Biological Monitoring of Ringed Seals in the Bering and Chukchi Seas

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Final Report

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Final Report

I. Project Identifiers

A. Award Number: NA16NMF4720079
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II. Project Summary

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 ringed seal population status and health in the Bering and Chukchi seas. Although listed as threatened under the Endangered Species Act in 2012, no recovery plan or recovery objectives have been developed for ringed seals. The purpose of this project was to address critical data gaps in understanding how ringed 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 annually. 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. This project sampled ringed seals only; bearded, spotted, and ribbon seals were sampled and reported under a concurrent NOAA Grant (Award No. NA16NMF390029). 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 1,135 ringed seals harvested by Alaska Natives for subsistence to evaluate ringed seal health and status. We found that (1) the diet of ringed seals included 19 major prey types (7 fish, 12 invertebrates) and seals consumed fewer Arctic cod, *Boreogadus saida*, during 2016–2020 than during 2000–2015, (2) we analyzed length at age data to detect birth years when conditions were good and poor; except for 2017, recent years have not been poor for ringed seal growth, (3) using blubber thickness as an index for body condition, 2017 and 2018 were below average, but 2019 was above average, a similar trend happened before and during the Unusual Mortality Event (UME) in 2010 and 2011 when body condition was below average, but 2012 was above average, (4) pregnancy rate for ringed seals during this project period was similar to other periods, however decreases in annual reproduction were detectable during 2010 and 2011, during the UME; age at maturity was lower in the 2000s than the 1970s and 1980s indicating higher productivity in recent years similar to the 1960s, (5) 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, (6) we expanded the understanding of a pinniped specific disease, *Brucella pinnipedialis*, relative to ice seals and found exposure to *Coxiella burnetii* in ringed seals and there was no increase in the prevalence of helminth parasites and no new parasite species, (7) harmful algal bloom (HAB) toxins were present in ringed seals although not as high as for bearded seals where the prevalence of domoic acid has increased in stomach contents to 100% between 2012 and 2019 in the Bering Sea. 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. Notably, several of these indices suggest a decrease in health in 2010 and 2011 coinciding with the UME with a subsequent return to average in 2012. The most pressing health concern we identified is the increase of HABs which should continue to be monitored.

III. <u>**Purpose of Project**</u>

Ringed (*Pusa hispida*, also *Phoca hispida*) and bearded seals (*Erignathus barbatus*) were listed as threatened under the Endangered Species Act (ESA) in 2012 because predicted changes in sea ice over the next century were considered likely to cause populations to decline (U.S. Federal Register 2012 a, b). The bearded seal listing was vacated by the U.S. 9th District Court in 2014 (Alaska 2014) leaving ringed seals the only listed ice seal species at the time of this NMFS Section 6 Program Opportunity, NOAA-NMFS-PRPO-2016-2004539 (RFP). In March 2016 (after the RFP), the decision to list ringed seals as threatened was vacated by the U.S. District Court (Alaska 2016). Subsequently, the vacated rulings for bearded seals (Circuit 2016) and ringed seals (Circuit 2018) were reversed; ringed and bearded seals are both listed as threatened as of September 2020. However, because ringed seals were the only listed species at the time of this award, ringed seals are the only species covered by this project.

In addition to ringed seals, three other species of ice seals, bearded, spotted (*Phoca largha*) and ribbon seals (*Histriophoca fasciata*), have been sampled and analyzed under a concurrent project with funding provided by NMFS (NA16NMF4390029). Each species uses different ice habitats and has different diet preferences and by sampling and studying them simultaneously we can better interpret the responses of ringed seals to a changing environment.

Little is known about the biology and ecology of ringed seals and how quickly changes in sea ice may affect the population. Ringed 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 ringed 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 ringed seal responses to rapid changes in sea ice and related environmental conditions.

By collecting and analyzing biological samples and harvest information from subsistence-harvested ringed seals at selected locations annually, we can assess the health and status of the 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 provided a means to detect and monitor the effects of such changes on ringed seals. This project provided the only long-term data available for ringed seals in Alaska and results allow NMFS to evaluate how ringed seals are responding to changes in the environment, the effect responses may have on the population, 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 ringed seals harvested for subsistence during 2016–2020 in the Bering and Chukchi seas.

2. Analyze samples collected during 2016–2020 to evaluate health and status of ringed seals.

   2a. Analyze stomach contents for diet

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

   2c. Analyze blubber thickness for body condition.

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

2e. Select tissues for contaminants testing and send to contract laboratory for analysis.

2f. Analyze blood for disease exposure.

2g. Analyze stomach or intestinal contents for domoic acid and saxitoxin.

2h. 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. Compare recent (including data collected in this study) with past data and provide a retrospective analysis to assess the most current status of ringed seals.

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 ringed 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 shared samples and data to maximize the use of the samples and what we can learn from them. The NSB contributed substantial financial support and in-kind match to make this project possible. In most locations, residents were 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 (formerly Barrow), Wainwright, and Point Lay contribute samples through the NSB. We process these samples and incorporate the data into our database; we also provide the 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 VI, Products and Publications).

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. <u>Results, Evaluation and Conclusions</u>

A.

Objective 1: **Collect morphometric data and tissue samples from ringed seals harvested for subsistence during 2016–2020 in the Bering and Chukchi seas.** During this 2016–2020 project period, we collected measurements and samples from 1,135 ringed seals from Utqiaġvik, Wainwright, Point Lay, Point Hope, Shishmaref, Nome, Gambell, and Hooper Bay. An analysis of the age structure of the sampled harvest (2000–2018) found that the average age for ringed seals was 3.7 years (SE = 0.13) and the maximum age was 42 years (Adam et al. 2020, see Section VI). This objective was fully achieved.

Objective 2: **Analyze samples collected during 2016–2020 to evaluate the health and status of ringed seals.**

2a. Analyze stomach contents for diet.
<u>Diet</u> – Stomachs from 1,173 ringed seals were processed in our laboratory during this project period of 2016–2020, some of which were collected in prior years. About 23% (265) of the stomachs were empty, probably because most prey are 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 relied on William Walker for uncommon or eroded fish

otoliths and cephalopod beaks; uncommon and difficult invertebrates were identified with the assistance of NRF Taxonomic Services and UAF's Institute of Marine Science.

Our previous analysis of ringed seal diet identified differences between season (open-water, June–October and ice-covered, November–May) and age class (non-pups [≥1 year old] and pups). Therefore, when sample sizes allowed, we summarized ringed seal diet by season and age class and identified changes in diet between the project period (2016–2020) and years prior (2000–2015). We assessed the diet of 606 ringed seals of known age collected during this project period and compared them to the diet of 1,068 ringed seals collected during 2000–2015 (Table 1).

Ringed seals are considered generalist foragers and since 2000, 19 major prey groups (7 fish and 12 invertebrate groups) had a frequency of occurrence (FO) ≥ 20% (Table 1). Using the otoliths found in ringed seal stomachs we identified 15,307 individual fish. Overall, changes in the consumption of fish between periods was not consistent. Non-pup ringed seals consumed rainbow smelt (*Osmerus mordax*) and saffron cod (*Eleginus gracilis*) more frequently during 2016–2020 than previous years during both seasons. Whereas Arctic cod (*Boreogadus saida*) was consumed less frequently by all age classes during 2016–2020; roughly 25% less during the ice-covered season. Although changes in invertebrate prey groups were not consistent, during the open-water season, ringed seals consumed crustaceans, especially shrimp, less frequently during 2016–2020 than previous years.

Otolith length can be used to determine the sizes of fish eaten by seals relative to seal sex, age, and harvest location. We measured 8,117 otoliths found in the stomachs of 745 ringed seals collected during 2014–2019. We photographed each measured otolith and archived the photographs (Fig. 1).

Otolith length is useful to determine if the size of species-specific fish consumed by seals has changed over time. A preliminary analysis of Arctic and saffron cod otolith lengths in ringed seal stomachs showed that average length of Arctic cod otoliths did not change from 2011 to 2018, however, average length of saffron cod otoliths increased significantly from 2011 (5.6 mm) to 2018 (7.9 mm) (Biderman et al. 2019, 2020, see Section VI).

This sub-objective has been achieved. We analyzed stomach contents for diet from 1,173 ringed seals during this project period and identified 15,307 fish. We measured 8,117 otoliths from 745 stomachs for use in estimating fish size. We found that ringed seals consumed fewer Arctic cod, *Boreogadus saida*, during 2016–2020 than during 2000–2015.

2b. Analyze body length and age to determine growth.
Morphometrics – We collected body length measurements of ringed seals to assess growth for comparisons by period (see Crawford et al. 2015, Quakenbush et al. 2011). Seals grow faster when they are young. They also grow longer relative to seals born in other years when conditions are good.

We examined residuals of growth (i.e., length given age) by period to determine if seals were on average longer or shorter during the award period (2016–2020) compared to prior years (2000–2015). Seals were harvested by 11 villages; pups were analyzed separately from non-pups (seals ≥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 (age would thus 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, 1958; Quakenbush et al., 2011). 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).

We assessed residual length measurements and paired them with ages of 2,085 ringed seals collected since 2000. With few exceptions, the residual growth of ringed seals did not vary from average in most birth years (Fig. 2). There is some evidence that ringed seal growth of non-pups was somewhat larger than average during 2007 and 2010 and less than average in 1993, 1994, 2006, and 2017 (Fig. 2a). However, residual length of pups revealed a somewhat contrasting pattern; pups were somewhat smaller than average in 2007, 2010, and 2017, which were followed by years with larger than average growth in 2008, 2009, and 2012 and average growth in 2018 and 2019 (Fig. 2b).

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, with the exception of 2017, conditions during recent years have not restricted growth.

2c. Analyze blubber thickness for body condition.

Morphometrics – We collected blubber thickness measurements to monitor body condition (see Crawford et al. 2015, Quakenbush et al. 2011). We compared 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 ringed seals occurred in February for adults (5+ years old) and subadults (1–4 years old, Fig. 3). We compared sternal blubber thickness for ringed seals harvested during 1972–2019.

We also looked at changes in blubber thickness over time and analyzed years where ≥15 samples were available. 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.

Data from all months and both age classes were included because variability in month and age was accounted for in the model. We found that sternal blubber thickness was below average in 2010 and

2011 (before and during the UME, returned to average during 2012–2016, was below average again in 2017 and 2018 (years of low winter ice and severe winter storms in the Bering Sea), but recovered to average in 2019 (Fig. 4).

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, average, and above average. Body condition was below average for ringed seals around the time of the UME in 2010 and 2011 and during this project period in 2017 and 2018. However, in both instances body condition rebounded quickly and was average or above by the following year; 2012 and 2019, respectively.

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

Productivity – We received, processed, and examined female reproductive tracts from 133 ringed seals during this project period for reproductive status and condition. We compared data collected during this project period 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 November 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.

Although pregnancy rate for ringed seals has varied some, no significant differences were identified between periods (Fig. 5). Pregnancy rate was lowest in the 1980s (76%) and highest in the 1960s (91%), during this project period it was 88%. During 2008–2017 and 2019 (years with a sample size of ≥7), the annual pregnancy rate ranged from 47% to 100% (Fig. 6). Annual pregnancy rate was notably low around the time of the UME in 2010 and 2011 at 65% and 47%, respectively. Pregnancy rates, however, increased to 82% by 2012 and 100% by 2013.

The average age of maturity for ringed seals was highest in the 1970s and 1980s (6.0 years old) and was lowest during the 1960s (2.9 years old); it was 4.5 years old during this project period (Fig. 7). As of 2019, we did not detect lower pregnancy rates or older maturation in ringed 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 one poster on ringed seal productivity through 2015 (Bryan et al. 2017, see Section VI), one through 2016 2016 (Bryan et al. 2019a, see Section VI), and two posters that compared the reproductive status of bearded, spotted, and ringed seals through 2018 (Bryan et al. 2019b, 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. Ringed seal pregnancy rates remain stable and have not changed much between periods. However, there was a decrease in the annual pregnancy rate in 2010 and 2011 around the time of the UME. Ringed seal age at maturity was lower in the 1960s and after 2000 than the 1970s and 1980s. Younger ages of maturity suggest that conditions are good for growth and females are able to grow quickly and begin to reproduce younger.

2e. Select tissues for contaminants testing and send to contract laboratory for analysis.

Contaminants – This project allowed us to accumulate sample sizes for many contaminants large enough to determine contaminant concentrations in multiple tissues of ringed seals. We will be able to explore the influence of tissue type, sex, age, and reproductive status 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 55 ringed seals collected during 2002–2007 and 2011–2016 have been determined in laboratories and are available to be analyzed (Table 2). Selenium is one of 14 other elements, some of which are essential elements (e.g., iron, calcium, magnesium) that were also tested. 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 ringed seals collected during 2011–2016 are now available for a comprehensive analysis (Table 2). Data are also available to compare changes in the concentrations of total mercury and selenium collected during two periods (2003–2007 and 2011–2016).

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 a UME. In addition to the 54 healthy ice seals listed above, we also have elemental concentrations for five UME ringed seals to use in these types of comparisons.

Organochlorine contaminant (OCs) concentrations from 47 ringed 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). Similar to the elemental concentrations, we also have OC's from five UME seals that can be compared to the healthy seals. 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 10 ringed seals and perflourinated contaminants (PFCs) were also determined in liver from 10 ringed 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 2008). 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.

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 achieved. Tissues to be analyzed for contaminants were selected and tested by a contract laboratory in adequate sample sizes for analysis.

2f. Analyze blood for disease exposure.
Disease Screening – Serum was collected from one ringed seal during this project period and archived at ADF&G for future disease screening. Whole blood was collected on filter paper for 42 ringed 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. Unfortunately, none of blood-soaked filter paper samples we collected resulted in positive readings when tested for *Coxiella burnetii*, therefore, more samples will need to be tested. Blood on filter paper can also be used to quantify mercury in whole blood (Hansen et al. 2014) and for carbon and nitrogen isotope values (O'Hara et al. 2018). 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 six ringed seals were tested for *C. 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. Two of the ringed seals (33%) were positive for *C. burnetii* antibodies; of those one tested positive for phase I antibodies and one for phase II.

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 known to cause reproductive failure and other symptoms and are zoonotic. 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 ringed 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).

Nasal swabs from five ringed 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 (VanWormer et al. 2019, see Section VI).

<u>Parasites</u> – The species and prevalence of parasites were predicted to change with the warming climate (Burek et al. 2008), therefore we analyzed internal parasites (helminths) from tissues (stomach, intestine, heart, liver, lung, gall bladder) collected during 2006–2015 from 141 seals (including 44 ringed seals) to evaluate changes in helminth load and species composition for 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. None of the helminths found were new to the Bering-Chukchi region and prevalence of known parasites has not increased. Therefore, as of 2015, no new parasite species were identified in any Alaskan ice seals including ringed seals, and the prevalence of endemic parasites has not increased. These data were presented as a poster at the Alaska Marine Science Symposium, AMSS (Bryan 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.

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 ringed seals. In addition to detecting disease using blood we expanded our disease analyses using other methods and addressed parasites.

2g. Analyze stomach or intestinal contents for domoic acid and saxitoxin.

Harmful Algal Blooms –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 (Lefebvre et al. 2016). 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 93 ringed seals, collected during 2007–2016 for DA and STX and analyzed additional data and found that for ringed seals, 61 of 289 (21%) had detectable concentrations of DA and 47 of 263 (18%) had detectable concentrations of STX. Of all the ice seal species, bearded seals had a higher prevalence of both toxins at 157 of 344 (46%) for DA and 96 of 404 (17%) for STX, however, ringed seals had the highest maximum concentration of DA at 1,740 ng/mL. Bearded seals prevalence of domoic acid in stomach contents increased from 0 to 100% between 2012 and 2019 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 trends in DA and STX for ice seals in the Bering and Chukchi seas from 2006–2019. 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 matrices is not appropriate. Because of these results, we have changed sampling protocols to collect future 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 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 2020, see Section VI), and have a second manuscript in preparation (Hendrix et al. *In prep*., see Section VI).

This sub-objective was achieved. We analyzed stomach contents from ringed seals for HAB toxins DA and STX and compared prevalence to toxins found in the other ice seal species. We detected both DA and STX toxins in ringed seal stomach contents, but prevenance was lower than what was detected in bearded seals.

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

The spring ringed 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 by period, can indicate poor years for pup production. By monitoring this proportion annually, we can detect strings of years that may indicate a trend. Ringed seal pups in the sampled harvest were lowest during the 1980s (6.1%), however few samples (n = 49) were available and few years were sampled (Fig. 8). The proportion of pups sampled during the 1960s (25.7%) and 1970s (22.4%) were not different from each other but were significantly lower than during the 2000s (55.4%) and 2010–2015 (50.1%), which were also not different from each other. The proportion of pups sampled was highest during 2016–2019 (74.5%) (Fig. 8a). Annually, pups comprised 32.8–86.3% of the ringed seal harvest from 2000–2019 (Fig. 8b). Interestingly, pup proportions were notably low in 2011 and 2012 which corroborates the low pregnancy rates seen in 2010 and 2011 corresponding to the UME years (see Objective 2d above). These data were presented on posters (Bryan et al. 2017, Bryan et al. 2019, Adam et al. 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 of the age, sex, or size of the seal harvested or the timing of the ringed seal harvest. A few hunters indicated that seals arrive earlier and the sea ice leaves earlier and freezers later, there are more winds, and the seals are harder to find. Although some hunters noted changes in seal condition citing hair loss, sores, and changes in blubber thickness; many others said the seals are healthy. About 48% of hunters said that ringed seals will haul out on land.

Objective 4: **Compare recent (including data collected in this study) with past data and provide a retrospective analysis to assess the most current status of ringed seals**. This sub-objective was completed. The comparisons of data among periods are included under the above individual objectives.

B. During our disease investigations using blood sera, we found that serology was ineffective in determining disease presence. We stopped analyzing sera and began exploring the use of polymerase chain reaction (PCR) methods 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 include:

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 ringed seal whiskers to examine temporal changes in cortisol concentrations and stable isotopes in sections of pinniped whiskers (Karpovich et al. 2018, see section VI).
2) 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).
3) 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).
4) Contributing samples of *longissimus dorsi* muscle collected from 10 ringed seals (and 41 bearded and 12 spotted 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.

5) 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).
6) Contributing tissues samples from ringed seals collected during this project period and previously were shared with Southwest Fisheries Science Center for DNA used for a genetic stock structure studies during this project period. 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) and a manuscript was prepared using DNA from these samples and is in review by the journal Endangered Species Research (Lang et al. *In review*, see Section VI).
7) Contributing ringed seal morphological and age and length at maturity data for an international effort to describe variation in ringed seal body size across the Arctic (Kovacs et al. *In Prep*., 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.

VI. Products and Publications

Products and publications are attached in the order that they appear below. Publications that are *In review*, *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.

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

Kovacs, K.M., J. Citta, R. Dietz, S. Ferguson, L. Harwood. E.V. Lea, C. Lydersen, L. Quakenbush, F. Riget, A. Rosing-Asvid, T.G. Smith, V. Svetochev, and O. Svetocheva. *In prep*. Variation in body size of ringed seals (*Pusa hispida*) across the circumpolar Arctic: evidence of morphs, ecotypes or simply extreme plasticity?

Lang, A.R., P. Boveng, L. Quakenbush, K. Robertson, M. Lauf, K.D. Rode, H. Ziel, and B.L. Taylor. *In review*. A re-examination of population structure in Arctic ringed seals using DArTseq genotyping. Endangered Species Research.

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

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. 2020. Age structure of subsistence harvested ice seals in Alaska 2000–2018. Alaska Marine Science Symposium, 27–30 January, Anchorage AK. (Poster)

Biderman, L., A. Bryan, J. Crawford, J. Citta, and L. Quakenbush. 2019. Occurrence of Arctic and saffron cod in the diet of ringed seals at Shishmaref, 1975–2016. Alaska Marine Science Symposium 28 January to 1 February 2019 Anchorage, AK. (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. Quakenbush, J. Crawford. 2017. Ringed seal productivity in Alaska using harvest-based monitoring, 1975–2015. Biennial Marine Mammalogy Conference, 22–27 October 2017, Halifax, Nova Scotia. (Poster)

Bryan, A., L. Quakenbush, J. Crawford, L. Biderman, and R. Adam. 2019a. Ringed seal productivity in Alaska using harvest-based monitoring, 1960s–1980s and 2000s–2010s. Alaska Marine Science Symposium 28 January to 1 February 2019 Anchorage, AK. (Poster)

Bryan, A., L. Quakenbush, J. Crawford, L. Biderman, and R. Adam. 2019b. 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)

Lang, A., L. Quakenbush, H. Ziel, K. Robertson, M. Lauf, and P. Boveng. 2017. Detecting population structure in bearded and ringed seals: current understanding and future challenges. Alaska Marine Science Symposium, 24–27 January 2017, Anchorage, AK. (Abstract)

Lefebvre, K.A., D. Marcinek, K. Burek Huntington, L. Quakenbush, A. Bryan, R. Stimmelmayr, G. Sheffield, H. Ziel, T. Goldstein, J. Snyder, T. Gelatt, F. Gulland, B. Dickerson, and V. Gill. 2017. Exposure risks and health effects of algal toxins in marine mammals using both environmental surveillance and biomedical laboratory models. Alaska Marine Science Symposium, 24–27 January 2017, Anchorage, AK. (Abstract)

Lefebvre, K., R. Stimmelmayr, L. Quakenbush, G. Sheffield, A. Hendrix, A. Bryan, A. Mounsey, M. Willis, E. Iversen, D. Kimmel, J. Duffy-Anderson, J. Murphy, K. Cieciel, E. Siddon, L. Eisner, E. Fergusson, S. Showalter, E. Yasumiishi, J. Gann, J. Grebmeier, J. Snyder, D.M. Anderson, E. Fachon. 2020. Algal toxins in Alaskan Arctic food webs: Seawater, zooplankton, bivalves, fish, ice seals, walruses, and whales. Alaska Marine Science Symposium, 27–30 January, Anchorage AK. (Abstract/Oral)

Nymo, I.H., K.A., Larsen, M. Tryland, G. Foster, K. Beckmen, L. Quakenbush, J. Godfroid. 2016. Brucella infections in phocids versus otariids in Alaska. European Wildlife Disease Association, Berlin, Germany. 27-31 August 2016.

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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 – Ringed seal, ice seals, Bering Sea, Chukchi Sea, disease, parasites, harmful algal toxins, contaminants, productivity, body condition, health indices

Table 1. Frequency of occurrence (%FO) of common prey items identified in the stomach contents of ringed 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	222	67	257	116	325	111	264	312
Fish	89.6%	97.0%	79.0%	75.9%	90.5%	93.7%	84.5%	80.1%
Osmerus mordax	27.5%	56.7%	22.6%	21.6%	33.8%	61.3%	14.0%	6.7%
Mallotus villosus	5.9%	20.9%	15.6%	25.0%	3.7%	12.6%	10.6%	25.6%
Gadidae	78.4%	83.6%	58.8%	42.2%	82.2%	86.5%	73.1%	57.7%
Boreogadus saida	43.7%	32.8%	28.8%	19.8%	53.2%	22.5%	53.0%	27.9%
Eleginus gracilis	65.8%	80.6%	44.0%	25.9%	61.8%	84.7%	38.3%	36.5%
Gadus chalcogramma	0.9%	1.5%	1.6%	3.4%	8.6%	8.1%	8.7%	10.6%
Gadus macrocephalus	0.0%	4.5%	0.0%	1.7%	2.2%	0.0%	1.5%	1.3%
Invertebrates	72.5%	64.2%	61.5%	59.5%	78.2%	81.1%	76.9%	78.5%
Crustaceans	68.9%	49.3%	58.0%	46.6%	74.2%	73.9%	72.7%	74.7%
Mysida	16.7%	3.0%	16.7%	21.6%	21.2%	18.0%	23.5%	33.0%
Mysidae	16.7%	3.0%	16.7%	21.6%	20.9%	18.0%	23.5%	33.0%
Neomysis sp	11.3%	3.0%	12.5%	21.6%	16.9%	18.0%	17.4%	31.4%
Amphipoda	27.9%	28.4%	29.6%	20.7%	34.5%	36.9%	42.4%	37.8%
Gammaridea	24.3%	25.4%	17.1%	11.2%	22.2%	30.6%	26.5%	33.0%
Euphausiidae	6.3%	7.5%	8.2%	10.3%	17.8%	0.9%	23.1%	3.2%
Decapod	54.5%	35.8%	30.7%	19.0%	54.5%	58.6%	39.0%	37.5%
Shrimp	54.1%	35.8%	30.0%	19.0%	53.8%	58.6%	38.6%	36.9%
Crangonidae	32.0%	29.9%	15.2%	12.9%	32.0%	38.7%	17.0%	19.2%
Crangon sp	27.5%	26.9%	11.7%	11.2%	25.2%	34.2%	11.0%	10.3%
Crangon alaskensis	22.5%	19.4%	8.2%	5.2%	20.9%	22.5%	8.3%	6.4%

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

	Ringed seal	
Analysis-tissue	2003-07	2011-16
Trace metals-liver	35	20
Trace metals-kidney	12	10
Trace metals-muscle	0	10
As-blubber	0	20
MeHg-liver	0	10
MeHg-kidney	0	10
MeHg-muscle	0	10

Table 3. Sample sizes of organochlorine contaminants (OCs) for ringed seals by contaminant, tissue, species.

	Ringed seal	
	2003-07	2011-16
OCs-blubber	27	20
PBDEs-blubber	6	10
PFCs-liver	17	10

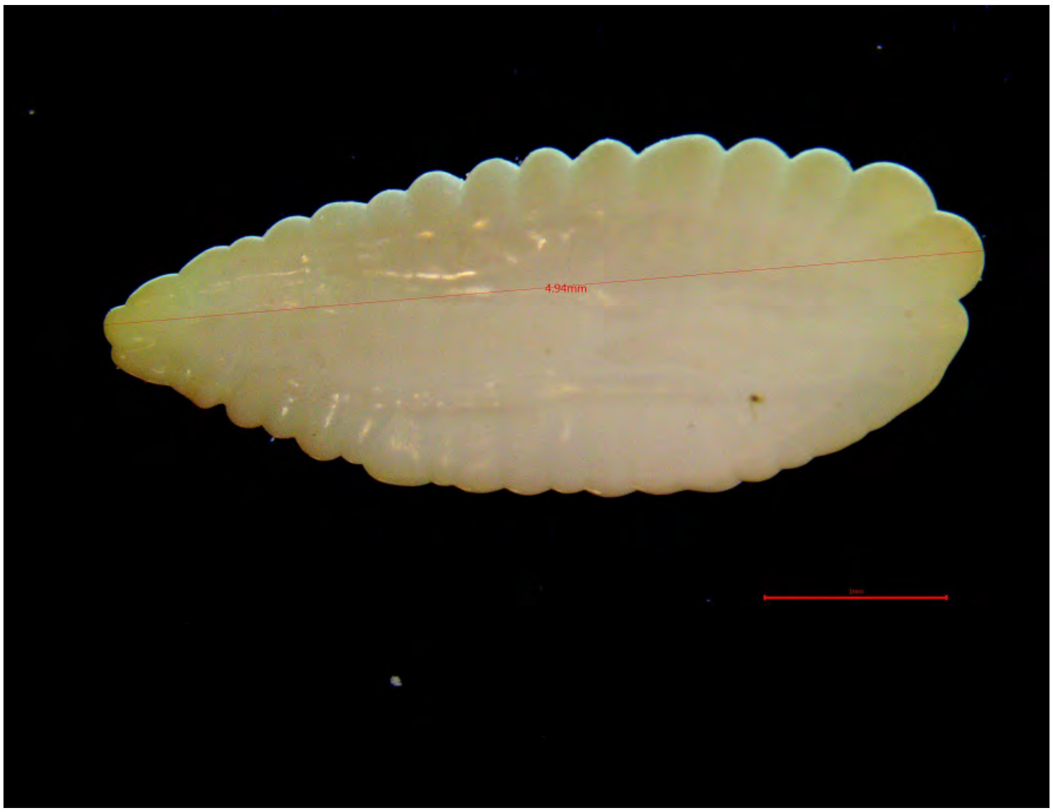


Figure 1. Saffron cod otolith from a seal stomach. Otolith length is measured electronically from rostrum to postrostrum in mm using MU1000 AmScope digital camera and software attached to a Leica M125 stereo microscope. This image is 20X magnification. The red line below the otolith represents 1 mm.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

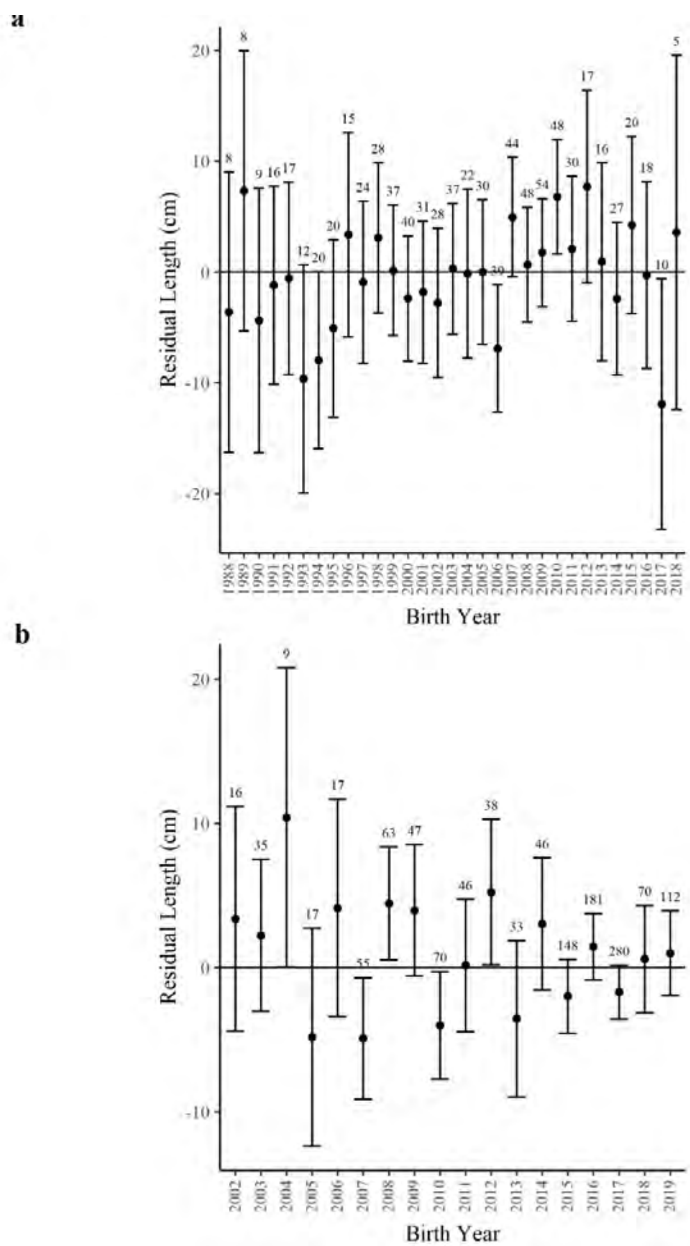


Figure 2. The mean residual length of (a) non-pup (≥1 year of age, n = 778) and (b) pup (n = 1,307) ringed seals, plotted by birth year. Seals were harvested for subsistence in Alaska (1998–2019). Residual lengths for each seal were calculated from difference between the measured standard length for 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.

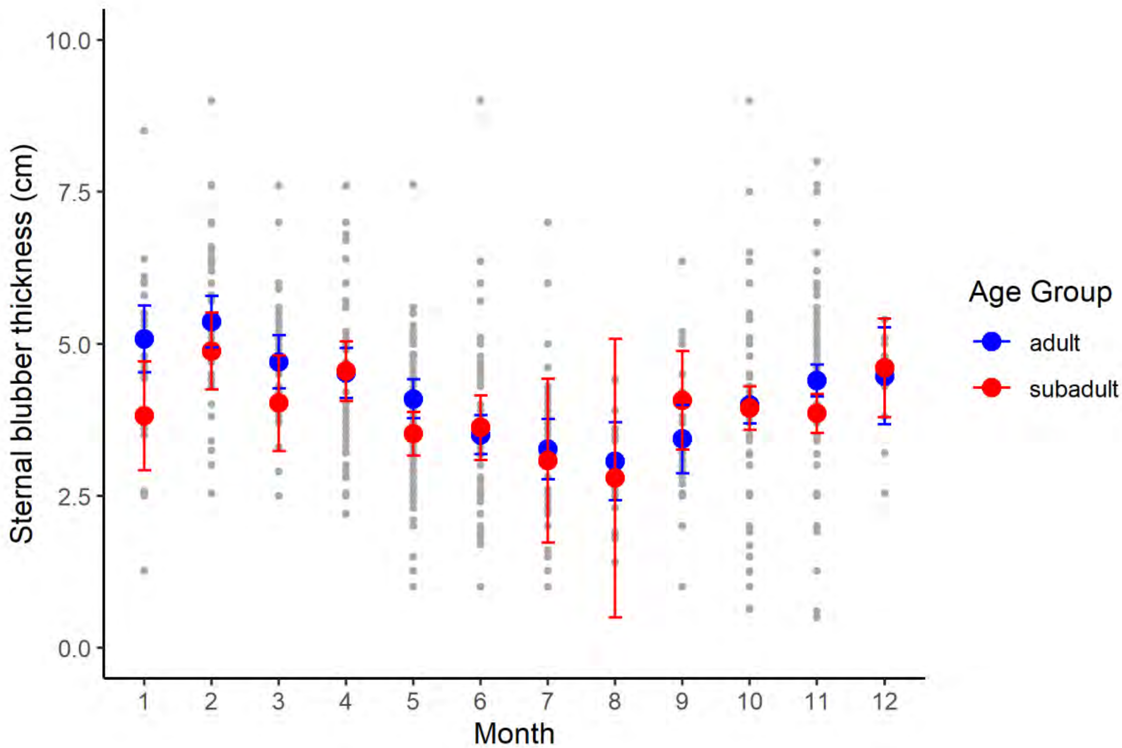


Figure 3. Mean monthly sternal blubber thickness for ringed seals, 1972–2019. Gray dots are raw data. Subadults were classified as 1–4 years old and adults were 5 years of age and older. The error bars represent the 95% confidence interval. The top model for monthly sternal blubber thickness included the month by age group interaction (F = 2.51, d.f. = 12, 957.22, p = 0.002) and standard length (F = 25.73, d.f = 1, 988.83, p < 0.001), which meant a dataset with 1,001 observations was used. Year was a random effect and all diagnostic plots showed the model was a good fit.

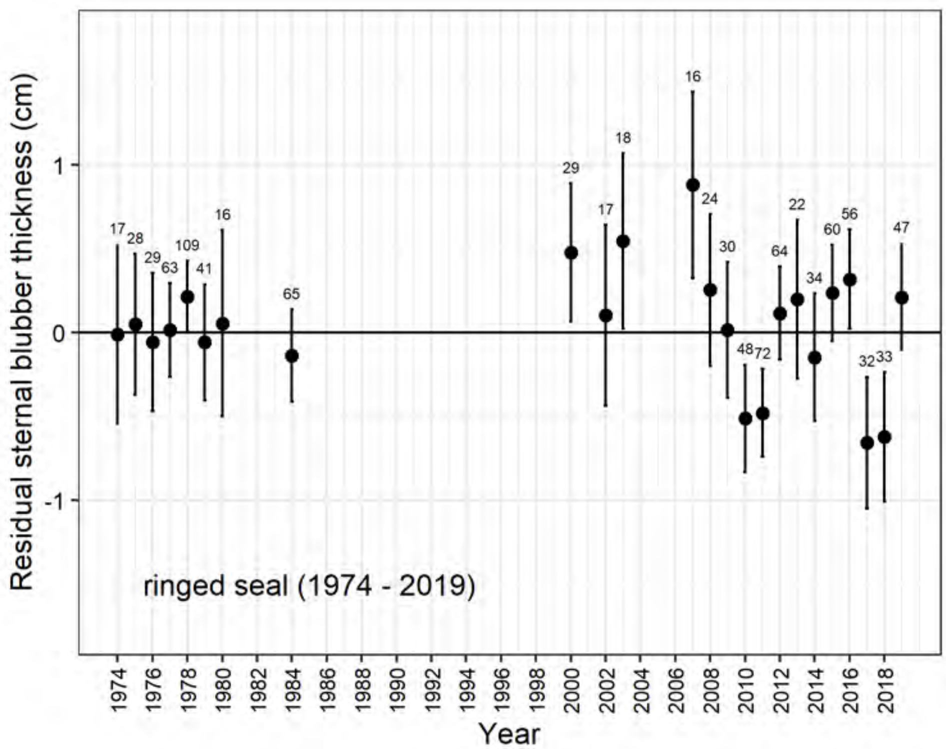


Figure 4. Mean residual sternal blubber thickness for ringed seals by year of harvest after accounting for month and age group. The error bars represent the 95% confidence interval. The number of seals analyzed each year is listed above the 95% confidence intervals. Only years with ≥15 samples were included in the analysis.

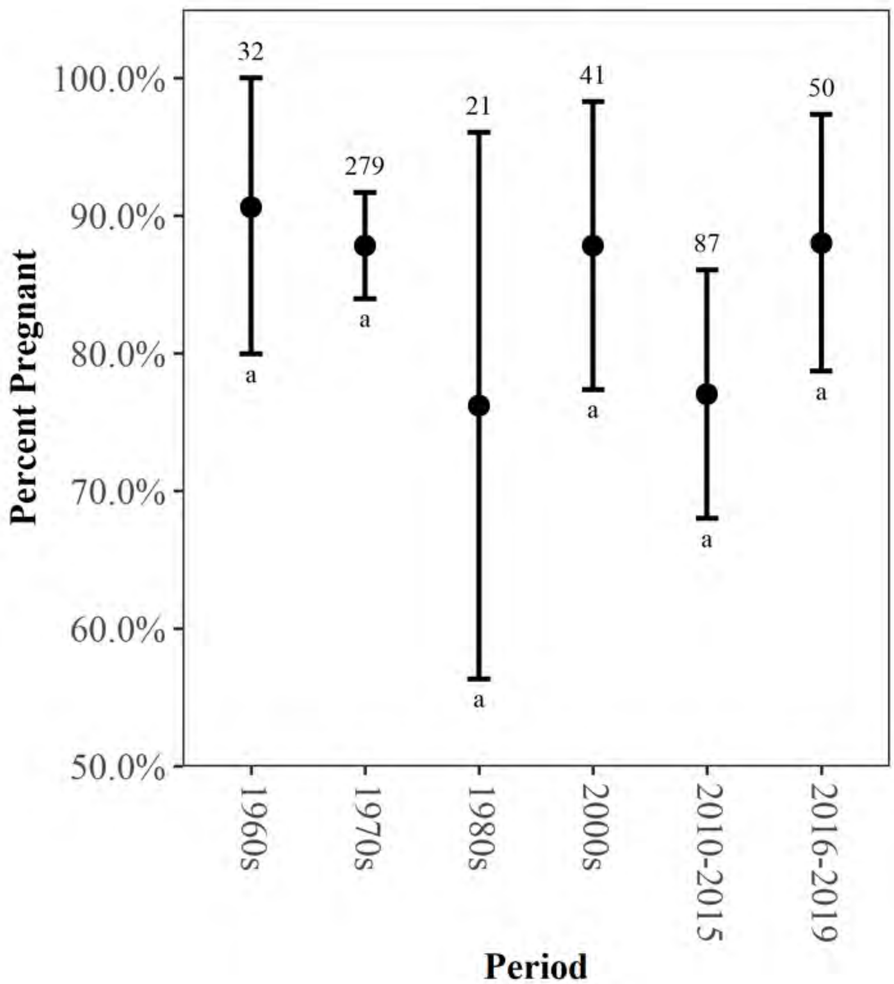


Figure 5. Pregnancy rates for mature ringed seals summarized by period. Seals were harvested for subsistence at 11 villages along the Bering, Chukchi, and Beaufort sea coasts in Alaska (1963–2019). The number of seals analyzed each period is listed above the 95% confidence intervals. There were no significant differences among periods ($p < 0.05$).

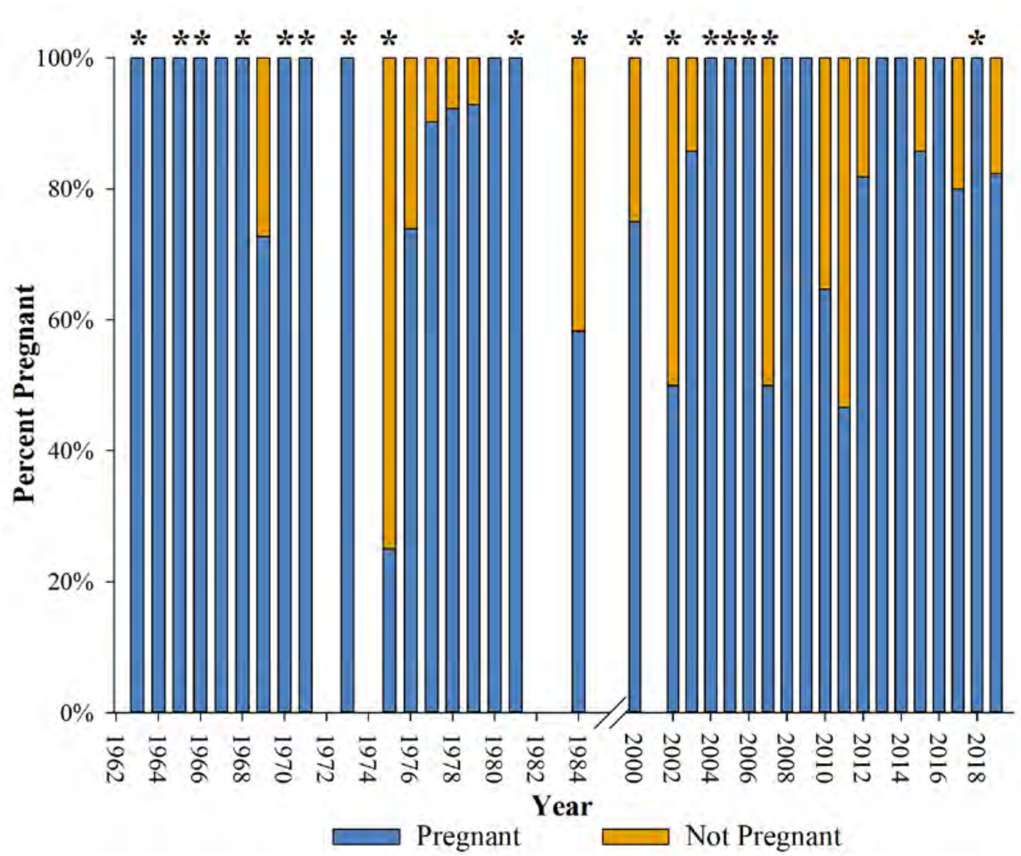


Figure 6. Annual pregnancy rate for mature ringed seals harvested for subsistence at 11 villages in Alaska along the Bering, Chukchi, and Beaufort sea coasts of Alaska (1963–2019). Asterisks ("*") designate years with samples sizes < 7 seals.

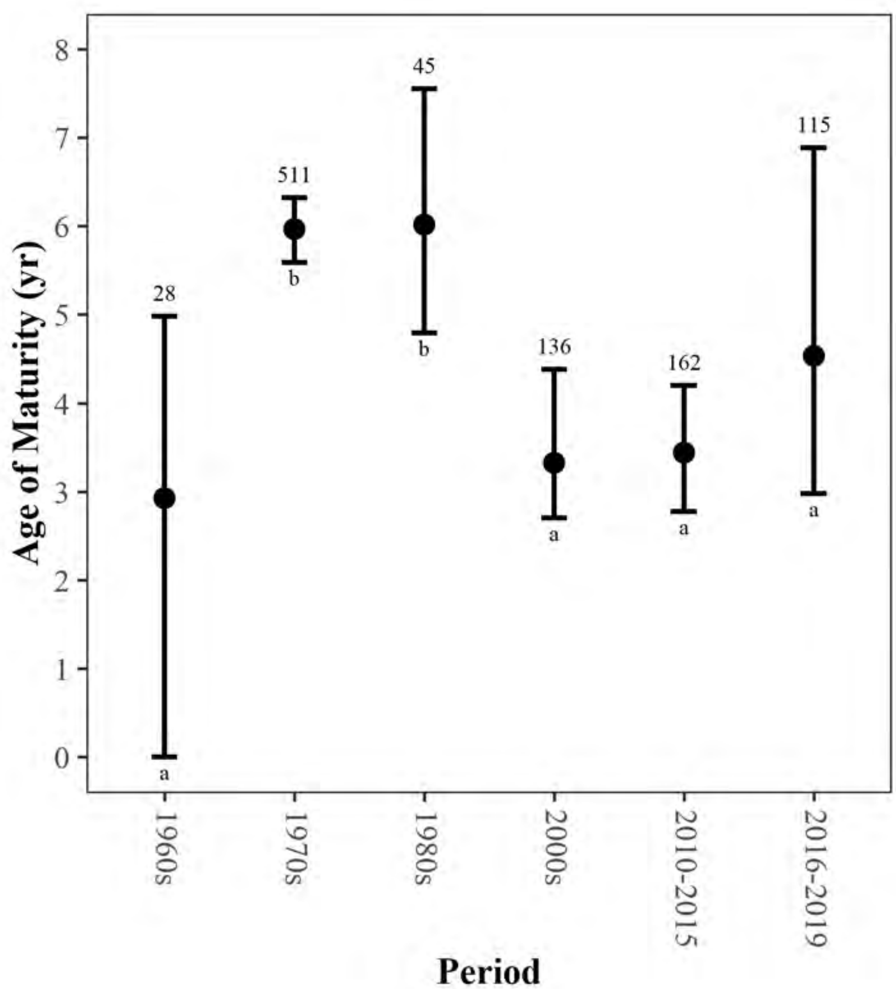
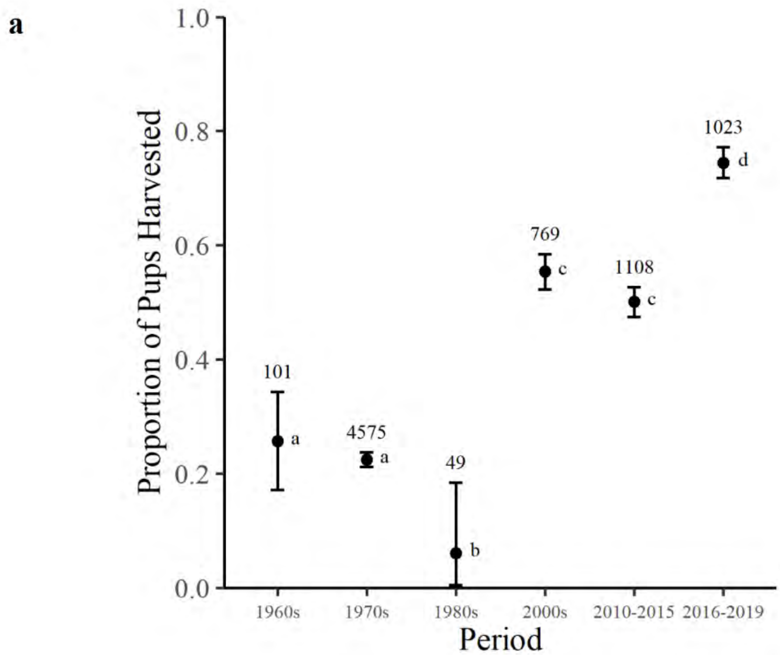


Figure 7. Mean age of maturity for ringed seals summarized by period. Seals were harvested for subsistence at 11 villages along the Bering, Chukchi, and Beaufort sea coasts in Alaska (1963–2019). The number of seals analyzed each period is listed above the 95% confidence intervals. Letters listed below indicate significant differences ($p < 0.05$).



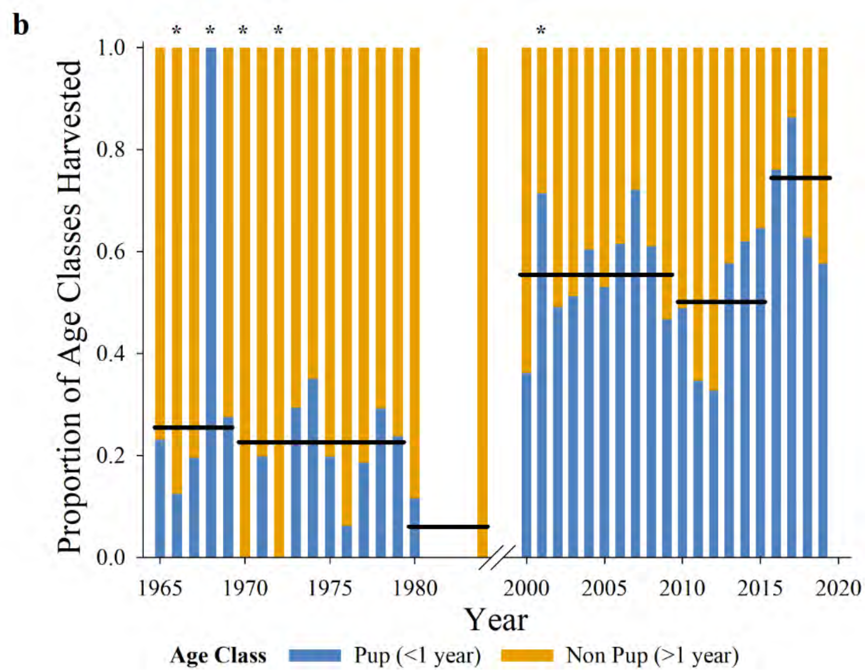


Figure 8. (a) The mean proportion of ringed seal pups harvested during the subsistence hunt at 12 villages along the Bering, Chukchi, and Beaufort sea coasts in Alaska, prior to (1960s, 1970s, 2000s, and 2010–2015) and during the project period (2016–2019). The number of seals analyzed each period is listed above the 95% confidence intervals. Letters listed next to means indicate significant differences ($p < 0.05$). (b) The annual proportion of age classes of ringed seals harvested. Asterisks ("*") designate the 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).