals in the southern Chukchi Sea select larger fishes. Regional differences in size distribution are known for these Arctic fish species (Gray et al. 2016, Helser et al. 2015). While harvest location is not necessarily equivalent to foraging location (e.g., phocid seals typically have a foraging range of up to 30 km in a 24 hr period; Lowry et al. 1998, Thompson et al. 1998, Quakenbush et al. 2011, Crawford et al. 2015), the short time that fish otoliths remain in a seal stomach (12-24 hours; Muring and Lavigne 1986, Lidster et al. 1994) suggests that foraging and harvesting locations are reasonably close. This indicates that seals could be valuable biosamplers of regional fish populations and otoliths in their stomachs could provide useful information about regional differences in fish size.

Adult ringed seals ate larger Arctic Cod than pups. This could occur if adult seals have more experience foraging, larger body size, and better diving capabilities (Noren et al. 2005), and prefer or encounter larger fish. Indeed, diving capability increases with age in phocid seals as oxygen storage capacity increases in blood and muscles (Lydersen et al. 1994, Noren et al. 2005). Such ontogenetic patterns are known from Arctic grey seals (*Halichoerus grypus*) where large (adult) individuals consume larger prey than smaller grey seals (Tucker et al. 2007). Older and larger gadids are known to inhabit deeper waters (Laurel et al. 2009) and would, thus, have a higher chance to be encountered by adult seals. While different size selection of forage fish in adults versus pups may be driven by physiological capacities and constraints, the ecological consequence from such spatial (feeding depth) separation is an example of effective resource partitioning among seal age classes, which may reduce intra-specific competition for resources (Field et al. 2005). Although bearded seals also are known to increase diving capability, both in number of long dives and duration of dives, with age (Lydersen et al. 1994), there was no evidence of bearded seal adults eating larger fish than pups in this study. Both pups and adults are benthic feeders and the sea floor is likely within reach of both age classes in our study area. Regardless, this improvement of diving capability with age supports the general effect of age on foraging capabilities between pups and adults of ice seals.

Fish lengths and weights estimated by OLFL relationships can be used to determine energy density of fishes eaten by ice seals. Fish length – fish weight relationships for 11 fish

species commonly consumed by ice seals have recently been characterized in the Alaska Arctic (Chapter 1). With these relationships, and stomachs from the subsistence seal harvest, changes can be tracked in fish species eaten and the size distributions of those species as the Arctic environment changes. Otolith length – fish length relationships will allow studies of foraging flexibility and a better understanding of the potential resilience of ice seals to changes in diet by determining sizes of fish ice seals target during foraging. Fish size and location could be useful to identify important seal habitat and to study resource partitioning among seal species and age classes. Thus, determining sizes of fish consumed by ice seals in the Alaskan Arctic will aide biologists in a better understanding of ice seal feeding ecology and habitat use.

The four fish prey species consumed by the three seal species are considered to be high energy forage fishes in the Alaskan Arctic (Capelin, Arctic Cod, Saffron Cod, and Pacific Sand Lance). Capelin, Arctic Cod and Pacific Sand Lance have the highest mean wet mass energy density among the four species (Van Pelt et al. 1997, Harter et al. 2013). Pacific Sand Lance from the North Pacific can have a mean energy content of 5.31 kJ gr^{-1} wet mass (Van Pelt et al. 1997); Capelin from the North Pacific averaged 4.11 kJ gr^{-1} wet mass (Van Pelt et al. 1997); and Arctic Cod from the Beaufort Sea averaged 3.9 ± 0.21 kJ gr^{-1} wet mass (Harter et al. 2013). Another fish species present in the Chukchi Sea commonly consumed by ice seals, Walleye Pollock (*Gadus chalcogrammus*), has an energy density of 2.73 ± 0.26 kJ gr^{-1} wet mass (Van Pelt et al. 1997). The diet overlap among seal species in high energy content fish may have implications for species competition and provide insight into habitat partitioning among seal species. Using the average wet energy density of Arctic Cod, one Arctic Cod of the size and weight consumed by bearded seals (19.03 g - 153.85 g, Table 2.1) would contribute 4.88 - 39.45 kJ to their daily diet. Ringed seals consumed Arctic Cod weighing 7.28 g to 155.83 g (Table 2.1) that would contribute 1.87 to 39.95 kJ. Spotted seals consumed Arctic Cod that were larger (15.18 g to 172.45 g, Table 2.1) and would contribute 3.91 – 44.22 kJ to their daily diet. To extend the estimation of energy value in fishes commonly consumed by ice seals, energetic values are needed for more Alaskan Arctic fish species that are commonly consumed; i.e., Saffron Cod, Arctic Staghorn Sculpin, Shorthorn Sculpin, Slender Eelblenny, Stout Eelblenny, Canadian Eelpout, Yellowfin Sole and Bering Flounder.

Climate change will affect the Arctic ecosystem. A longer open water season will likely change fish species diversity and size distributions and, in turn, may influence ice seal distribution. For example, if fish shift the boundaries of their ranges northward in response to warming temperatures (Mueter and Litzow 2008), concurrent changes in the growth rates and size distribution of these fishes (Perry et al. 2005) may have important consequences for foraging ice seals. By understanding how ice seals are foraging now, scientists will be able to better predict how ice seal diets may change in the coming years.

Figures

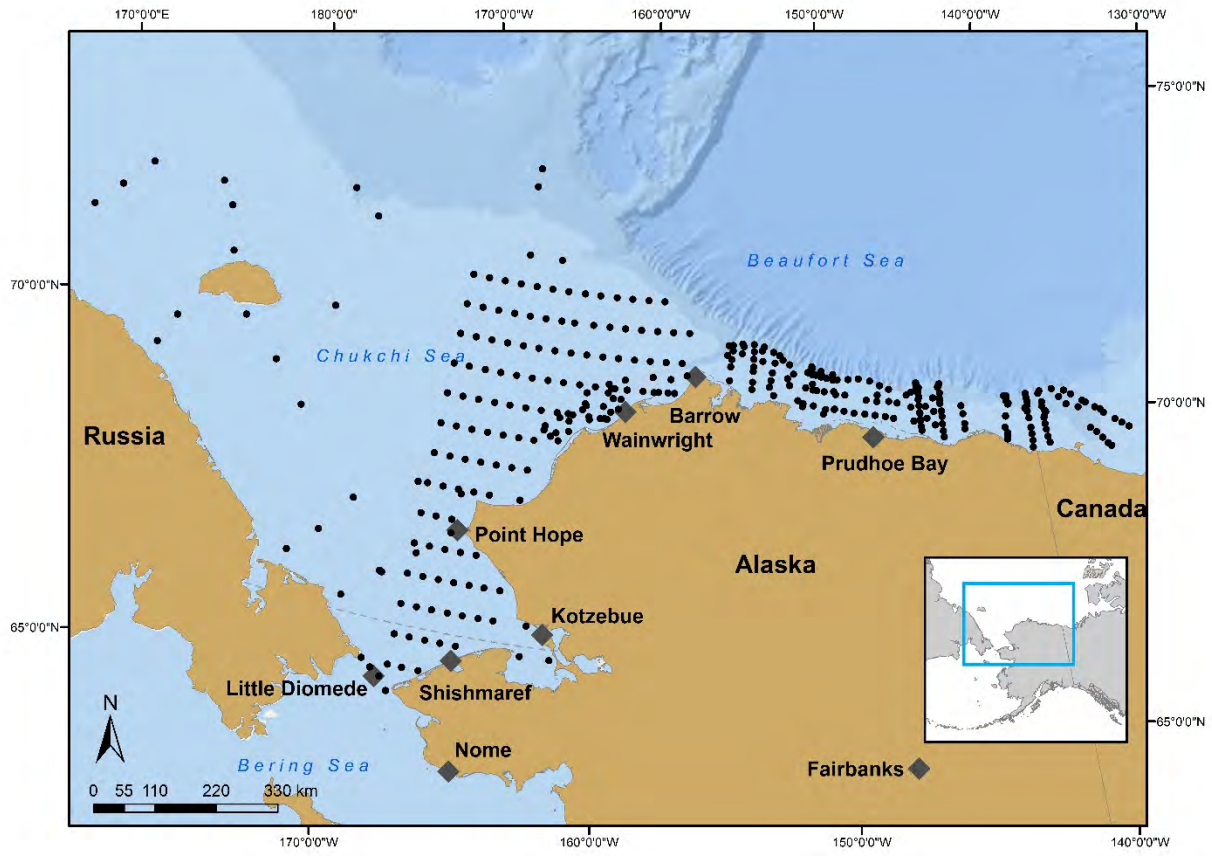

Figure 2.1. Study area. Diamonds (except for Prudhoe Bay and Fairbanks) indicate seal harvest location, black dots indicate fish sampling locations used to establish otolith length – fish length relationships (Chapter 1).

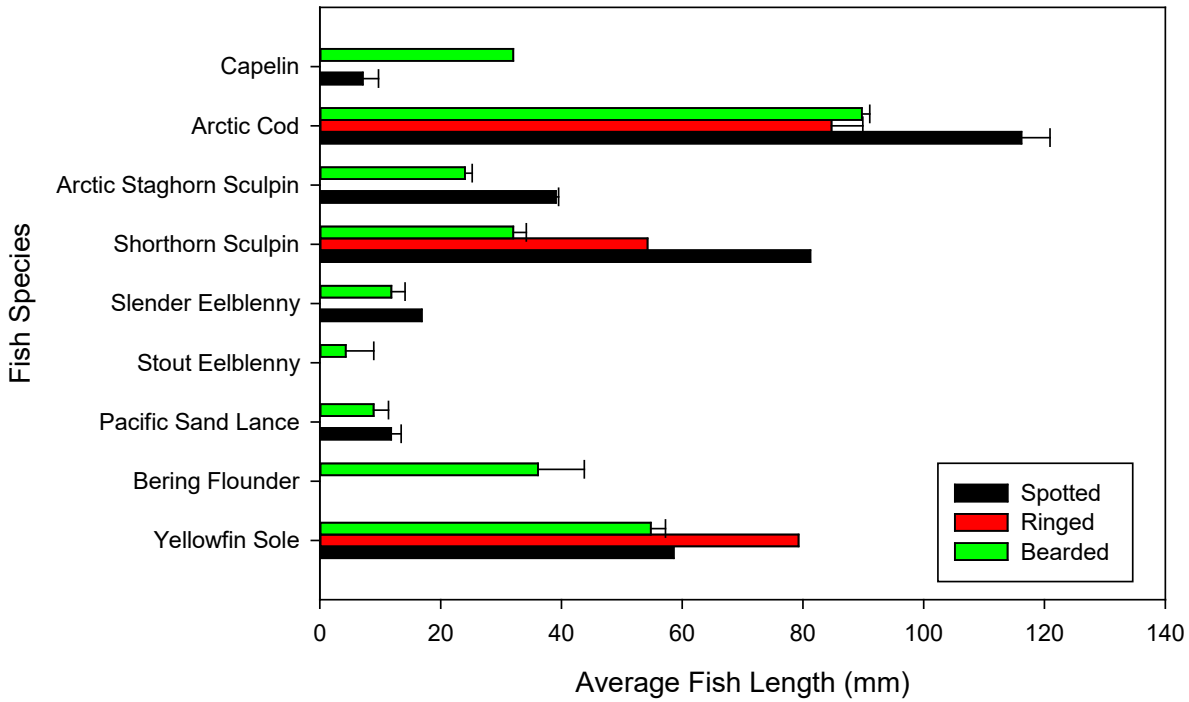

Figure 2.2. Average (± standard error) fish length (mm) of species consumed by three seal species (bearded, spotted and ringed seals) that had otolith lengths within the range of the otoliths used to develop the existing linear otolith length – fish length relationships. This figure does not include larger fish of these species or any Saffron Cod and Canadian Eelpout, which were also eaten by seals.

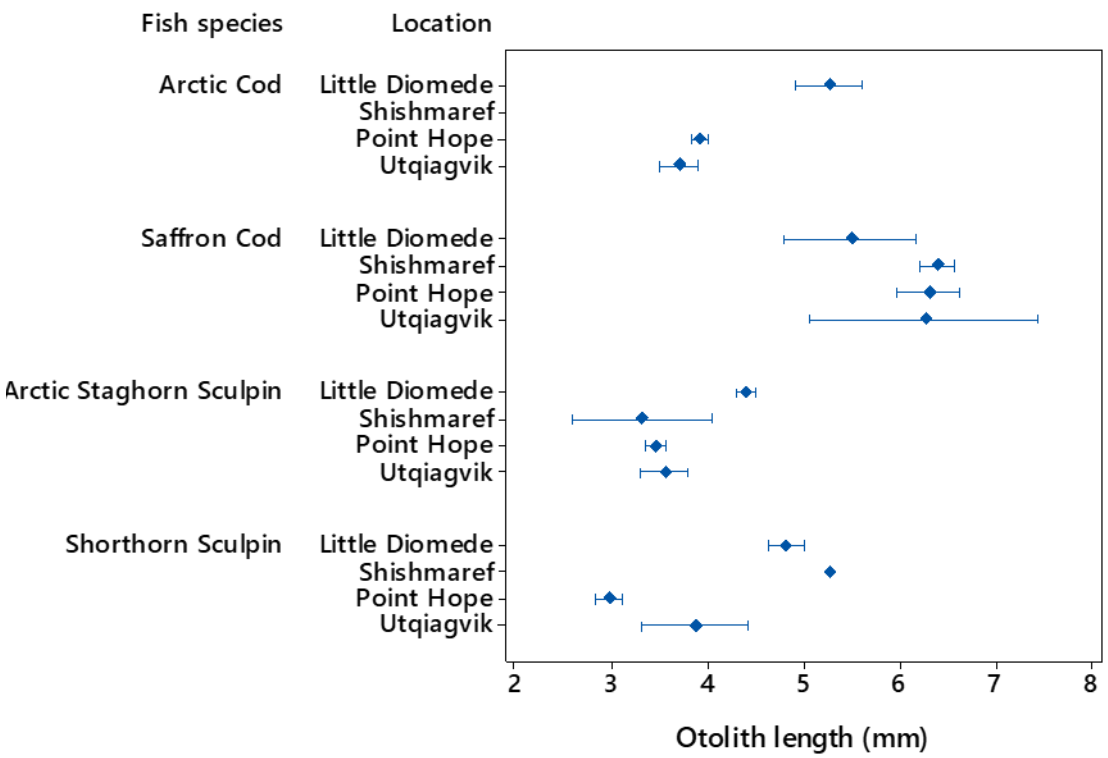

Figure 2.3. Interval plot of significant differences in otolith length among harvest locations for bearded seals. The distance between points indicates differences (non-overlapping error bars) in otolith length among harvest locations.

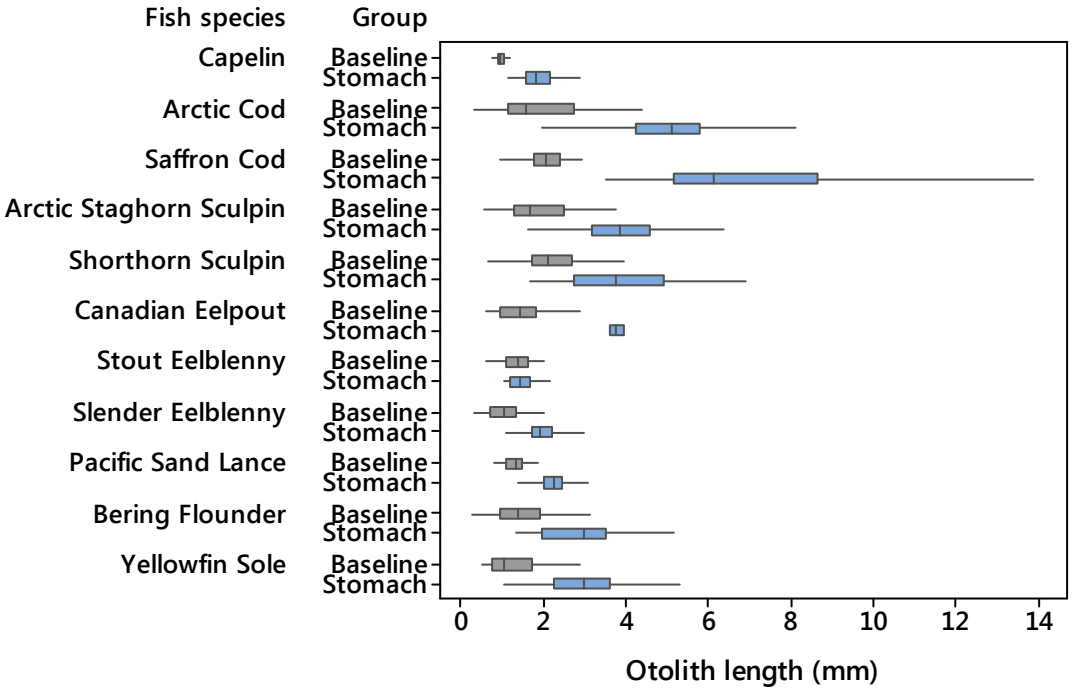

Figure 2.4. Box plot of otolith lengths (mm) of fishes consumed by ice seals (blue boxes, labeled as "Stomach") vs fishes in Chapter 1 use to create otolith length - fish length relationships (grey boxes, labeled as "Baseline"). Box plot includes median line, interquartile range box and whiskers that encompass more than 95% of data points. No outliers are shown.

Tables

Table 2.1. Average fish lengths (mm) and weights (g) of fish species consumed by Alaskan Arctic three seal species. The number of otoliths (n) used for length estimation is included.

	n	Average Fish Length	Minimum Fish Length	Maximum Fish Length	Average Fish Weight	Minimum Fish Weight	Maximum Fish Weight
Bearded Seal							
Capelin	1	110	110	110	6.28	6.28	6.28
Arctic Cod	242	229	143	284	85.14	19.03	153.85
Arctic Staghorn Sculpin	221	116	64	143	24.02	3.01	44.87
Shorthorn Sculpin	150	128	74	181	32.02	4.94	83.35
Slender Eelblenny	81	163	109	195	11.82	3.99	18.08
Stout Eelblenny	23	108	78	155	4.28	1.47	10.78
Pacific Sand Lance	21	142	112	159	8.89	3.82	12.92
Bering Flounder	31	146	83	202	36.12	4.32	79.53
Yellowfin Sole	161	150	59	200	54.77	2.27	119.92
Spotted Seal							
Capelin	2	113	109	118	7.08	6.03	8.14
Arctic Cod	99	242	129	285	116.18	15.23	172.45
Arctic Staghorn Sculpin	2	137	132	142	39.06	34.27	43.86
Shorthorn Sculpin	1	179	179	179	81.24	81.24	81.24
Slender Eelblenny	1	190	190	190	16.87	16.87	16.87
Pacific Sand Lance	8	155	136	161	11.81	7.44	13.36
Yellowfin Sole	1	160	160	160	58.60	58.60	58.60
Ringed Seal							
Arctic Cod	331	214	104	285	73.05	7.28	155.83
Shorthorn Sculpin	1	158	158	158	54.24	54.24	54.24
Yellowfin Sole	1	176	176	176	79.28	79.28	79.28

Table 2.2. ANOVA results for each seal species with significant results ($p < 0.001$) denoted by *.

Bearded

	Sum Sq	Mean Sq	DF	F.value	Pr(>F)
Location	41.309	13.7696	3	14.1511	7.03e-8*
Age Class	4.329	2.1646	2	2.2246	0.1127
Sex	5.093	2.5467	2	2.6172	0.0768

Spotted

	Sum Sq	Mean Sq	DF	F.value	Pr(>F)
Location	24.1102	24.1102	1	16.6509	0.0001*
Age Class	3.0004	1.5002	2	1.0361	0.3611
Sex	1.7071	0.8535	2	0.5895	0.5578

Ringed

	Sum Sq	Mean Sq	DF	F.value	Pr(>F)
Location	16.22	16.22	1	18.6924	3.15e-5*
Age Class	11.5777	5.7888	2	6.6712	0.002*
Sex	3.3978	1.6989	2	1.9579	0.1456

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General Conclusions

Otolith length – fish length (OLFL) relationships are known to be species specific, but they may also be regionally specific within species, necessitating caution in widespread applications. Fish growth varies by environmental conditions, including temperature, salinity and food supply, which account for regional differences in OLFL relationships (Mosegaard et al. 1988). For example, Arctic Cod (*Boreogadus saida*) found in the northern Bering and southern Chukchi seas are larger than their counterparts in the northern Chukchi Sea (Helser et al. 2015). If the Bering Sea has an environment more conducive for fishes such as Arctic Cod to grow larger than their counterparts, it is very likely that their OLFL relationships will differ among these three regions.

OLFL relationships were developed for 11 common Arctic fish species to further diet studies of marine piscivores in the Alaskan Arctic. A limitation of these study was that despite the generally linear relationship between otolith length and fish length, the relationship may change slightly for much smaller or much larger otoliths than those used for creating the linear function. In other words, the size range of fishes used to develop the original OLFL relationship may not be representative of the entire fish population or of the fraction of the fish population commonly consumed by ice seals or other predators. Using a larger range of fish otolith sizes than was available here to create more extensive OLFL relationships in future studies could result in a larger number of otoliths from seal stomachs to which size analyses could be applied. In addition, OLFL relationships only accurately apply to undamaged otoliths. Otoliths erode once exposed to digestive acids in a predator's stomach, making it imperative that OLFL relationships only be applied to otoliths that retained their species-specific shape. This risk is generally reduced because of the relatively short time (12 to 24 hours; Murie and Lavigne 1986) that otoliths remain in seal stomachs. Eroded otoliths will not produce accurate length estimates compared with fresh and undamaged otoliths, however they will always be biased low and could provide a minimum size of fish eaten, which may be acceptable in some studies. Despite these limitations, OLFL relationships are still vital in furthering piscivore diet studies and are key in future energetics studies of piscivorous marine mammals in the Arctic.

Although bearded, spotted, and ringed seals generally differed in the fish composition of their diet, all three species consumed Capelin (*Mallotus villosus*), Arctic Cod, Saffron Cod (*Eleginus gracilis*), and Pacific Sand Lance (*Ammodytes hexapterus*). These four fish species are very high in energy content (Van Pelt et al. 1997, Harter et al. 2013), likely making them highly valuable prey for seals. It is possible that some of these differences and similarities in diets among the three seal species are influenced by their foraging locations. Seals that were harvested in more southern locations (Shishmaref and Little Diomede) consumed larger fish of the same species than seals harvested in northern locations (Utqiagvik and Point Hope), likely reflecting differences in the fish size structure in these different locations. Future shifts in fish populations or seal foraging locations thus could influence prey size availability to seals. Intraspecific competition may be modulated by different fish size preferences by seal age. Older seals within a species generally consumed larger fishes than younger seals, likely driven by physiological diving and foraging constraints for pups (Noren et al. 2005). Size separation, however, provides effective resource partitioning and reduction of competition between adult seals and their offspring. The overlap in prey species for all three seal species could lead to interspecific competition of prey resources. There was a trend, although not statistically significant, that different seal species consumed different sized Arctic Cod, possibly reducing competition among species for this key link in the Arctic marine food web (Hop and Gjøsæter 2013).

These relationships provide an essential stepping stone for the investigation of bioenergetics of seal and other top fish predators. Building on this prey fish size information from the OLFL relationships, energetic value of the prey can be calculated from known fish size-biomass relationships and energetic values per fish biomass. Thus, applying OLFL to otoliths from seal stomachs, biologists will be able to determine the energetic importance of various fish species to an ice seal's or other piscivore's diet. These results provide an important basis to evaluate ramifications of possible changes in fish distributions or size structure based on the ongoing environmental changes in the Arctic (Perry et al. 2005). Similarly, there may be changes in seal foraging behavior that may result in different use of fish prey (Moore and Huntington 2008, Kovacs et al. 2011).

The Alaskan Arctic is just one of many environments being affected by climate change. The results of this study will help biologists better understand the predator-prey relationships of ice seals and fishes. If climate change affects the distribution and size structure of important fish species, it will simultaneously affect the distribution and/or the diet of ice seals. As a next step, it would be useful to determine energetic values by region of the fish prey of ice seals so that energetic values of prey species can be assessed for important top predators of the Arctic marine ecosystem. Developing a better knowledge base about ice seals and their fish diet preferences is essential to determining the future of these species in the Arctic.

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