

ENVIRONMENTAL IMPACTS ON REPRODUCTIVE RESPONSES OF PACIFIC WALRUSES
(*ODOBENUS ROSMAREUS DIVERGENS*) AND SUBSISTENCE USERS OF ST. LAWRENCE ISLAND

By

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A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy

in

Fisheries

University of Alaska Fairbanks

August 2020

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Abstract

An interdisciplinary approach is used in understanding change and resiliency in St. Lawrence Island (SLI) resources and resource users throughout this dissertation. Historically SLI inhabitants have relied on the Pacific walrus (*Odobenus rosmarus divergens*) for their survival and this resource is still highly valued for cultural and dietary purposes. The responses of Pacific walruses and SLI subsistence users to environmental change was analyzed. In walruses, reproductive capacity was analyzed using an anatomical approach as well as reproductive plasticity which was determined using a physiological approach to characterize their estrus cycle. A suite of anatomical measurements were developed to characterize reproductive capacity of walruses by analyzing ovaries from three distinct time frames during a 35-year period. Reproductive capacity was reduced during time frames when carrying capacity (K) was reached and when large environmental changes occurred in the Bering Sea, including years of very low sea ice extent. Reproductive capacity was high in times when K was lower and harvest levels were greater. Our results explained how perturbations in K and environmental changes may have influenced reproductive capacity of the population in the past. Endocrine techniques were used in ovarian tissues to determine if progesterone and total estrogens are useful indicators of female reproductive status in walruses harvested during the spring hunt. Progesterone and total estrogen concentrations were greater in the reproductive tissues of unbred and pregnant females than postpartum females, however neither hormone could distinguish between pregnant and unbred animals. These results provide the first physiological evidence for pseudopregnancy in this species, rather than a postpartum estrus. Lastly, discussions were held with SLI community members to determine changes in key subsistence resources and community resiliency with regard to food security. Walruses ranked highest among key resources. Stakeholders reported limited access and increased effort to hunt walruses, changes in crab abundance, and increases in commercially exploitable fish stocks. Changes were attributed to loss of sea ice, “bad” weather, and climate change. In order for SLI communities to continue their subsistence-based way of life, inhabitants may need to expand their diet to include less-preferred food items in place of harvested

ice-associated species. In conclusion, loss of sea ice and rapid environmental changes in the Bering Sea have the potential to greatly impact walrus reproduction and the marine subsistence way of life that is practiced by SLI stakeholders.

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Acknowledgements

My first thanks go to the communities of Gambell and Savoonga; without them this work would have never been possible. I thank all of the hunters and participants that have contributed with biological sampling, time, knowledge and stories that you have shared with me. I am deeply thankful to the residents of Gambell who have shared with me their food, language and landscape. Thank you for taking me on Honda rides and encouraging me to participate in your Eskimo dances. A special thank you goes to Petu Koonooka for being my friend and inviting me into your home to enjoy tea or even a meal of polar bear, and for always replying to my emails and messages with your help and insight. I hope to continue working with these St. Lawrence Island communities for years to come.

This research would not have been possible without the guidance and support of my advisor (Shannon Atkinson) and committee members (Corey Fugate, Gordon Kruse and Nick Pyenson) and co-author (Sarah Wise). Shannon, your positive, flexible demeanor and can-do attitude is one I hope to embody as a scientist, mentor and person throughout my career. Thank you for approving so many of my ambitious ideas ranging from learning Russian, to taking on a social science project, to starting my own business, and my many other jobs and adventures, all while being your student. Corey, thank you for assisting me with laboratory work and serving as such a wonderful mentor. Your passion for science is evident and I have greatly enjoyed working with you. Gordon, I appreciate all of your statistical help, your patience and kindness with which you teach others and the thorough edits you have always provided me. Nick, thank you for sharing a cab with me to the airport in Anchorage so that I could tell you all about walrus ovaries. Had we not met in Sitka I doubt I would have upgraded my project to a doctoral one. Thank you for believing in me and making me feel capable of great things.

Many other collaborators have been instrumental to my Ph.D. I would like to highlight the College of Fisheries and Ocean Sciences staff Gabrielle Hazelton and Chris Brooks for navigating the challenges of forms, programs and computer software. Gratitude is owed to the Atkinson Lab including Angie Steeves as well as Kendall Mashburn for her wisdom in endocrine techniques. I would like to thank

the group at the Alaska Fisheries Science Center (Sarah Wise, Lisa Hiruki-Raring, Maggie Mooney-Sues, Kim Sparks and Lyle Britt). Thank you for hosting me, assisting me with funding opportunities, connecting me with contacts and resources and providing me with feedback on my work. I especially thank Sarah Wise who introduced me to qualitative social science and assisted me with sampling design, data collection, data analysis and much more for my third chapter. I have loved this process and cannot wait to use this new skill set as an interdisciplinary researcher. I would also like to thank collaborators at US Fish and Wildlife Service (Jonathan Snyder and Brad Benter) for their assistance in permitting, inviting me to annual walrus hunter meetings, fieldwork and replying to my every email regarding animal ages, harvest numbers and literature. Brad, thank you for introducing me to the many contacts in Gambell whose respect you have gained with more than 20 years of experience. I appreciate you teaching me how to use a snow machine. I am sorry that I threw you off the Honda, please hold on next time. Additional gratitude is owed to the Museum of the North, especially Link Olsen and Aren Gunderson. Thank you for loaning me your ovaries, providing me with data and transporting many buckets of formalin on my behalf.

A great variety of funding sources have made this work possible. This publication is the result in part of research sponsored by the Cooperative Institute for Alaska Research with funds from the National Oceanic and Atmospheric Administration under cooperative agreement NA13OAR4320056 with the University of Alaska. I would like to thank the Rasmuson Fisheries Research Center and its board members for two years of student support. Other funders included: the Resilience and Adaptation Program, the Erich Follman Memorial Student Research Support Scholarship, NSF's EPSCoR Internship, an Internship at the Smithsonian, the North Pacific Research Board Graduate Student Research Award, the Coastal Marine Institute/Bureau of Ocean Energy Management and the Marine Mammal Commission (Grant #: MMC19-172). Additional support has been provided by two Teaching Assistantships through the University of Alaska Southeast under the guidance of Dave Tallmon and Sherry Tamone. Additional sources of student support were provided by the Alaska Department of Fish and Game and the College of

Fisheries and Ocean Sciences for which I thank Mandy Keogh and Casey McConnell respectively for hiring me and training me in laboratory and further field sampling techniques.

Additional thanks is owed to those that have supported me personally throughout this journey. Thank you to my lab mates Kelly Cates, Valentina Melica and Dan Olsen with whom I have attended conferences, shared hotel rooms and bounced ideas off of—thank you for making me smile, laugh and dance. Thank you to my brother Jared, our time on the Hardship will always be savored as a favorite summer that we shared. Thank you for the help, support and many adventures. To my husband Mattheus, thank you for always supporting me, for being forgiving of my messiness and encouraging me to get work done while you went off to do fun things. Thank you for being my lifeline, both figuratively and literally. I look forward to spending more of my evening and weekend hours with you when this commences.

Lastly, I acknowledge and thank my parents who have cultivated my love of the natural world from a young age. Dad, my earliest memories with you are being strapped to your back taking in cattails over our heads as you tromped through wetlands in pursuit of waterfowl. I have become a hardy field biologist thanks to these outings with you, and your slogan “dress for it,” which rings in my ears every wet, cold, blustery day I find myself outside. Mom, thank you for your steadfast support for all things in life, but most especially for believing in me and assisting me in pursuing this dream. Thank you for taking me on field trips, proof reading applications, letting me attend a university a thousand miles away and all of the sacrifices you have made so that I could make this passion my career. This volume is dedicated to you mom and to my sweet Lidia Lou whose companionship and loyalty has been unwavering.

General Introduction

The Bering Sea provides habitat for nearly 30 marine mammal species (Ray et al. 2014), many of which serve as important dietary resources to coastal Alaska Native communities including: bowhead whales (*Balaena mysticetus*), belugas (*Delphinapterus leucas*), walruses (*Odobenus rosmarus divergens*), bearded (*Erignathus barbatus*), ringed (*Phoca hispida*), ribbon (*Phoca fasciata*), and spotted seals (*Phoca largha*), and to a lesser extent polar bears (*Ursus maritimus*). St. Lawrence Island (SLI) is the largest island in the Bering Sea and is home to two Alaska Native communities: Gambell and Savoonga. The inhabitants of SLI are Siberian Yupik (hereafter Yupik) who rely heavily on marine resources from the Arctic and sub-Arctic as part of their dietary needs and cultural identity. Among these various marine resources, walruses have been tightly coupled with SLI people for nourishment, housing, equipment and overall survival for at least the last 2000 years (Hill 2011). Rapid reductions in sea ice and increases in air and seawater temperatures have been well documented in the Arctic in recent decades (Onarheim 2018, Stroeve et al. 2007, 2012, Polyak et al. 2010, Steele et al. 2008, Comiso 2012, Stroeve & Notz 2015, 2018, Hamilton & Stroeve 2016). Of concern in the Bering Sea, are warming waters and a continuing trend in decreasing sea ice formation. For the first time on record, data from a 2018 National Oceanic and Atmospheric Administration (NOAA) trawl survey in the southeastern Bering Sea showed a lack of the “cold pool,” a thermal barrier that isolates fish and invertebrate species from transiting between the northern and southern Bering Sea (Stabeno et al. 2019). This may have widespread impacts on productivity and predators feeding on benthic prey such as walruses, bearded seals and grey whales (*Eschrichtius robustus*), as well as top trophic level predators such as polar bears and killer whales (*Orcinus orca*).

For walruses, the impacts of sea ice loss could be many. The Pacific walrus relies on sea ice for resting, birthing and nursing platform and as a means of passive transportation to extensive feeding grounds in the Bering and Chukchi Seas (Fay 1982). Historically, the entire Pacific walrus population wintered in the Bering Sea and during the summer months, although most males and females with young

were geographically segregated (Ray et al. 2006). Males utilized terrestrial haulouts along the coast of the Bering Sea, while juvenile males and adult females and their young continued to employ ice floes and were consequently transported to new feeding grounds in the Chukchi Sea during summer (Fay 1982). There is now concern about the health and population status of this species as large herds of both adult males and females with their young are being observed hauling out at terrestrial sites during late summer in the Northeastern Chukchi Sea (Fay & Kelly 1980, Ovsyanikov et al. 2008, Fischbach et al. 2009). While this observation has made a large splash in public news recently, this trend was first documented by scientists and inhabitants of the Arctic some 35 years ago (Fay et al. 1990). Historically, this change in haulout location was attributed to extensive sea ice covering the Chukchi Sea and restricting open water access to prey items (Fay et al. 1989). However, the current understanding of this behavior is that it is due to a lack of sea ice in the Arctic (Polyak et al. 2010, Jay et al. 2012). Of great concern during these large terrestrial haulout events is the trampling of calves and juveniles by adult animals during mass movement of the herd, often the result of disturbance (Fay & Kelly 1980, Fischbach et al. 2009).

In addition to increased calf and juvenile mortalities, the lack of ice floes in the Bering and Chukchi Seas could result in several consequences for Pacific walruses regarding foraging and prey items. Walruses feed on a wide variety of benthic organisms including both invertebrates, such as bivalves, gastropods and polychaetes, and, less commonly, vertebrates such as fishes, sea birds and seals (Sheffield et al. 2001, Sheffield & Grebmeier 2009). As sea ice melts and more walruses are recorded hauling out on land, there is potential that they will (1) deplete the food resources close to these land haulouts (2) forage further distances from their haulouts; and/or (3) alter their diet to attempt to meet their metabolic needs. These changes could affect the productivity of the walrus population, the productivity and biomass of shellfish and other prey species populations, and possibly other pinniped populations. Any of these changes alone or any combination could affect the energy balance, life history and/or fitness of Pacific walruses, and, consequently, could affect the reproductive success and carrying capacity of Pacific walruses.

While recent studies have estimated population dynamics and reproductive rates using a statistical modeling approach (Taylor et al. 2018, Udevitz et al. 2013, Taylor & Udevitz 2014), these estimates do not include measures of reproduction from an anatomical approach. The majority of literature published on Pacific walrus reproduction prior to Garlich-Miller's effort in the 1990s (Garlich-Miller 1997, Garlich-Miller et al. 2006), occurred in the 1970s and 1980s (Fay 1982, Fay & Stoker 1982, Fay 1985). As Arctic habitat has undergone drastic changes since these studies were conducted, we hypothesized that the carrying capacity of the Pacific walrus has decreased and that this decrease is associated with the loss of sea ice in the Bering, Chukchi and Beaufort Seas. At this point it is not clear if the changes in foraging and energy requirements associated with loss of sea ice will be manifested in changes in age-specific survival or reproductive rates. Reproductive rates of this species are lacking and, therefore, current management practices do not account for reproductive rates in models used to estimate population size (MacCracken et al. 2017). Quantifying reproductive success in the past may be useful to understand how the population responds to changes in both their environment and carrying capacity and may be used to make predictions.

Further, little is known about the reproductive physiology of the Pacific walrus. Fay (1982) determined that mating occurs December-March and calving 15-16 months later, the following April-June. Yet there have been several documented reports of calves being born outside of this established birthing window (Fay 1982). It is unknown if these instances were premature births or they provide evidence of animals successfully mating and birthing outside of the established breeding window. If walruses exhibited plasticity in the time in which they mate and give birth, this ice-obligate species might be able to cope with the loss of ice by giving birth earlier in the year, when sea ice platforms are still abundant. Fay (1982) used histology to determine the timing of male spermatogenesis and gross dissections of ovaries to determine the timing of estrus in females. His studies found that some females exhibited corpora lutea (CL) indicative of ovulation following the birthing window, demonstrating that this species may exhibit a postpartum estrus as is seen in most other pinnipeds (Atkinson 1997). Yet the

function of this estrus is mysterious as Fay found his sample of males to be infertile during this window. Today, the field of endocrinology provides new tools to further investigate the hormones that govern reproduction, making it a useful method for determining whether this species has plasticity in their breeding and birthing windows, which could be advantageous in responding to environmental change.

Reproductive failures due to loss of sea ice may have large consequences on walrus and Alaska Native communities that rely on this resource for dietary and cultural purposes. Already, literature shows that climate change has impacted Alaska Native hunters' ability to access this resource. The total number of walrus harvested across Bering Sea communities has plummeted in recent years (Krupnik & Benter 2016) and SLI hunters have reported earlier and shorter ice-break ups in the spring (Metcalf & Krupnik 2003). Poor harvest levels are cause for concern in communities like Gambell and Savoonga where subsistence foods are relied on for food security and well-being.

The goals of this dissertation are to: 1) better understand the reproductive responses and resiliency of the Pacific walrus to environmental change; and 2) understand how the people of SLI are perceiving and responding to perturbations in this region with a primary focus on Pacific walrus. In the first two chapters of my dissertation I assess reproductive capacity using anatomical and physiological approaches. The third chapter uses a human dimensions approach to understand how sea ice loss is affecting the ability of SLI inhabitants to harvest marine resources.

Chapter 1 uses walrus ovaries from three distinct time frames to analyze how the reproductive capacity of the species has fluctuated with changes in their environment and carrying capacity. A suite of anatomical measurements was developed to assess reproductive capacity. These measurements included using ovarian weights and volumes, total corpora counts and measuring the proportion of reproductively active females within the sample. Our results found reproductive capacity was limited when the population was at or near carrying capacity and that reproductive capacity may be influenced by environmental drivers and stressors. This study demonstrates that this suite of measurements is a useful

tool for determining the reproductive capacity of the species and suggests its utility for continued monitoring of reproduction in the already established Walrus Harvest Monitoring Program (WHMP).

Chapter 2 uses a physiological approach to determine whether hormone concentrations can be used to determine reproductive status of harvested female walrus and assesses reproductive plasticity in the timing of estrus. Enzyme immunoassays and radioimmunoassays were used to profile total estrogens and progesterone in ovarian tissues. This is the first study to validate the use of these reproductive hormones in tissues of free-ranging walrus. Our results shed light on the reproductive physiology of this species and provide evidence that endocrinology is a promising tool for determining reproductive status.

Chapter 3 focuses on the perceptions that SLI community members have on how sea ice loss is impacting marine resources harvested for subsistence. This chapter explores the key marine resources valued on SLI today and the changes that these resources are undergoing from the perspective of the resource users. The way participants are responding to change is documented and used to determine the resiliency of SLI inhabitants and make predictions about the future of this subsistence-based culture.

In summary, this dissertation focuses on change and resiliency in the Pacific walrus, the key resource utilized by SLI residents, and SLI communities themselves. Our work employs new techniques to measure reproductive capacity and plasticity in female walrus, which have been used to make management recommendations and encourage continued monitoring. Important insights into the perturbations of the social ecological system on SLI are also documented and it is our hope that this information will also inform policy of needed actions to improve the resiliency of those that live there.

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Chapter 1. Pacific walrus (*Odobenus rosmarus divergens*) reproductive capacity changes in three time frames during 1975-2010 ¹

1.1 Abstract

An anatomical approach was used to assess changes in the reproductive capacity of Pacific walruses (*Odobenus rosmarus divergens*). Ovarian weights and volumes, corpora lutea diameter, total number of corpora lutea and albicantia, and the percent of females ovulating in their current cycle were used to evaluate reproductive capacity. Ovaries were collected from walruses hunted for subsistence purposes during three distinct time frames over a 35-year period: 1975, 1994-99 and 2008-10. A pilot study was conducted to assess effects of preservation method; no differences existed. There were no differences in ovarian weights or percent of quiescent females between 1975 and 2008-10. Ovaries from 1994-99 were significantly heavier, exhibited more corpora and all females from this time frame were ovulating at the time of harvest. Reproductive capacity was limited during 1975, due to known density-dependent stressors. Reproductive capacity increased during 1994-99 as harvests increased and more resources became available. Walruses sampled during 2008-10 were as reproductively limited as those of 1975. The cause for this reduction in reproductive capacity is unknown, but may be a result of an increase in population size, habitat changes, including sea ice loss or cumulative stressors relating to contaminants and anthropogenic impacts. This study highlights the use of long-term community-based monitoring programs to understand reproductive responses to changes in the environment and carrying capacity of this species.

¹ Larsen Tempel J, Atkinson S. 2020. Pacific walrus (*Odobenus rosmarus divergens*) reproductive capacity changes in three time frames during 1975-2010. Prepared for submission to Polar Biology.

1.2 Introduction

Pacific walrus (*Odobenus rosmarus divergens*) are important dietary and cultural resources for Alaska and Russian Natives and make up approximately 90% of the world's walrus population (Fay 1982). The general range of the Pacific walrus population includes the continental shelf of the Bering and Chukchi Seas, occasionally including the Alaskan Beaufort Sea and westward into the East Siberian Sea (Garlich-Miller et al. 2011, USFWS 2014). Rapid reductions in sea ice and increases in air and seawater temperatures have been well documented in the Arctic in recent decades (Serreze et al. 2007, Stroeve et al. 2007, 2011, 2012, Polyak et al. 2010, Steele et al. 2008, 2010, Comiso 2012, Stroeve & Notz 2015, Hamilton & Stroeve 2016). Of concern is that much of the reduction in sea ice has occurred over the Chukchi Sea and continental shelves along Arctic marginal seas in Alaska and Russia, where Pacific walrus do the majority of their foraging (Serreze et al. 2007, Steele et al. 2008, Stroeve et al. 2011, 2012, Comiso 2012).

Sexually mature females are likely to be most vulnerable to changes in sea ice associated with foraging, as the energy budgets of lactating females are twice that of non-reproductive females (Noren et al. 2012, Jay et al. 2012). Females normally give birth to one calf. Twinning is extremely rare and has been reported only three times in over 1000 births reported by both Russian and US scientists; there are no confirmations of females successfully rearing twins (Fay 1982). Female walrus become sexually mature between ages 4-10 years old (Fay 1982) and the age of first birth varies with population abundance and animal health (Eberhardt & Siniff 1977). Sexual maturity is reached when a female ovulates, indicated by the presence of a corpus luteum (CL) within the ovary (Perrin and Reilly 1984, Tetsuka et al. 2004, Tarpley et al. 2016, Kesselring et al. 2017). CL form regardless of conception and eventually regress into corpora albicantia (CA), hard, fibrous scars within the ovary (Iwasa & Atkinson 1996). In walrus CA are known to be long-lasting and, therefore, counting the total number of corpora (CL and CA) within both ovaries can serve as an index of the number of reproductive events during a female's lifespan (Boyd 1984, Iwasa & Atkinson 1996). If a female produced fraternal twins her ovaries

would have an additional corpora, however the instances of twinning are so rare we considered the probability of sampling a female bearing twins to be close to zero. Corpora have been used a measure of reproductive activity in other marine mammals including: bowhead whales (*Balaena mysticetus*) (Tarpley et al. 2016), minke whales (*Balaenoptera acutorostrata*) (Tetsuka et al. 2004) and California sea lions (*Zalophus californianus*) (Colegrove et al. 2009).

Reproductive studies have been conducted on Pacific walrus in previous decades to calculate reproductive rates and success (Fay & Stoker 1982b, Fay et al. 1989, Garlich-Miller 1997) as well as in Atlantic walrus (Garlich-Miller & Stewart 1999, Born 2001); yet, it has been two decades since reproductive success has been measured in this population using an anatomical approach. In this study we measure reproductive capacity which we define as a more encompassing measurement of reproductive success than reproductive rates. It is important to assess reproductive capacity using a multi-decadal comparative evaluation to better understand how reproductive capacity of female walruses is impacted by rapid changes in their environment and carrying capacity. We define reproductive capacity as a female's ability to conceive.

The aim of this study was to assess how walrus reproductive capacity has changed over a span of 35 years analyzing ovaries from three time frames: 1975, 1994-99 and 2008-2010. Information on the abundance (Speckman et al. 2011, MacCracken et al. 2014) and environmental conditions and haulout behavior of walruses (Kavry et al. 2008, Metcalf & Robards 2008, Semenova et al. 2010, Fischbach et al. 2009, Fischbach et al. 2016), differs during these time frames making them distinct. To assess reproductive capacity, ovarian weights and volumes were measured, and the number of CL and CA present in pairs of ovaries were determined, along with the percent of females ovulating in their current cycle. Measurements of ovarian weights and volumes relate to reproductive capacity as indicators of a follicular reserve or ovulatory activity. In human (*Homo sapiens*) research there is a positive correlation between ovarian volumes and non-growing follicles in pre-menopause females ages 25-51 and ovarian volumes steadily decline thereafter (Kelsey et al. 2013). In cervid ovaries, ovarian weights and volumes

have been shown to indicate reproductive activity. Gastal et al. (2017) found that the ovarian weight and volume of sexually mature white-tailed (*Odocoileus virginianus*) does was two times and 1.6 times greater, respectively, than in female fawns. We hypothesized that Pacific walrus experienced significant changes in reproductive capacity during 1975-2010 in response to changes in population abundance, density dependence, and rapid environmental changes in their habitats.

1.3 Materials and methods

1.3.1 *Sample collections*

Ovaries analyzed in this study were provided by the University of Alaska (UA) Museum of the North and were obtained from the Walrus Harvest Monitoring Program, managed by the US Fish and Wildlife Service (USFWS). A list of museum specimens has been provided in the appendix (Appendix 1.1). Samples were collected in the Alaskan communities of Gambell and Savoonga on St. Lawrence Island, as well as Little Diomed Island (hereafter Diomed) (Fig. 1.1) by Alaska Native subsistence hunters. Despite the large range of the Pacific walrus, the population is genetically homogenous (Scribner et al. 1997, Shitova et al. 2017). Ovaries from hunted females were removed onsite. Samples were stored frozen until transported to the laboratory.

Ovaries selected for analysis were based on completeness of the sample, which included an animal identification number, the presence of both ovaries intact, and age of the female; the number of complete samples varied by time frame. A list of complete samples from each of the three time frames - 1975, 1994-99 and 2008-10 - was compiled and a random number generator selected samples from each group. An effort was made to collect ovaries from the spring hunts in 2015 and 2016 on St. Lawrence Island; however, the hunts were poor and 2015 was one of the worst on record. Thus, not enough samples were collected to be able to compare the reproductive capacity of these animals to those of former time frames, rather these samples were used in the pilot study to determine differences in tissue distortion due to various preservation methods.

Age was estimated by counting growth layers in the cementum of teeth, and was made available by the Alaska Department of Fish and Game (ADF&G), USFWS and museum records. Age classes were divided into 4 groups: 8-11, 12-15, 16-19 and 20+ years of age. Non-calf females younger than 7-8 were rarely taken, thus they were excluded from this study. Fay (1982) determined that approximately 10% of female walruses reach sexual maturity as early as 4 years old, 68% ovulate for the first time by their 6th year, and 100% are fertile by age 10. Females are thought to decrease in reproductive activity at 16-18 years of age (Burns et al. 1990). Our selected age classes aimed to target even numbers of females at age of first reproduction, females at peak reproductive activity, and females with diminished reproductive activity per time frame (Table 1.1).

The communities of Gambell and Savoonga are known to select for mother-calf pairs, which are considered a delicacy. However, hunter bias did not likely impact our sample selection as reports from the WHMP over the past 45 years indicate that hunter selection bias has remained fairly consistent in the Bering Strait region (Dickerson et al. 1996, Garlich-Miller et al. 2006) and hunters likely select for the healthiest segment of the population. Furthermore, our sampling of females of similar ages across timeframes would remove any age-related biases.

1.3.2 Laboratory analysis

In the present study we defined an ovary containing a CL as the active ovary and the ovary lacking a CL as the inactive ovary. Ovaries were sectioned and analyzed following Fay & Stoker (1982a), with the exception that ovaries were not soaked in water prior to handling. Most ovaries were sectioned into approximately 2-3 mm slices as is consistent with other published literature on the species (Fay & Stoker 1982a, Garlich-Miller & Stewart 1999, Born 2001) as well as other marine mammals (Garde et al. 2015, Chambellant et al. 2012). Ovaries were generally sectioned longitudinally, though some were sectioned latitudinally. Laboratory analysis of each pair of ovaries involved making gross observations of each section of tissue and counting the number of CL and CA, which were added together to obtain a total corpora count for each animal. Total corpora counts served as an index of the number of ovulations of a

female over her adult lifespan, which in addition to corpora diameters, ovarian volume and percent of the females that were reproductively active, we defined as reproductive capacity. To keep corpora counting methods consistent, the same researcher observed all ovaries and counts were conducted a single time. The corpora of samples from 1994-1999 were previously counted and recorded (Garlich-Miller 1997). The counts of corpora from individual females in this study were compared to those recorded by biologists in 1994-1999 and no significant differences were found between corpora counts of the same animals between studies (Wilcoxon test $Z = -0.72$ $df = 2$, $p = 0.4698$). This gave us confidence that counting error was minimal.

Both ovarian structures, CL and CA, were grossly determined by several morphological criteria. CL: distinguished by their large size (often taking up 1/3 to 1/2 of the ovarian size), spherical shape, firm texture and often yellowish color; CA: small in size, fibrous in texture, inconsistent in shape and usually white in color. CL diameter was measured in mm across its longest plane in a single longitudinal section using dial calipers. A female not reproductively active in her current cycle lacked a CL and was considered quiescent. Our definition of a reproductively active female does not include females that may have had a nursing calf from the previous year as this information was not available. Accordingly, we limit the term “reproductively active” only to females with CL present in their ovaries. Length, width and depth of the ovary were measured to the nearest millimeter using the dial calipers; weight was recorded to the nearest tenth of a gram. Length (L), width (W) and depth (D) of the ovaries were used to determine volume, which was calculated using the prolate ellipsoid formula ($L \times W \times D \times 0.523$), developed for human research (Pavlik et al. 2000) as ovarian shape and structure is similar across mammalian species (Yao & Bahr 1998).

Several accounts exist of newborn calves being observed outside of the typical birthing window, April-June (Fay 1982). A female would have to be ovulating and breeding beyond the winter months in order to give birth later or earlier in the year. To determine if our samples may have been from females ovulating late, we conducted a subjective size assessment on developing follicles of all females. Follicle

diameter was measured in mm across its longest plane using dial calipers and was recorded as small (≤ 1 mm), medium (2-3 mm) or large (≥ 4 mm), as these size classifications have been used in pinnipeds and sheep (*Ovis aries*) (Atkinson et al. 1986, Atkinson et al. 1994).

1.3.3 Pilot study

Two different ovary preservation techniques have been used. Ovaries from the 1994-99 and 2008-10 time periods were fixed and preserved in 10% buffered formalin. Ovaries from 1975 were preserved in 70% ethanol, but may have been originally fixed in formalin; however, records were lacking. Fixation in formalin stabilizes proteins allowing tissues to retain the characteristics of the living specimen, yet it is common for researchers to preserve specimens directly in ethanol without formalin fixation (Gaston et al. 1996). Qualitative differences in color and size between the ovaries from the oldest time frame and the two newer time frames were apparent. The ovaries preserved in ethanol from 1975, were noticeably smaller, more brittle and typically darker in color (Fig. 1.2). A pilot study was conducted using fresh/frozen ovarian tissues to determine whether preservation method was the cause for obvious differences in ovary size.

Two trials were conducted in the pilot study to test for effects of two different preservation methods. For each trial, ovaries were remeasured approximately 2 weeks, 5-8 weeks and 20 weeks after preservation began. The first trial measured ovarian tissues fixed in 10% buffered formalin, then transferred to 70% ethanol for preservation. Twelve pairs of ovaries from the 2016 spring hunt on St. Lawrence Island were stored frozen at -20°C . Fresh/frozen ovaries were thawed, weighed and measured, then submerged in 10% buffered formalin and allowed to fix for 19 days. Afterward, ovaries were removed, excessive liquid was removed and they were reweighed and remeasured prior to being transferred to 70% ethanol. Ovaries were reweighed and remeasured after 33 and 146 days in ethanol. In the second trial, nine ovarian tissues from animals hunted in 2011, 2015 and 2016 were used. The thawed tissues were weighed and measured prior to being preserved in 70% ethanol for 14, 57 and 143 days, at which time they were remeasured and reweighed.

1.3.4 Statistical analysis

Most ovaries sampled in 1975 were from walrus hunted near Diomede, whereas most samples from the latter two time frames were from walrus hunted near St. Lawrence Island (Table 1.2). Differences in the timing of hunts were due to availability of walrus and conditions appropriate for boating, both of which were linked to the extent of sea ice and open water. Diomede is located in the northern Bering Sea (Fig. 1.1), resulting in the spring breakup of sea ice being several weeks later than near St. Lawrence Island. To assess ovarian volume and weight across time we needed to adjust for variation in harvest date as it related to parturition. Hunters in Gambell, on St. Lawrence Island, typically observe the first wave of the walrus migration during the first and second weeks of May, with the peak of the migration around May 15th (Ray et al. 2016). Accordingly, the median hunt date for samples from both 1994-99 and 2008-10 was May 17th and the median hunt date for 1975 was 3 weeks later (June 7th). Ovarian sizes decrease after parturition as the CL regresses and becomes a CA (Iwasa & Atkinson 1996). Thus, it is likely that ovarian sizes were larger in May than June as peak calving for Pacific walrus is mid-May (Fay 1982) coinciding with estimated peak migration date. To adjust for weight and volume loss within ovaries, simple linear models were fit using least squares to both volume and weight versus hunt date in the forms:

$$W_a = a + bt \text{ and } V_a = a + bt$$

where W_a is the adjusted ovarian weight (g) at the median hunt date, V_a is the adjusted ovarian volume (cm^3) at the median hunt date, a is the original ovarian weight (g) or volume (cm^3), b is the slope expressed as the mean ovarian weight (g) or volume (cm^3) lost per day and t is the time (days) of hunt from the median hunt date, May 17th. This was conducted separately for active and inactive ovaries and resulted in four different regression lines with four different slope values, all of which were decreasing (Fig. 1.3). This linear model operated under the assumption that ovarian weight and volume decreases consistently across females regardless of harvest period. This is convincing as the Pacific walrus is one

homogenous stock and there is no documentation that birthing times differed during the 35 years in which animals were sampled for this study.

Statistical analyses and plots were performed in RStudio, version 3.6.1 (R Studio Team 2019). Ovarian weights and volumes used in the pilot study failed to meet normality assumptions for parametric tests, even when log transformed, therefore Friedman tests were conducted to compare the effects of formalin and ethanol preservation. Because the main point of interest was to determine how ethanol and formalin fixation may have affected the original ovarian weights and volumes, only the first and last measurements of each trial are presented in the results. These pairwise comparisons were assessed using Durbin-Conover tests. Data for ovarian weights, volumes and corpora counts also failed normality assumptions for parametric tests, even when log transformed, thus data were examined using Kruskal-Wallis one-way ANOVA on ranks for non-normal data (Zar 1974). Dunn's multiple comparison tests were applied to each dataset to identify differences among groups. A $p \leq 0.05$ was used to determine significance for all statistical analyses.

Fisher's Exact Test was used for determining whether differences existed between the proportion of quiescent females within each time frame (Zar 1974). Differences among time frames were readily apparent and post hoc analyses were performed to determine the significance of these differences. A Chi-squared test was performed comparing 1975 vs 2008-10 samples, and combined samples from the 1975 and 2008-10 time frames were compared to the 1994-99 samples using Fisher's exact test (Zar 1974). The criterion for use of Fisher's test was whether more than 20% of the expected values had a frequency less than 5.

1.4 Results

1.4.1 Pilot study

There were no statistically significant differences between the weight measurements of the fresh/frozen ovaries and the formalin fixed/ethanol preserved ovaries in the first trial (Durbin-Conover

test, $p = 0.561$); there were however significant differences in their volumes (Durbin-Conover test, $p < 0.001$), with the formalin fixed/ethanol preserved ovaries being larger than the fresh/frozen ovaries. In the second trial, ovaries fixed and preserved in ethanol showed no significant weight (Durbin-Conover test, $p = 0.059$) nor volume changes (Durbin-Conover test, $p = 0.632$) compared to the fresh/frozen ovaries.

1.4.2 Ovarian weights, volumes, CL diameter and number of corpora

Ovarian weights ranged from 6.4-60.3 g across all three time frames for both hunt date-adjusted and unadjusted ovaries. Once adjusted for hunt dates, median ovarian weights of ovaries from 1994-99 were significantly larger than those from the other two time frames and no difference was detected between ovarian weights in 1975 and 2008-10 (Fig. 1.4). Active ovaries from females in 1994-99 were 5.2% larger in volume and 19.7% heavier in weight than those of females in 1975. Differences in ovarian weights were significant across time frames for active (Kruskal-Wallis test, $H = 59.47$, $df = 2$, $p < 0.0001$) versus inactive ovaries (Kruskal-Wallis test, $H = 42.11$, $df = 2$, $p < 0.0001$) (Fig. 1.5). Differences in weights between time frames were also significant after adjusting for hunt date for both active (Kruskal-Wallis test, $H = 7.89$, $df = 2$, $p = 0.019$) and inactive ovaries (Kruskal-Wallis test, $H = 8.47$, $df = 2$, $p = 0.015$) (Fig. 1.5). For adjusted weights, Dunn's pairwise multiple comparison tests showed that no difference existed for 1975 vs 2008-10 samples for active (Dunn's test, $p = 0.210$) nor inactive ovaries (Dunn's test, $p = 0.472$). However, 1994-99 active ovarian weights were significantly heavier than 1975 (Dunn's test, $p = 0.003$) and 2008-10 (Dunn's test, $p = 0.026$) samples. Similarly, 1994-99 inactive ovarian weights were greater than both 1975 (Dunn's test, $p = 0.006$) and 2008-10 (Dunn's test, $p = 0.007$) samples, while no difference existed between 1975 and 2008-10 (Dunn's test, $p = 0.472$).

Volumes of unadjusted ovaries ranged from 7.2-66.7 cm³ across all time frames and 8.4-66.7 cm³ for ovaries adjusted for hunt date. Prior to adjusting, volumes of active ovaries were significantly different among all time frames (Kruskal-Wallis test, $H = 30.23$, $df = 2$, $p < 0.0001$) (Fig. 1.6). Inactive ovaries showed differences between 1994-99 vs 1975 (Dunn's test, $p = 0.0002$) and 1994-99 vs 2008-10 (Dunn's test, $p = 0.0016$). When adjusted for hunt date, no significant difference in volumes was observed

in active ovaries (Kruskal-Wallis test, $H = 5.09$, $df = 2$, $p = 0.079$). The adjusted inactive ovaries of females from 1994-99 were significantly larger than 2008-10 females (Dunn's test, $p = 0.003$) and 1975, though not significantly (Dunn's test, $p = 0.055$). No differences existed between adjusted inactive ovaries from 1975 and 2008-10 (Dunn's test, $p = 0.136$).

There was a significant difference in the size of CL diameter across time frames (Kruskal-Wallis test, $H = 14.86$, $df = 2$, $p = 0.0006$). CL diameters from 1975 were significantly smaller than those of 1994-99 (Dunn's test, $p = 0.002$) and 2008-10 (Dunn's test, $p = 0.0001$). No significant differences were found between the median number of corpora across time frames (Kruskal-Wallis test, $H = 1.48$, $df = 2$, $p = 0.476$), which varied from 4 (1975 and 2009-10) to 5 (1994-99).

1.4.3 Proportion of quiescent females

A significant difference existed between the proportions of females quiescent among all time frames: 1975, 1994-1999 and 2008-2010 (Fisher's Exact test, $p = 0.03$). No significant difference existed between samples from 1975 vs 2008-10 (Fisher's Exact test, $p = 1.0$). Hence, frequencies from 1975 and 2008-10 samples were combined and tested against 1994-99; females between these two groups of timeframes were significantly different (Fisher's exact test, $p = 0.016$) (Table 1.3), with females from 1994-99 reproductively more active than those from the other time frames.

1.5 Discussion

1.5.1 Pilot study

No published studies on the effects of different preservation methods were found on walrus or marine mammal ovaries. However, limited research has been conducted on other mammal species (Kanerva et al. 1983, Fraser 1985). Kanerva et al. (1983) assessed differences in weights of rat (*Rattus norvegicus domesticus*) organs. Changes from fixation varied across organs but were uniform in each organ for both male and female rates. Ovaries were 6.8% heavier after 72 hours of fixation in 10% neutral buffered formalin. Fraser (1985) assessed changes in ovarian weights in rabbits (*Oryctolagus cuniculus*)

after fixation in 10% formalin. The greatest changes in tissue distortion occurred in the first 24 hours, after day 32 changes in weight compared to fresh/frozen became statistically insignificant. Contrary to the results from Kanerva et al (1983), the rabbit ovaries decreased in weight over time. The effects of various preservatives on length and weight of tissue varies on a case-by-case basis (Cunningham et al. 2000, Wetzel et al. 2005), and are likely the result of the physiological parameters that differ between species including differences in lipids and salinity, as well as specimen life stage and condition, sampling procedure and tissue types. In the present study, the lack of differential effects of preservative on walrus ovary weight and volume allowed us to compare ovary characteristics over time without regard to preservation method.

1.5.2 Proportion of quiescent females

Of those females that were considered quiescent across all timeframes, only three exhibited large follicles (≥ 4 mm in diameter). Because this is only 2% of our sampled animals, we considered them to be operating outside of the norm of the population's reproductive cycle. We did not consider these individuals as reproductively active as it was unknown if they were truly gearing up for a late ovulation or they were individuals undergoing a follicular wave that would not result in estrus. Furthermore, three reproductively active individuals from the 1990s showed large follicles in addition to a present CL. Therefore, follicle size alone was not a good indicator of a late ovulation and was not included in our definition as a reproductively active female.

1.5.3 Reproductive capacity as a proxy to understand population fluctuations and environmental change

In this study we examined a suite of anatomical measurements to assess reproductive capacity of female Pacific walruses over a span of 35 years in the Bering Sea, but did not analyze direct causes for the variation in reproductive capacity of the population during these timeframes. Rather, this study used an anatomical approach to better understand the reproductive capacity of the population through time, which may not have been captured in other methods, such as in statistical modelling approaches.

Here we review known ecological and anthropological drivers that may impact reproductive capacity and could provide explanations for the fluctuations in reproductive capacity of the Pacific walrus population during 1975-2010. Some of the known drivers in the region that may have impacted reproductive success during our sampling periods include: changes in population size as it relates to carrying capacity (Taylor et al. 2018), changes in age structure of the population (Garlich-Miller et al. 2006), contaminants and pollutants (Towell et al. 2006, Reiner et al. 2016, Lefebvre et al. 2016), disease outbreaks (Burek et al. 2008), fluctuations in removal (Fay et al. 1989, Garlich-Miller et al. 2011), quality and quantity of nutrition, and changes in the quality of habitat used for various life history stages (Grebmeier et al. 2006, Grebmeier 2012, Ray et al. 2016). Other factors that are poorly understood but may impact reproductive capacity include: chronic stress resulting from anthropogenic factors such as noise, fragmentation of habitat and disturbance, immune system function and the effects of a warming climate on animal physiology. Here we review the potential impacts of changes in population size, fluctuations in removal, stressors and contaminants based on the available literature.

The present study found that female walrus reproductive capacity was limited in 1975, increased in 1994-99 and in 2008-10 decreased to the same level as that of 1975. Likely, the cause for low reproductive capacity in 1975 was due to density dependent factors. Previous studies support the notion that the population was at or near carrying capacity in 1975 (Krogman et al. 1979, Fay et al. 1989, 1997, Speckman et al. 2011, MacCracken et al. 2014, Taylor & Udevitz 2015, Taylor et al. 2018). Alaska Natives and scientists witnessed signs of an increasing population during this time period, including: large terrestrial haulouts coupled with extended time spent on haulouts, shifts in prey species selection with observations of an increased presence of fish associated parasites, reduced size of bivalves commonly consumed, increased proportion of higher trophic level prey (seals) and reduced calf production (Fay & Stoker 1982*a,b*, Lowry & Fay 1984, Fay et al. 1989, Fischbach et al. 2016). Stress from overcrowding at haulouts and competition for food resources may have hampered reproduction during the mid-1970s. The primary hormone produced in response to stress in mammals is cortisol (Atkinson et al. 2015). Wasser et

al. (2004) found cortisol levels peak in grizzly bears (*Ursus arctos*) during summer berry season when there is competition with other bears for food resources. The study also found regional correlation in that the population segment with the lower cortisol levels had higher progesterone levels, an indication of greater reproductive potential, as progesterone production reflects ovarian activity and possible pregnancies. Furthermore, decreases in food availability have been linked to poor body condition in mammals (Cattet et al. 2002, Stewart et al. 2005) and poor body condition is known to impair reproductive output (Atkinson & Ramsay 1995, Guinet et al. 1998, Harwood et al. 2000, Stewart et al. 2005, Tollefson et al. 2010). In African fur seals, decreased body condition index linked to poor food availability yielded higher proportions of non-pregnant females and failures in lactation (Guinet et al. 1998). While we do not have measures of body condition, this would explain why a greater proportion of females in 1975 were quiescent and had reduced ovarian capacity compared to those from 1994-99.

Ovaries from females in 1994-99 were the largest and heaviest (Figs. 1.3 & 1.5) and demonstrated a significantly smaller proportion of females that were reproductively quiescent during this time frame compared to both 1975 and 2008-10 (Table 1.3). Additionally, females from 1994-1999 experienced a median increase of one more ovulation over their life spans compared to females from the 1975 and 2008-2010 time frames. While these findings were not statistically significant, we would expect females from 1994-1999 to have more corpora present, as this matches the trends seen in our other measures of reproductive capacity. This increase in reproductive capacity may have been attributed to increases in the subsistence hunt during this time. The total annual removal of the Pacific walrus population saw significant increases in both Russia and Alaska during the 1980s (Fay et al. 1989, Garlich-Miller et al. 2011). The mid-1980s yielded the highest subsistence harvest numbers since monitoring subsistence harvest began in the 1960s; in 1985 it was estimated that more than 16,000 animals were taken (Garlich-Miller et al. 2011). This increase in harvest may have removed a large proportion of adults that had a high index of reproductive fitness (heritable reproductive traits). In the late 1980s, Fay et al. (1989) projected that increasing catches, paired with low reproduction and survival rates, the natural

mechanisms controlling population growth, could result in another period of depletion of the population. More recent studies using Bayesian modeling also support a decreasing population during the 1990s (Taylor & Udevitz 2015, Taylor et al. 2018). Results from the present draw similar conclusions and indicate that density dependent pressures were removed in 1994-99, such that reproductive capacity of the population was found to be significantly greater in this time frame than two decades prior. This was evidenced by active ovaries from females hunted in 1994-99 which were 5.2% larger in volume and 19.7% heavier in weight than those of females hunted in 1975. Inactive ovaries from those respective time frames were 7.6% larger in volume and 12.8% heavier in weight. Reproductive parameters of female walrus also provide evidence for a trend of decreasing population from 1975 to the mid-1990s. Garlich-Miller (1997) found the mean age of first birth of 10 primiparous females from 1994-1996 to be seven years of age, a reduction from 10 years of age for females from 1975-1985. This change is regarded as an indicator of a population reaching carrying capacity (Eberhardt & Siniff 1977) and is thought to be the result of decreased body growth (Laws 1956) due to limited nutrient intake (Fay et al. 1989).

Ovaries of females from 2008-10 were not statistically different than those of 1975 in size, weight or median number of corpora. The percent of quiescent females also reverted to a level similar to that of animals hunted in 1975 (Table 1.3). This reduction in reproductive capacity in 2008-2010 may have been due to a variety of factors, including changes in population size, environment and anthropogenic impacts; here we review the supportive evidence that may have led to a decline in reproductive capacity in females hunted during 2008-2010. Since 2007, declining availability of sea ice in the Chukchi Sea during the summer has impacted the haulout behavior of Pacific walrus. Greater numbers of walrus have been using terrestrial haulouts during the late summer in both Alaska and Chukotka (Fischbach et al. 2009). Additionally, new and historical haulouts formed or were reoccupied on the Alaskan and Russian coasts starting in 2007 (Kavry et al. 2008, Semenova et al. 2010, Fischbach et al. 2016), which caused increases in mortality from 2007-2009, most of which were young animals (Kavry et al. 2008, Kochnev et al. 2008, Kochnev 2010, Ovsyanikov et al. 2008, Semenova et al. 2010). Walrus responded to these conditions of

low sea ice very differently than in previous years (Jay et al. 2012). On the Alaskan coast in 2007, unusual sightings of emaciated females and abandoned calves were observed along the Beaufort Sea, a location where historically walrus rarely come ashore (Metcalf & Robards 2008). It is estimated that in the fall of 2007 alone, 3000-5000 walrus died along the Russian coast of the Chukchi, with additional mortalities reported in Anadyr Bay in the Bering Strait (Kochnev et al. 2008). While the majority of terrestrial summer haulouts are used by males, Anadyr Bay is the exception in that hundreds to thousands of females and dependent young use the haulout in ice-free months (Fischbach et al. 2016). Therefore, mortalities in this region have large impacts on reproductively active females and result in high mortalities of young due to trampling associated with disturbance. High disturbance at haulouts along the Russian coast due to machinery work, feral packs of dogs, helicopters and live captures of calves for oceanariums and zoos, also likely stressed the population and may have contributed to limited reproductive capacity (Kavry et al. 2008, Kochnev 2004, 2010). Predators, including brown bears (*Ursus arctos*), polar bears (*Ursus maritimus*), wolverines (*Gulo gulo*) and killer whales (*Orcinus orca*) were also cause for disturbance leading to panic and stampedes, resulting in stress and mortalities (Kochnev 2002, 2004, 2010, Kochnev et al. 2008, Semenova et al. 2010). Like stressors such as decreased nutrition and resource competition, anthropogenic stressors are known to cause chronic stress and impact reproduction (Atkinson & Dierauf 2018).

An increasing amount of research has occurred on the prevalence of contaminants in the Arctic in recent years. Contaminants can be naturally occurring such as biotoxins produced by cyanobacteria and diatoms or can be the result of manmade products which include pesticides, flame retardants and industrial compounds, known collectively as persistent organic pollutants (POPs). POPs are known to impact immune and endocrine functions as well as impair development and reproduction in mammals (Muir & de Wit 2010, Reiner et al. 2016) and the Arctic has been shown to be a sink for POPs. In the Bering Sea it has been hypothesized that POP levels in northern fur seals (*Callorhinus ursinus*) may be linked to population declines (Towell et al. 2006, Reiner et al. 2016) as there is evidence that POPs have

lowered reproductive rates in other pinnipeds (Hutchinson & Simmonds 1994). Reiner et al. (2016) sampled northern fur seals from 1987-2007 and found that while phased out POPs either decreased or remained constant with time, their replacements: polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs) and perfluorinated alkyl acids (PFAAs) increased with each successive sampling period, with 2007 showing the greatest concentrations of new POPs. Walruses exhibit a different feeding ecology than northern fur seals and generally are found further north in the Bering Sea. POP levels have not been measured in Pacific walrus and tend to be site and species specific, however, we cannot rule out that POPs may have contributed to the lower reproductive capacity witnessed in the 2008-10 time period.

Naturally occurring contaminants have also shown increases in Arctic waters in recent years. Two of the most common harmful algal bloom toxins on the west coast of North America are domoic acid (DA) and saxitoxin (STX). Both affect the central nervous system, causing paralysis which may lead to death. In California sea lions (*Zalophus californianus*) DA has been shown to be transferred to fetuses via the placenta leading to reproductive failure and likely impacting survival of successfully delivered offspring as DA continues to be delivered to pups via lactation (Brodie et al. 2006, Rust et al. 2014). Lefebvre et al. (2016), found DA and STX to be present in humpback whales (*Megaptera novaeangliae*), bowhead whales, ringed seals (*Phoca hispida*), bearded seals (*Erignathus barbatus*), northern sea otters (*Enhydra lutris kenyoni*) and walruses. Walruses were sampled in the years 2012-13 and had the greatest concentrations of all tested animals for both DA and STX (Lefebvre et al. 2016). The concentrations of both contaminants were within the range of concentrations known to cause neurological effects on California sea lions. Because this is the first-time walruses have been tested for the presence of DA and STX it is unknown if these are new trends or if these contaminants have been present within the ecosystem for decades. Though walruses used in Lefebvre et al. (2016) are more recent than samples from our study, it is possible that increasing levels of DA and STX may have impacted reproductive capacity during the 2008-10 time frame.

1.6 Conclusion

Our study showed the reproductive capacity of Pacific walrus was 1) limited in 1975 which was likely a density dependent response, 2) rebounded in 1994-99 potentially due to increased harvests and greater prey availability and 3) decreased in 2008-10 to level similar to that of 1975. While we cannot elucidate a clear culprit for this more recent decline in reproductive capacity, it is possible that the population was again increasing in abundance. Additionally, several other suspects that are known to impair reproductive performance may have also contributed to this reduction, including: chronic stress and increased mortalities linked reductions in sea ice habitat, POP prevalence and increasing concentrations of DA and STX may be involved. We recognize that additional data on abundance, such as a time series of abundance estimates, and consistent records of environmental factors, would be beneficial in making statistical comparisons to our results. However, such data are unavailable due to the difficulty in surveying the range of the Pacific walrus, thus we recommend that future research on the population focus on addressing these shortcomings. Continued monitoring through multiple measures of reproductive capacity of the Pacific walrus during these times of rapid warming in their environment is essential.

1.7 Acknowledgements

All samples were collected by USFWS in accordance with Federal regulations 50 CFR 18.23(b)(2). This work would not have occurred without the Bering Strait island communities of Diomede, Gambell and Savoonga providing walrus specimens from their subsistence hunts and we thank them. Additional walrus specimens (spanning the 2008-10 time frame) were graciously provided by Jonathan Snyder of USFWS, Alan Springer and Suzann Speckman. We thank our collaborators at the University of Alaska Museum of the North, especially Link Olson and Aren Gunderson, US Fish and Wildlife Service and the Eskimo Walrus Commission. We acknowledge the contributions from colleagues Gordon Kruse and Gay Sheffield, as well as Philip Ganz and Wendel Raymond for assistance in statistical analyses and figure preparation, and Anatoloy Kochnev for kindly sharing his research. This

manuscript was improved by the helpful comments of R. Stimmelmayer, J. Gilbert and an anonymous reviewer. Funding was provided by the North Pacific Research Board Graduate Research Award, the Resilience and Adaptation Program at the University of Alaska Fairbanks, the Rasmuson Fisheries Science Center, Alaska EPSCoR and the Erich Follman Memorial Student Research Support.

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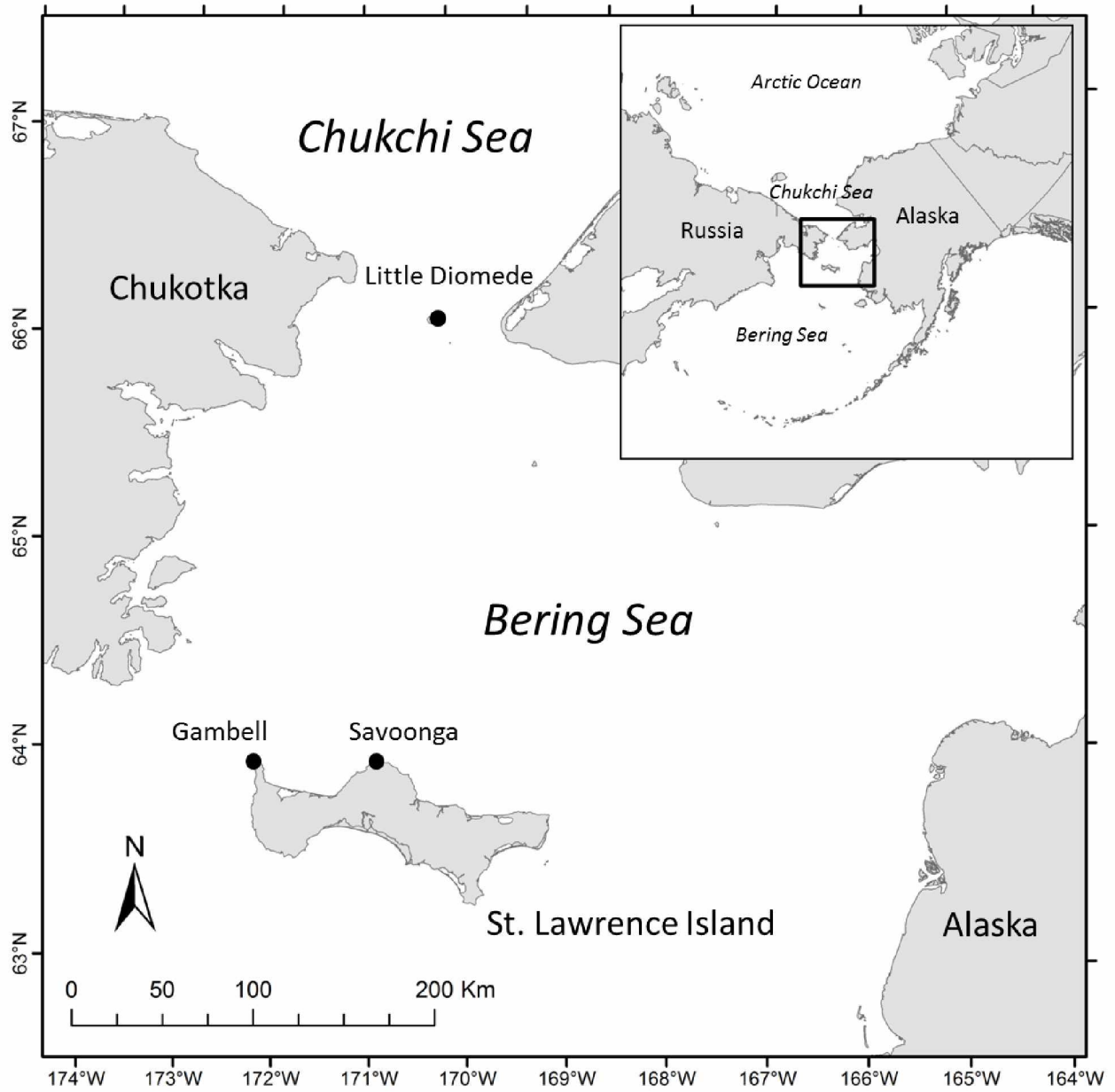


Figure 1.1. Location of Alaskan villages from which ovaries were collected from subsistence hunted Pacific walrus (*Odobenus rosmarus divergens*) during 1975-2010

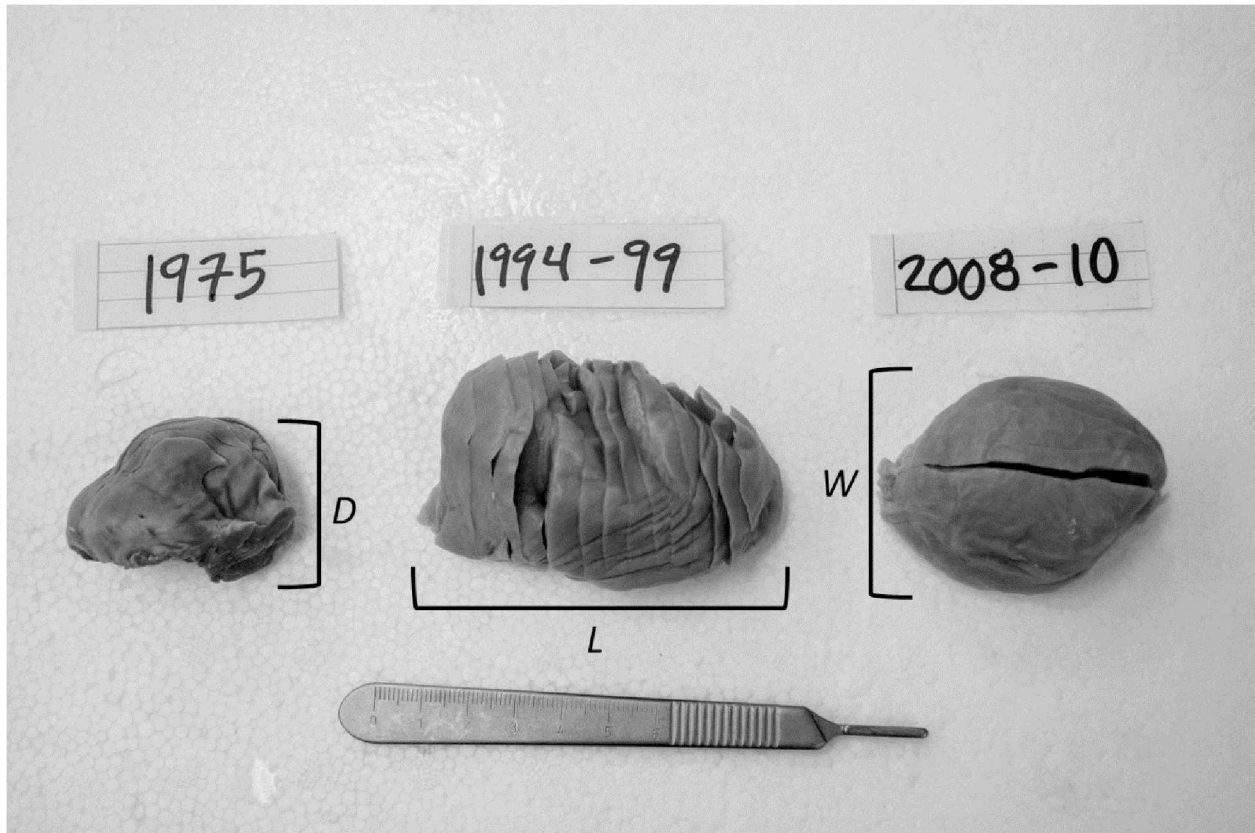


Figure 1.2. Photograph of the typical size of an active Pacific walrus (*Odobenus rosmarus divergens*) ovary from each time frame. Samples from 1994-99 exhibited the largest ovaries, whereas 1975 exhibited the smallest ovaries. Letters indicate measurements used to calculate ovarian volume: *length (L) x width (W) x depth (D) x 0.523*. Note that the 1975 ovary is on its side to illustrate the depth measurement

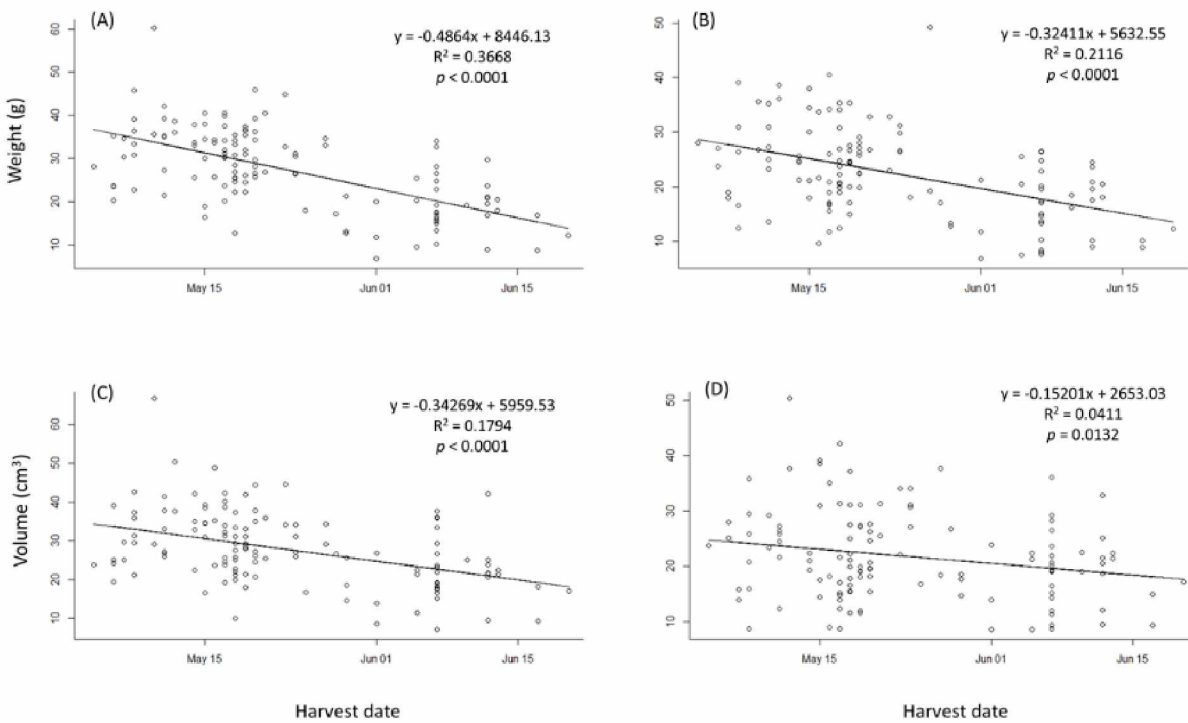


Figure 1.3. Relationships between ovarian weight (g) and volume (cm³) loss per day for Pacific walrus (*Odobenus rosmarus divergens*) for (A) weight of active ovaries, (B) weight of inactive ovaries, (C) volume of active ovaries, and (D) volume of inactive ovaries

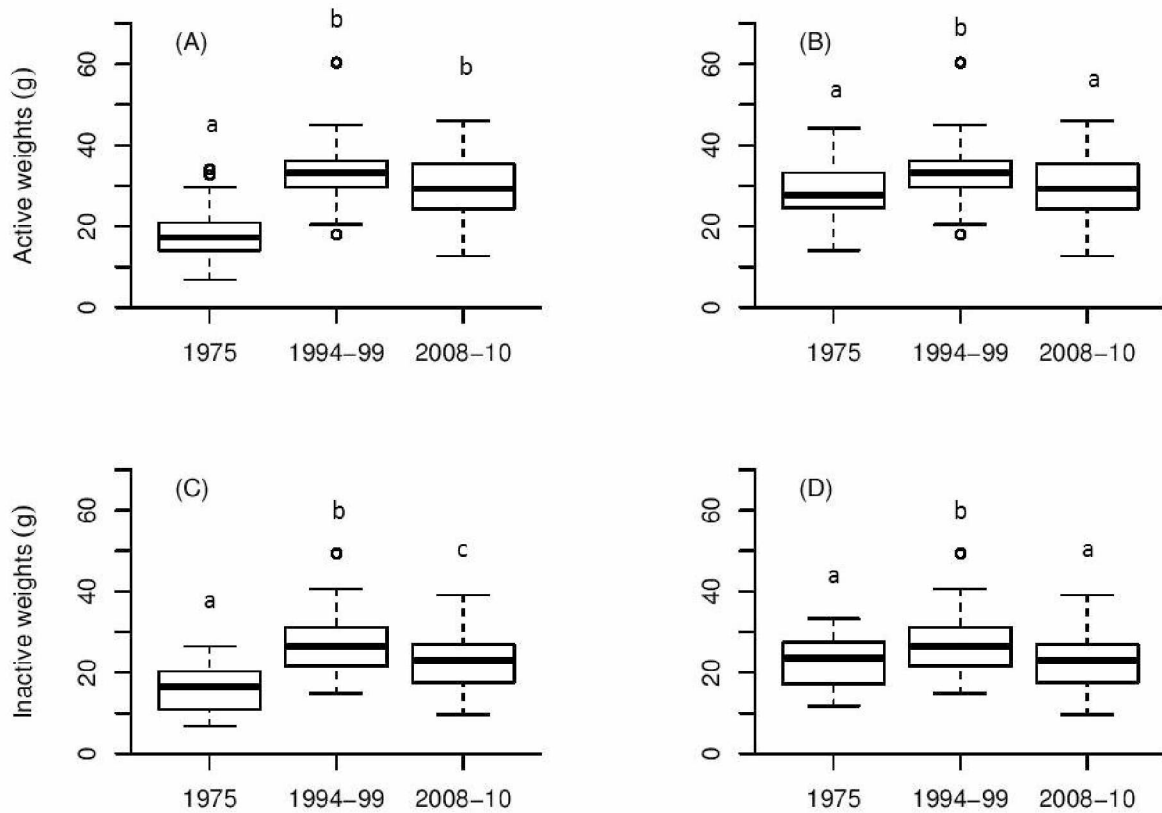


Figure 1.4. Boxplots of ovarian weights (g) of Pacific walrus (*Odobenus rosmarus divergens*) from 1975 ($n = 45$), 1994-99 ($n = 46$) and 2008-10 ($n = 48$) for (A) active ovaries, (B) active ovaries adjusted for hunt date, (C) inactive ovaries, and (D) inactive ovaries adjusted for hunt date. Different letters (a, b, c) indicate significant differences between different time frames ($p \leq 0.05$). The heavy bar in the middle of the box is the median value; the end of box shows the upper and lower quartiles; the whiskers represent the range of values excluding outliers; hollow circles indicate outliers.

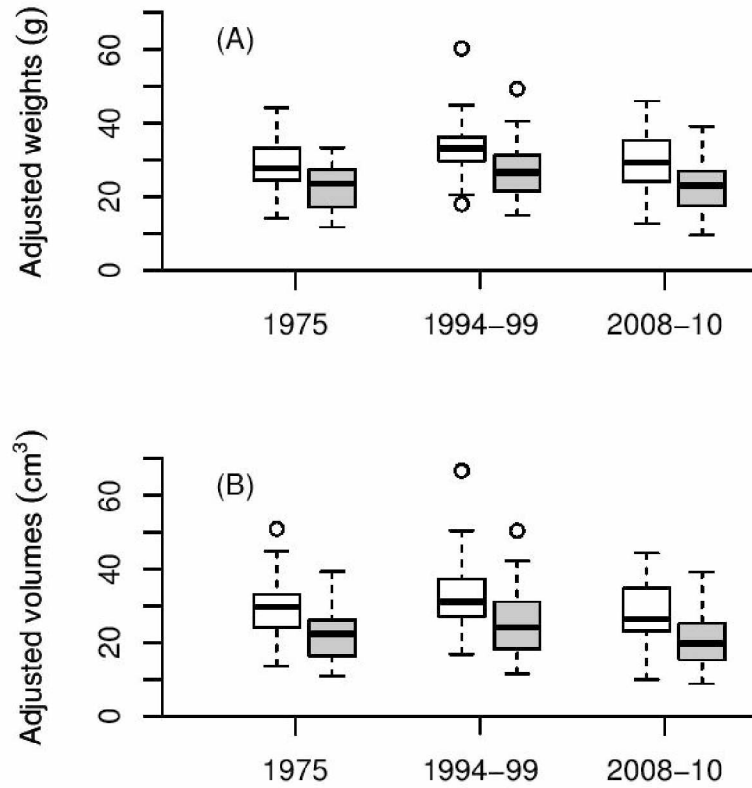


Figure 1.5. Boxplots comparing the active (white) and inactive (grey) (A) adjusted weights (median \pm SE g) and (B) adjusted volumes (cm^3) of Pacific walrus (*Odobenus rosmarus divergens*) ovaries sampled in 1975 ($n = 45$), 1994-99 ($n = 46$) and 2008-10 ($n = 48$). Weights and volumes have been adjusted for hunt date. The heavy bar in the middle of the box is the median value; the end of box shows the upper and lower quartiles; the whiskers represent the range of values excluding outliers; hollow circles indicate outliers.

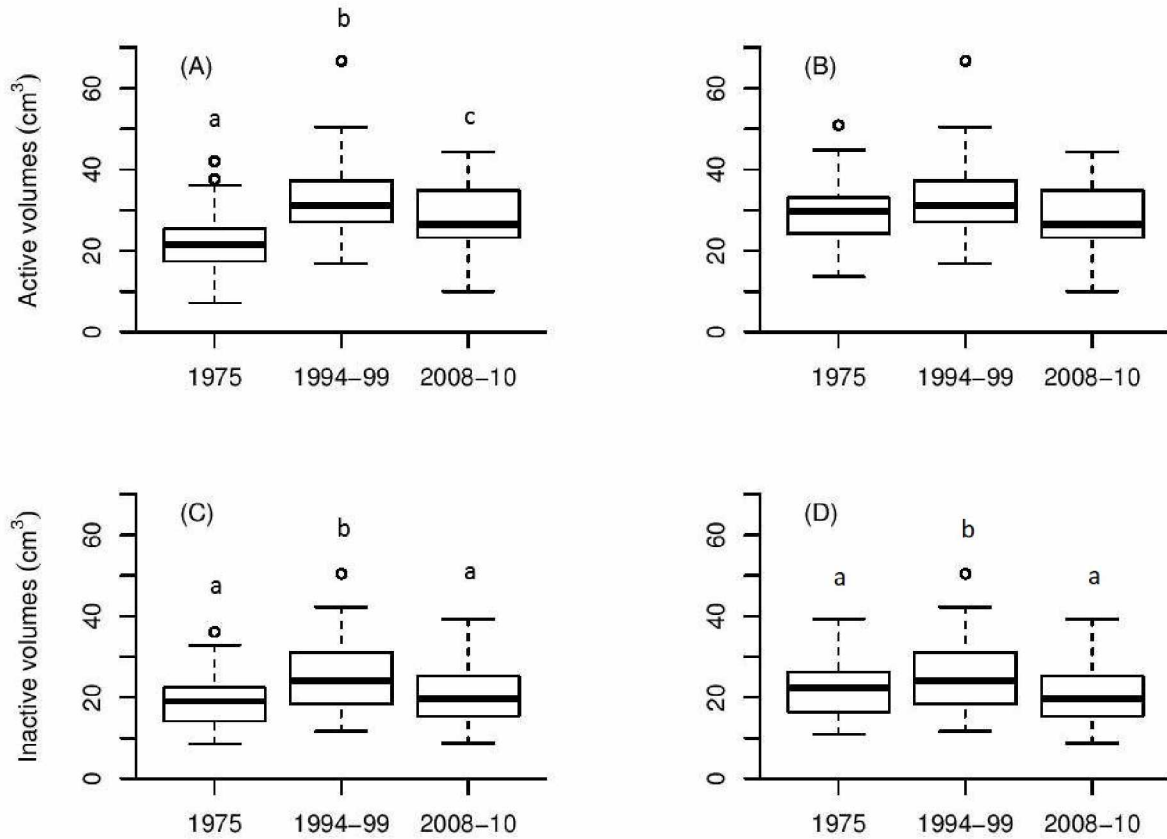


Figure 1.6. Boxplots of ovarian volumes (cm^3) of Pacific walrus (*Odobenus rosmarus divergens*) from 1975 ($n = 45$), 1994-99 ($n = 46$) and 2008-10 ($n = 48$) for (A) active ovaries, (B) active ovaries adjusted for hunt date, (C) inactive ovaries, and (D) inactive ovaries adjusted for hunt date. Different letters (a, b, c) indicate significant differences between time frames ($p \leq 0.05$). The heavy bar in the middle of the box is the median value; the end of box shows the upper and lower quartiles; the whiskers represent the range of values excluding outliers; hollow circles indicate outliers.

1.10 Tables

Table 1.1. The number of pairs of Pacific walrus ovaries (*Odobenus rosmarus divergens*) per time frame, per age class in years used in the study

Age of animals	Time frame		
	1975	1994-99	2008-10
	Pairs of ovaries		
8-11 years	12	11	11
12-15 years	15	17	23
16-19 years	10	9	9
20+ years	8	9	5
Total <i>n</i>	45	46	48

Table 1.2. Timeframes, sample sizes, hunt dates ranges, walrus age ranges (in years) and number of specimens (*n*) by community (Gambell, Savoonga, Little Diomed) from which Pacific walrus (*Odobenus rosmarus divergens*) ovaries were provided in this study

Time frame	<i>n</i>	Hunt dates	Ages (years)	Number of animals by location		
				Gambell	Savoonga	Diomed
1975	45	5/25-6/20	8-36	0	2	43
1994-99	46	5/4-6/5	8-30	25	3	18
2008-10	48	5/6-5/21	8-34	31	17	0

Table 1.3. Mean Pacific walrus (*Odobenus rosmarus divergens*) corpora lutea diameters, % females quiescent and % active in their current reproductive cycle for each time frame. **Bold** text indicates significant differences from the other groups in the same column

Time frame	<i>n</i>	Mean CL diameter	% quiescent	% active
1975	45	28.38 ± 1.04	11.1%	88.9%
1994-99	46	28.70 ± 0.93	0%	100%
2008-10	48	29.18 ± 1.12	12.5%	87.5%

1.11 Appendix. List of Pacific walrus ovaries from University of Alaska Museum of the North used in this study.

Museum ID	Year Collected
UAM:Mamm:138323	1996
UAM:Mamm:108180	1995
UAM:Mamm:108500	1999
UAM:Mamm:109384	1996
UAM:Mamm:109700	1998
UAM:Mamm:128827	2009
UAM:Mamm:128828	2009
UAM:Mamm:127964	2008
UAM:Mamm:127968	2008
UAM:Mamm:128008	2009
UAM:Mamm:108422	1995
UAM:Mamm:138328	1994
UAM:Mamm:128831	2009
UAM:Mamm:129177	2010
UAM:Mamm:127976	2008
UAM:Mamm:138327	1999
UAM:Mamm:138321	2010
UAM:Mamm:138322	1995
UAM:Mamm:108346	1994
UAM:Mamm:107966	1995
UAM:Mamm:109493	1996
UAM:Mamm:109702	1998
UAM:Mamm:138329	1994
UAM:Mamm:128801	2009
UAM:Mamm:127974	2008
UAM:Mamm:131464	2010
UAM:Mamm:138318	1994
UAM:Mamm:108134	1995
UAM:Mamm:109179	1996
UAM:Mamm:107279	1995
UAM:Mamm:128856	2009
UAM:Mamm:128802	2009
UAM:Mamm:129174	2010
UAM:Mamm:127967	2008

UAM:Mamm:127969	2008
UAM:Mamm:127970	2008
UAM:Mamm:128010	2009
UAM:Mamm:128017	2010
UAM:Mamm:128029	2010
UAM:Mamm:128035	2010
UAM:Mamm:131492	2010
UAM:Mamm:36308	1994
UAM:Mamm:138320	1995
UAM:Mamm:108181	1995
UAM:Mamm:109336	1997
UAM:Mamm:108653	1999
UAM:Mamm:110229	1995
UAM:Mamm:110243	1995
UAM:Mamm:128023	2010
UAM:Mamm:128028	2010
UAM:Mamm:108162	1995
UAM:Mamm:108326	1996
UAM:Mamm:107624	1995
UAM:Mamm:109881	1999
UAM:Mamm:138330	1995
UAM:Mamm:138331	1996
UAM:Mamm:128804	2009
UAM:Mamm:128833	2009
UAM:Mamm:129155	2010
UAM:Mamm:129156	2010
UAM:Mamm:128006	2009
UAM:Mamm:128011	2009
UAM:Mamm:138324	1998
UAM:Mamm:138325	1999
UAM:Mamm:138326	1999
UAM:Mamm:108133	1995
UAM:Mamm:107671	1996
UAM:Mamm:108266	1996
UAM:Mamm:108562	1999
UAM:Mamm:109310	1996
UAM:Mamm:109841	1999
UAM:Mamm:127971	2008
UAM:Mamm:127977	2008

UAM:Mamm:128018	2010
UAM:Mamm:128021	2010
UAM:Mamm:128026	2010
UAM:Mamm:128031	2010
UAM:Mamm:128032	2010
UAM:Mamm:138319	1995
UAM:Mamm:108340	1995
UAM:Mamm:108173	1995
UAM:Mamm:108556	1999
UAM:Mamm:109606	1994
UAM:Mamm:109845	1998
UAM:Mamm:109712	1994
UAM:Mamm:128855	2009
UAM:Mamm:128858	2009
UAM:Mamm:128859	2009
UAM:Mamm:128803	2009
UAM:Mamm:128829	2009
UAM:Mamm:127965	2008
UAM:Mamm:128005	2009
UAM:Mamm:128015	2009
UAM:Mamm:128033	2010
UAM:Mamm:128037	2010

Chapter 2. Endocrine Profiling of Reproductive Status and Evidence of Pseudopregnancy in the Pacific Walrus (*Odobenus rosmarus divergens*)²

2.1 Abstract

Endocrine profiling is an increasingly utilized tool for detecting pregnancies in wild populations of mammals. Given the difficulty in calculating reproductive rates of Pacific walruses (*Odobenus rosmarus divergens*) the use of endocrine techniques for determining pregnancy rates could be particularly useful for management of the population. The goals of this study were to determine whether: 1) progesterone and total estrogen concentrations in ovarian tissues of female walruses could be used to determine reproductive state; and 2) walruses undergo a functional postpartum estrus, as is seen in other pinnipeds. Ovaries were collected from female walruses ($n = 14$) hunted in subsistence hunts by Alaska Native communities in Alaska. Females were categorized as postpartum, pregnant or unbred. The pregnant group included one female in embryonic diapause and one female that was full-term pregnant. Total estrogen concentrations were significantly greater in unbred ($n = 2$) and pregnant ($n = 2$) than in postpartum females ($n = 8$). Progesterone concentrations were also nominally larger in unbred ($n = 2$) than pregnant ($n = 2$) and postpartum ($n = 9$) animals, though differences were not statistically significant. However, neither estrogen nor progesterone profiles alone could distinguish between unbred and pregnant females. Samples in this study did not reflect the presence of a postpartum estrus; rather, unbred animals were in a state of pseudopregnancy. The progesterone profiles in late and early pregnancy were lower than expected and fell within the range of the postpartum females (36-210 ng/g), suggesting low production of the hormone by the corpus luteum during these phases in pregnancy. This is the first study to show physiological support for pseudopregnancy in this species. Profiling reproductive hormones in free-ranging walruses demonstrates that an endocrine approach may be a valuable tool for determining

²Larsen Tempel J, Atkinson SA. Pregnancy Detection and Evidence of Pseudopregnancy in Pacific Walrus (*Odobenus rosmarus divergens*). Prepared for submission to PLoS ONE.

reproductive statuses of females, however time of year must be considered to accurately separate pregnant versus pseudopregnant individuals.

2.2 Introduction

Reproductive hormone profiles can be used to detect pregnant and non-pregnant animals, as has been demonstrated in multiple marine mammal species [1,2,3]. For this reason, endocrinology has gained traction as a tool for determining pregnancy in captive animals, as well as in wild populations. Most of the research on female reproductive hormones in wild populations has focused on cetacean species, including common bottlenose (*Tursiops truncatus*) [4], long-beaked (*Delphinus capensis*) [5], short-beaked common (*Delphinus delphis*), Northern right whale (*Lissodelphis borealis*), Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) [6], and pilot (*Globicephala melas*) [4], minke (*Balaenoptera acutorostrata*) [7-8], fin (*Balaenoptera physalus*) [9], blue (*Balaenoptera musculus*) [10-11], humpback (*Megaptera novaeangliae*) [12] and bowhead whales (*Balaena mysticetus*) [13]. Fewer studies exist for pinnipeds and many of them have been carried out on captive animals, which may not follow the seasonal reproductive patterns witnessed in species in their native environments. Studies on progesterone and estrogen concentrations in pinnipeds have shown that these endocrine markers are able to detect pregnancy and sometimes estrus in Hawaiian monk seals (*Monachus schauinslandi*) [14], harbor seals (*Phoca vitulina*) [15], and Steller (*Eumetopias jubatus*) [16], California (*Zalophus californianus*) [17], and Galapagos sea lions (*Zalophus wollebaeki*) [18].

Endocrine profiles of reproductive hormones are useful in determining reproductive status because they reflect ovarian activity. In female mammals two main hormones govern the reproductive cycle: estrogens and progesterone. There are three primary types of estrogen: estrone, estradiol-17 β , and estriol [19]. Estradiol is the principle product produced during the follicular phase, however estrone sulfate or “total” estrogens are often easier to measure [14, 20]. Estradiol is the estrogen associated with ovulation and it is responsible for cellular proliferation and hydration. Both progesterone and estrogens are produced in the ovaries. Estradiol is produced in the thecal and granulosa cells within the ovarian follicles as they enlarge in preparation for ovulation [20]. Estradiol levels spike prior to ovulation and then quickly decline. Following ovulation, the newly formed corpus hemorrhagicum develops into the

corpus luteum (CL) and begins producing progesterone whether or not the animal is pregnant. The role of progesterone is to prepare the uterus and sustain the pregnancy. If the female is not pregnant, the CL will regress, and progesterone production will decrease. However, if pregnancy occurs, progesterone will remain high throughout gestation [21-22]. Yet, some species exhibit high levels of progesterone as well as behavioral indicators of pregnancy when they are not pregnant [23]. This “false pregnancy” has been termed pseudopregnancy and occurs primarily in copulation-induced ovulators [23].

To our knowledge, no published studies have profiled reproductive hormones in the tissues of free-ranging walrus. The Pacific walrus (*Odobenus rosmarus divergens*) has a unique reproductive cycle among pinnipeds, most of which have an annual breeding cycle which includes a postpartum estrus in spring or summer followed by an embryonic diapause [20]. In brief, the reproductive cycle of female walrus is characterized by mating during December-March, embryonic diapause until June-July, and a 15-16-month total gestation period, during which 8-11 months are active gestation [24] (Fig 2.1). There have been several reports of calves being born outside of this “established” window, during winter months [24]. It is unclear if these were premature births or if these were rare instances in which females were breeding beyond December-March. If these females were following a 15-16 month gestation, it would require breeding in May-July, coinciding with parturition. Based on anatomical studies finding CL within the ovaries of females as late as August, Fay [24] determined the species to have a postpartum estrus. However, he recognized that this postpartum estrus was likely outside the fertility window of breeding males, therefore this diestrus cycle could be considered functionally a monoestrus cycle. However, these determinations were made solely using an anatomical approach from harvested ovaries. Slightly newer literature is at odds with Fay [24] and reports that walrus are among the only pinnipeds that lack a postpartum estrus [20]. We investigated for the first time whether females show signs of estrus following parturition using an endocrinological approach.

In the present study, Pacific walrus were opportunistically sampled from reproductive tracts collected by Alaska Native hunters, to determine if endocrine profiles in ovarian tissues were indicative of

the known reproductive status of the harvested female. The objectives were to: 1) examine whether females exhibit endocrine indicators of a postpartum estrus or functional diestrus; 2) profile progesterone and total estrogen concentrations and percent lipids in CL of female Pacific walrus in four known reproductive states; and 3) determine whether follicular activity is a good indicator of ovarian activity by analyzing the number, size and endocrine profiles of follicles. This study adds to our knowledge of walrus reproductive ecology by ascertaining whether endocrine profiles are useful indicators for determining reproductive status of adult female walrus. In addition, the use of hunted specimens allows for anatomical confirmation of the endocrine measurements.

2.3 Materials and methods

2.3.1 *Ethics Statement*

All ovarian tissues were obtained from female walrus harvested by Alaska Native subsistence hunters in the communities of Gambell and Savoonga on St. Lawrence Island, Alaska, during May of 2011, 2015 and 2016. All samples were collected by US Fish and Wildlife Service (USFWS) in accordance with Federal regulations 50 CFR 18.23(b)(2) per the Walrus Harvest Monitoring Program (22).

2.3.2 *Sample collection*

A total of 15 sexually mature females were sampled, 13 of which contained ovarian samples. Females were harvested in the month of May during which they could be categorized as one of four different reproductive states: postpartum ($n = 9$), full-term pregnant ($n = 1$), embryonic diapause ($n = 1$) and unbred ($n = 2$) (Fig 2.1, Table 2.1). The sample was not unbiased as hunters on St. Lawrence Island typically select for female-calf pairs. This skewed our samples toward sexually mature, breeding females, which was appropriate for our study as we sought to compare the endocrine profiles of pregnant and non-pregnant adult females. Morphometrics were taken including: tract weight, placental zone width, uterine horn width, and CL weight. Tracts were stored frozen and allowed to thaw before analysis. Excess water

was absorbed from the tracts and they were weighed on a hanging scale to the nearest 0.5 lbs and converted to kg. The width of the zonary placental scar was measured inside the uterine horn, along the dissected section, within the horn to the nearest 0.5 cm. Uterine horn width was measured from the proximal edge of the broad ligament directly outward to the greatest width of each uterine horn; widths were recorded to the nearest 0.5 cm. Ovaries were removed from the reproductive tract. Ovaries were examined for the presence of CL and follicles. CL were identified by their size (taking up to $\frac{1}{3}$ to $\frac{1}{2}$ of the ovarian size), spherical shape, firm texture and often yellowish color [24]. The CL were removed, trimmed of adjacent ovarian tissues and weighed to the nearest 0.05 g. Follicles were located on the serosal surface of the ovary and ranged from <1 mm to 5 mm. Antral follicles were spherical shape and the antrum was filled with a translucent fluid. The number of follicles per ovary were counted, excluding those ≤ 1 mm, as at that size they are no longer grossly distinguishable. Follicles were measured with calipers to the nearest millimeter. The following size classes were used: small (≤ 1 mm), medium (2-4 mm) and large (>4 mm). Those that were of medium or large size were aspirated with a syringe and analyzed for total estrogen concentrations. For this reason, more than one follicle per female was analyzed as some animals yielded multiple medium and large sized follicles, while others contained only small follicles. In addition to follicular fluid, ovarian tissue samples were analyzed separately for hormone concentrations and excluded follicles in the medium size class or larger. Ovarian tissues were sampled from both active and inactive ovaries.

Reproductive states were determined using information provided by hunters about each animal harvested and by gross dissection of the ovaries and reproductive tracts when available. Hunters were trained by USFWS personnel and given field notebooks to document each animal hunted. Sexual maturity was determined by presence of a CL within the ovary [25-28]. Ovaries indicated if the female was reproductively active during her current cycle by presence of the CL, whereas ovaries lacking a CL were termed inactive. The presence of an active CL two or more months post-mating season indicated a physiological pseudopregnancy. Hunter observations indicated if a newborn calf and/or yearling was

present with the adult and if she was lactating. Females that were neither pregnant, nor with a newborn calf, nor lactating were considered unbred. When only ovaries were present, a female documented with a newborn calf and lactating was considered postpartum; when a reproductive tract was present a fresh zonal scar from placentation confirmed hunters' observations. All hunter observations of females with newborn calves were confirmed when reproductive tracts were present ($n = 7$), providing confidence in using hunter knowledge of reproductive status when full reproductive tracts were not available ($n = 2$) (Table 2.1). One female was categorized as full-term pregnant based on the hunter observation of a fully developed fetus in the uterine horn of the female, and that the timing of the hunt on St. Lawrence Island coincides with peak calving for Pacific walruses [24]. The reproductive tract of the full-term pregnant animal was not collected. One reproductive tract was collected that contained an amniotic sac indicative of a female in embryonic diapause. Any unbred female with a CL during the sampling period (in May) was considered to be exhibiting diestrus as the breeding window generally occurs December-March.

2.3.3 *Total estrogens radioimmunoassays*

A double antibody radioimmunoassay (RIA) kit (MP Biomedicals, Solon, OH) was validated for total estrogens in ovarian tissues of walruses. A pool of CL tissue including females of all reproductive states was used in the validation. Serial dilutions of the pooled tissues (neat to 1:80) yielded displacement parallel to that of the total estrogen standard curve. Samples were run in duplicate following manufacturer instructions with the exception that volumes were halved and an additional standard (one-half of the lowest standard) was added to the curve to increase sensitivity [17, 18, 29]. Recovery of added total estrogens was 87.04% ($y = 0.725x + 0.1698$, $r^2 = 0.983$). Interassay coefficient of variation for low, medium and high controls were 4.4%, 13.6% and 12.9%, respectively, and intra-assay coefficients of variation was <10%. Samples with an intra-assay coefficient of variation >10% were re-diluted as appropriate and re-assayed. Mean sensitivity was 0.3 ng/g \pm 0.04 SE ($n = 5$). Total estrogen concentrations were profiled from CL ($n = 12$), ovarian tissues ($n = 9$) and follicles ($n = 12$). Table 2.1

provides sample sizes for each tissue relative to reproductive status. All samples except one had a mass of ≥ 100 mg.

2.3.4 Progesterone enzyme immunoassays

Progesterone concentration was determined via enzyme immunoassay (EIA) kits purchased from Enzo Life Sciences (ADI-900-011). Standard concentrations were determined according to kit instructions and samples were run in duplicate. Progesterone was extracted following the methods of Mansour et al. and Kellar et al. [6-7] as modified by Atkinson et al. [10]. As with the total estrogens, a pool of CL tissue including females of all reproductive states was used in the validation for progesterone. Serial dilutions of the pooled tissues (1:200 to 1:3200) yielded displacement parallel to that of the total progesterone standard curve. Recovery of added progesterone was 72.04% ($y=1.00x + 5.3914$, $r^2=0.988$). Interassay coefficient of variation for low, medium and high controls were 17.5%, 7.7% and 11.2% respectively and intra-assay coefficient of variation was $<10\%$. Samples with an intra-assay coefficient of variation $>10\%$ was re-diluted as appropriate and reran. Mean sensitivity was $6.58 \text{ ng/g} \pm 0.23 \text{ SE}$ ($n = 5$). Progesterone concentrations were profiled from CL ($n = 13$). All samples had a mass of ≥ 200 mg.

2.3.5 Lipid analysis

Percent lipids of ovarian tissues were determined using a modified Folch method [30]. Tissue samples weighed 5-40 mg. Each sample was combined with 5 mL of hydromatrix and compacted into an ASE cell (Thermo Dionex ASE 350). The remaining volume in each ASE cell was filled with sand and cells were loaded into the ASE machine. A two-part chloroform: one-part methanol solvent was created and all samples were rinsed twice with the solvent. Afterwards, the volume of the samples was calculated and 0.88% potassium chloride was used to separate the organic fraction. Potassium chloride was added to each sample in a volume that was one-fourth of the sample's volume after the rinse. The aqueous layer was discarded and the organic layer was collected carefully so that no amount of aqueous solution contaminated the sample. A Rotovap (Heidolph Laborota 4011/HB Digital) was used to concentrate the

sample to just under 1 ml using a rotation speed of 165-200 rpms. Samples were pipetted into labeled tin pans and dried overnight in a vacuum oven. Percent lipids were calculated using the dried weights of the samples, using the equation:

$$\% \text{ Lipids} = \frac{(X_1 - X_2)}{X_3} \times 100,$$

where X_1 is the weight of the organic fraction plus the pan weight, X_2 is the weight of the empty pan, and X_3 is the weight of the original tissue.

2.3.6 Data analysis

All statistical analyses were performed in RStudio, version 3.6.1 [31]. The full-term pregnant and pregnant diapause samples were combined and categorized as pregnant, as the estrogen and progesterone concentrations of these animals were similar. Pooling these two samples together allowed for statistical comparisons between reproductive states. Normality of data were tested using Shapiro-Wilk tests. When data violated the normality assumption, the Wilcoxon rank sum test was used as a non-parametric alternative. The postpartum and pregnant female groups were normally distributed and compared using a Student's t-test. Progesterone and total estrogen data for CL were log transformed using \log_{10} for each female but did not pass normality tests so medians were compared using Kruskal-Wallis ANOVAs. Ovarian tissues analyzed for total estrogen concentration also did not pass normality tests so Kruskal-Wallis ANOVAs were used. When statistically significant differences were found, a Dunn's test was used for post hoc analysis to determine differences among groups. For data that were normally distributed, means are reported \pm standard deviation; for data that were not normally distributed, medians are reported \pm standard deviation; when only 2 samples are present the range of the two samples is presented. $P \leq 0.05$ was used as the significant level.

2.4 Results

2.4.1 *Corpora lutea*

Total estrogen profiles were significantly different between reproductive states ($H = 7.69$, $df = 2$, $P = 0.02$) (Fig 2.2). Differences were found to be significantly lower in postpartum ($n = 8$) than pregnant females ($n = 2$) ($P = 0.039$) and postpartum ($n = 8$) than unbred females ($n = 2$) ($P = 0.0069$); there was no statistical difference between pregnant and unbred females ($P = 0.289$). Median concentrations for total estrogens for unbred females were 868.87 ng/g (368.92 - 1368.82 ng/g) ($n = 2$), pregnant 199.57 ng/g (193.70 - 205.44 ng/g) ($n = 2$), and postpartum 13.25 ± 8.51 ng/g ($n = 8$). Differences in progesterone concentrations were not significant among females in the three reproductive states ($H = 5.07$, $df = 2$, $P = 0.079$). However, unbred females yielded the highest progesterone profiles at 1770.10 ng/g (1533.01 - 2007.18 ng/g) ($n = 2$), whereas pregnant females were 99.42 ng/g (58.53 - 140.30 ng/g) ($n = 2$), and postpartum females were 47.72 ± 64.65 ng/g ($n = 9$). Unbred females could not be distinguished from pregnant females using either estrogen or progesterone profiles, providing evidence of a physiological pseudopregnancy in the unbred females in our sample. There were no differences among percent lipids of the three reproductive states ($H = 4.45$, $df = 2$, $P = 0.107$); however, median values were greater for both pregnant 3.06% (2.20 - 3.91%) ($n = 2$) and unbred females 3.34% (1.99 - 4.69%) ($n = 2$) than postpartum females $1.52 \pm 0.58\%$ ($n = 9$).

Nine samples contained both the ovaries and full reproductive tracts (S110004, G110035, S110021, G110286, S110002, G110044, S110008, G160045, and G110290); of these, seven were postpartum females (Table 2.1) yielding placental scaring. There was a significant, positive correlation with percent lipids in the CL and the horn width in the pregnant horn of the postpartum females ($y = 0.1787x - 9.9532$, $r^2 = 0.7072$, $P = 0.018$) (Fig 2.3). For these females, the percent lipids in the CL did not have any correlation with the harvest date, animal age, CL weight, CL dimension, reproductive tract weight or placental zone width ($P > 0.05$).

2.4.2 Ovarian tissues

In ovarian tissues there were no significant differences in estrogen profiles of females in differing reproductive states ($H = 3.97$, $df = 2$, $P = 0.137$). Median estrogen concentrations for ovarian tissues from unbred females were 24.54 ng/g (5.31-43.77 ng/g) ($n = 2$), pregnant females 10.73 ng/g (5.09-16.37 ng/g) ($n = 2$) and postpartum females 4.02 ± 3.55 ng/g ($n = 5$). Only one unbred female tissue sample was available for lipid analysis. Postpartum and pregnant females were compared for percent lipids, and no significant differences were found ($T = 1.513$, $df = 3.57$, $P = 0.213$). Percent lipids were variable in postpartum females, and ranged from 0.78-2.32%. Mean percent lipids for postpartum, pregnant and unbred females were $1.60 \pm 0.71\%$ ($n = 5$), 0.95% (0.66-2.66) ($n = 2$), and 2.66% ($n = 1$) respectively.

2.4.3 Follicular activity

Estrogen concentrations in follicular fluid were significantly greater in unbred ($n = 2$) females than postpartum females ($n = 10$) ($T = -3.64$, $df = 5.517$, $P = 0.005$) (Fig 2.4C). The median concentration for unbred females was 24.85 ng/ml (21.18-28.51 ng/g), whereas for postpartum females it was 4.89 ± 14.41 ng/ml. There were no significant differences between estrogens and follicle size ($n = 10$, $P = 0.670$) (Fig 2.4A), nor estrogen concentrations in follicles in active versus inactive ovaries ($n = 12$, $P = 0.246$) (Fig 2.4B), nor in the number of follicles in active versus inactive ovaries ($n = 17$, $P = 0.875$) (Fig 2.4D).

2.5 Discussion

Profiling hormone concentrations in the tissues of walruses demonstrates that endocrine analyses are potentially useful indicators in determining reproductive states of this species. While this has been done in multiple species with many types of matrices [2, 3], no studies using ovarian tissue of marine mammals for endocrine analyses were found in published literature. The tissues used are unique in that they are the source location in which progesterone and estrogens are produced, rather than the more commonly sampled matrices used in endocrinology studies of marine mammals such as blood, blubber, and feces [2, 3]. This allowed the measurement of sex steroid hormones from the animal at the time of

sampling and directly from the production site. Studies on cetaceans have found the rate at which progesterone is transferred from blood serum to blubber is variable by species. In bowhead whales, progesterone may take weeks to months before concentrations in blubber reflect that of blood [13], however, in bottlenose dolphins progesterone concentrations in blubber may reflect blood serum concentrations just days prior to sampling [32]. Ultimately, using CL tissues allowed for the assessment of females that may be in estrus, which due to the rapid nature of the preovulatory estrogen surge might be missed when sampling other matrices. Thus, this study adds new information to the unique and understudied reproductive cycle of free-ranging walrus.

None of the postpartum females in the present study exhibited physiological signs of estrus. Postpartum animals yielded total estrogen concentrations lower than both pregnant and unbred females. The total estrogen concentrations in CL of pregnant and unbred females were significantly greater than that of postpartum females. Total estrogens in CL were 59 times greater in unbred than postpartum animals. One unbred female (G110290) yielded 1368.82 ng/g total estrogens, whereas the median total estrogen concentration for postpartum females was 13.25 ng/g. Unbred female G110290 did not have a dominant follicle present (≥ 19 cm), meaning the CL was functionally biosynthesizing estrogens and producing aromatase to stimulate this biosynthetic pathway. This spike in total estrogens might be interpreted as the preovulatory surge that accompanies estrus, yet, there was no statistically significant difference in estrogen profiles of the pregnant versus unbred females owing to the small sample size in this study. Unbred female G150005 had 368.92 ng/g of estrogens in her CL. This is slightly higher than the two pregnant females, yet interestingly the unbred female G110290 exhibited a concentration 3.7 times greater. Estrogen concentrations of the unbred females were not statistically distinguishable from those of pregnant females. This may be due to our small sample size, as the median concentration for total estrogens of pregnant females is nominally smaller than that of unbred females. Estrogen concentrations in the full-term pregnant female and the female in embryonic diapause were similar.

Interestingly, progesterone concentrations in the CL among the three reproductive states were not statistically significantly different, though unbred females yielded the highest progesterone levels. Again, this is likely due to small sample sizes, as two of the nine postpartum females were considered outliers and had high levels of progesterone. These two females (S110008 and G110286) had some of the largest placental scar zone widths (both 13.5 cm) and heaviest tract weights (12.47 kg and 12.70 kg respectively), and they also had few follicles compared to other postpartum females. S110008 had 11 total follicles and G110286 had 7 follicles, whereas the mean for postpartum females was 28.2 follicles. While it is unknown whether or not the uterine lining produces progesterone, the large zone of placentation may have contributed to suppressing folliculogenesis and/or sustained progesterone production from the CL of the pregnancy. These factors indicate that these females were hunted very close to parturition, as Fay [24] found that placental scar widths of females that gave birth within 24 hours were 15 cm or greater. It appears that these CL were either still producing progesterone at the level of pregnancy, or possibly not metabolizing it during the time these females were hunted. The CL of the two pregnant females had progesterone concentrations similar to the postpartum group, 99.42 ng/g and 77.49 ng/g, yet the two unbred females yielded progesterone concentrations 77 times greater than the postpartum females. It was expected that the greatest progesterone concentrations would be found in pregnant females as progesterone concentrations have been used as an accurate indicator of pregnancy in other marine mammals, though in different matrices such as blubber and serum [2-3]. Of the two female walrus in the present study that were pregnant, G160045 was in embryonic diapause and her CL had 140.30 ng/g progesterone, whereas G160053 was full term pregnant and had 58.53 ng/g progesterone. Both females had levels similar to postpartum individuals which ranged from 36.12-209.83 ng/g (median = 47.72 ng/g). In the female in embryonic diapause (G160045), this may indicate that the progesterone concentration remains low during embryonic diapause until nidation occurs. In that walrus, both of her reproductive hormone profiles mimicked that of the full-term pregnant female suggesting she was likely very close to implantation. An estrogen surge has been recorded to occur prior to nidation in several pinniped species

including northern fur seals (*Callorhinus ursinus*) [33], harbor seals [15], and grey seals (*Halichoerus grypus*) [34], and may therefore be a signal of the end of embryonic diapause and re-activation of embryonic growth. Unfortunately, the reproductive tract of the full-term pregnant animal was not collected, not allowing for an estimate of time from parturition using cervical dilation measurements.

Pseudopregnancy has been defined as a state in which a non-gravid female shows clinical symptoms of pregnancy [35], during which the CL produces progesterone, making pregnant and non-pregnant individuals endocrinologically indistinguishable [15,16,20]. Acknowledging the small sample sizes in this study, in both the total estrogen and progesterone profiles the pregnant and unbred females were indistinguishable, fitting the definition of pseudopregnancy. Pseudopregnancy has been recorded in multiple pinniped species including Steller sea lions [16], harbor seals [15] and harp seals (*Phoca groenlandica*) [23]. The duration of pseudopregnancy has been determined to be equivalent to that of embryonic diapause in other pinniped species [20,23,36,37]. In the Pacific walrus, embryonic diapause is estimated to last 6 months, meaning pseudopregnancy symptoms could persist in individuals beginning in December (Fig 2.1). Assuming signs of gravidity appear after breeding commences in March, nonpregnant females may have elevated concentrations of progesterone April-September, however samples are needed during these months of the year to confirm this.

In the case of the full-term pregnant female walrus (G160053), this reduced level of progesterone production (in comparison to the pseudopregnant females) may indicate that at this phase in the pregnancy the placenta maybe producing the required progesterone, however this has yet to be documented in pinnipeds. On the contrary, in ribbon seals (*Phoca fasciata*), spotted seals (*Phoca largha*) and Steller sea lions the placentae lack the enzymes capable of synthesizing progesterone, and it was determined that the CL is responsible for its production throughout the entire pregnancy [38, 39]. Studies on terrestrial carnivores including Japanese black bears (*Ursus thibetanus japonicus*), [22] and domestic dogs (*Canis lupus familiaris*) [40] have shown that the CL is the sole producer for progesterone even in late gestation, leading to the hypothesis that progesterone production in the placenta of carnivores may

not be possible. There are several possible explanations as to why the two pregnant females exhibited progesterone profiles lower than their pseudopregnant conspecifics. In pregnant bitches there is a rapid decrease in progesterone 12-24 hours prepartum [40]. It could be that the full-term pregnant female was just hours away from parturition and her CL was already down-regulating progesterone production. It is also possible that pseudopregnant females generally have greater levels of CL progesterone than pregnant females because pregnant females are removing the available progesterone produced by the CL and it is being circulated to where it is needed, such as in the increasing and growing uterine, placenta and mammary glands. Concannon et al. [40] found that progesterone-releasing implants in nonpregnant bitches yielded concentrations 3 times greater than that of pregnant females with the same implants.

There was a significant, positive relationship between the percent lipids found in CL and uterine horn width in the seven postpartum female walruses. Because the fetus develops in the uterine horn of this species [24] as in other pinnipeds, horn widths are greatest closest to parturition and diminish after birth with uterine involution. This supports the notion that percent lipids in CL may be a good indicator of time since parturition. Other studies that analyzed percent lipids in ovarian tissues of marine mammals were not found in the published literature, however percent lipids have been used to differentiate between reproductive classes of striped dolphins (*Stenella coeruleoalba*) in blubber [41] and their results showed that pregnant females had the largest mean percent lipids. Therefore, it could be expected that pregnant female walruses would have greater percent lipids than either postpartum or unbred females. When analyzing CL tissues, pregnant and unbred females had approximately twice the mean lipid content of postpartum females, though statistical significance was not found between groups, potentially due to the small sample sizes available for this study. Again, the unbred females were indistinguishable from the pregnant females, providing further support for pseudopregnancy in the two unbred animals. It is worth noting that one of the unbred females had the largest percent lipid content in ovarian tissues of the three reproductive states, which would be characteristic of a capital breeder, where energy reserves are stored in preparation for and during pregnancy. Surprisingly, when analyzing lipid content for ovarian tissues,

postpartum females had twice the median value of percent lipids as their pregnant counterparts. It is likely that after pregnancy, lipids in the CL disperse outward into the surrounding ovarian tissue and are being mobilized for lactation. Again, small sample sizes precluded us from making a strong statistical comparison and these results should be interpreted with caution as there was large variability in the percent lipids of postpartum females. This study supports that analyzing CL tissues for lipid content may be a more useful technique than analyzing ovarian tissues for measuring time since parturition or determining reproductive status during this time of year. More samples are needed to determine if percent lipid content can differentiate among females in differing reproductive states.

Follicular activity was measured by comparing estrogen profiles within the follicles of large versus medium follicle sizes and in active and inactive ovaries, as well as the number of follicles per active and inactive ovary. No significant differences were found between groups, though inactive ovaries tended to have slightly more follicles and slightly increased levels of estrogens. It was not surprising that inactive ovaries had more follicles than active ovaries as this is likely due to FSH suppressing follicular activity in ovaries containing active CL. Active ovaries have been documented to have less follicles than inactive ovaries in other pinnipeds [33]. It was expected that larger follicles would yield greater estrogen concentrations, however, Fay [24] found that follicles were not mature until they reached a diameter of 19 mm, meaning all of the follicles in our sample were too small to be nearing ovulation and subsequent increased progesterone production. This is yet another indicator that the females in our sample were not exhibiting postpartum estrus, nor were they in a phase nearing estrus.

In summary, the use of endocrine profiling to distinguish female walruses in various reproductive states is in its infancy but holds great potential. Specifically, profiling total estrogens of CL were useful in determining postpartum from non-postpartum females. This study presents the first documented evidence for pseudopregnancy in walruses. CL tissues were a better indicator than ovarian tissues for determining reproductive status and percent lipids may also be a promising tool for determining reproductive status and time since parturition for postpartum females. Studies on captive walruses have been carried out to

determine if pregnancy and ovarian activity could be determined using a variety of matrices, including saliva [42], vaginal fluid [43], and serum [44], though with limited success. These studies demonstrated that the reproductive hormones of captive animals were not contiguous with the breeding cycle of their free-ranging conspecifics. Future directions for these methods include estimating reproductive rates. Progesterone assays have been used in creating pregnancy rate estimates for cetacean species [10,12,45,46]. This technique could be extremely useful for walrus and should be validated in blubber and feces. Walrus are a particularly difficult species for obtaining reproductive rates because of their vast distribution, wary nature and harsh habitat to survey. Estimates for recent reproductive rates are lacking for this species [47]. It has been nearly three decades since reproductive rates have been quantified and current management practices do not account for reproductive rates in models used to estimate population size [47]. However, in order to determine pregnant from nonpregnant females during the potential pseudopregnancy phase in free-ranging walrus, we suggest 1) testing blubber samples and CL tissues when sampling in the spring, as blubber may acquire reproductive hormone signals more slowly than production sites and 2) sampling during fall and winter months, particularly from the end of September- end of November, when unbred females are less likely to be in a phase of pseudopregnancy (Fig 2.1). This study was limited by the sampling time frame and sample size; continued year-round sampling could determine the length and duration of pseudopregnancy, as well as determine the percent of the female population that experiences the pseudopregnant phase. Further, sampling programs that collect female reproductive tracts from subsistence hunted, free-ranging animals already exist and could be utilized to assess reproductive rates of this hard-to-study species that is experiencing rapid environmental changes.

2.6 Acknowledgements

We thank the Alaska Native hunters from Gambell and Savoonga that provided samples; the staff at USFWS, specifically Jonathan Synder and Brad Benter, for assistance obtaining samples, including fieldwork and permitting; Kendall Mashburn and Corey Fugate for laboratory assistance and Gordon

Kruse for statistical assistance and providing feedback on this manuscript. Fig 2.1 was created by Kierstin & Eric Keller of Golden Spiral Studios.

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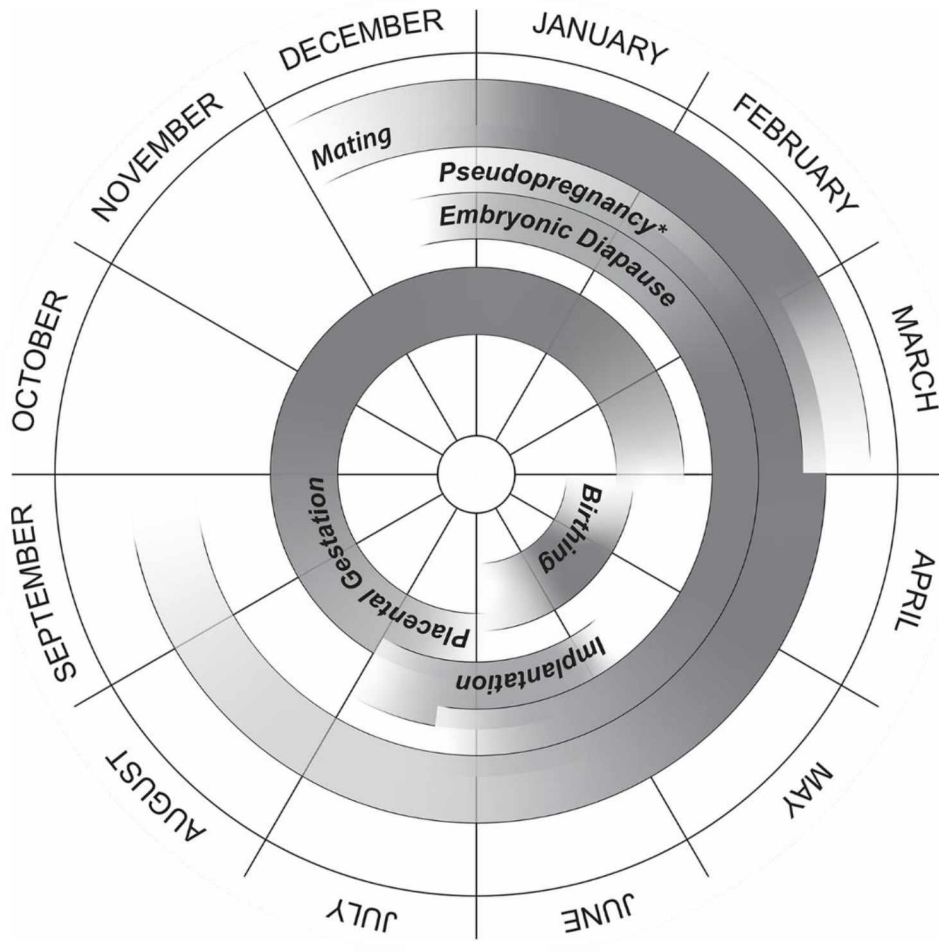


Figure 2.1. The reproductive cycle of the female Pacific walrus (adapted from Fay 1982). Findings from the present study that some females may experience a pseudopregnancy if they do not successfully breed (indicated by an asterisk). It is unknown if this is the case for the majority of the population and how long this phase lasts. Pseudopregnancy lasts a duration similar to that of embryonic diapause in other pinnipeds, which ranges from 1-5 months depending upon the species (Atkinson 1997); we expect that it may last between 3-6 months long in walruses.

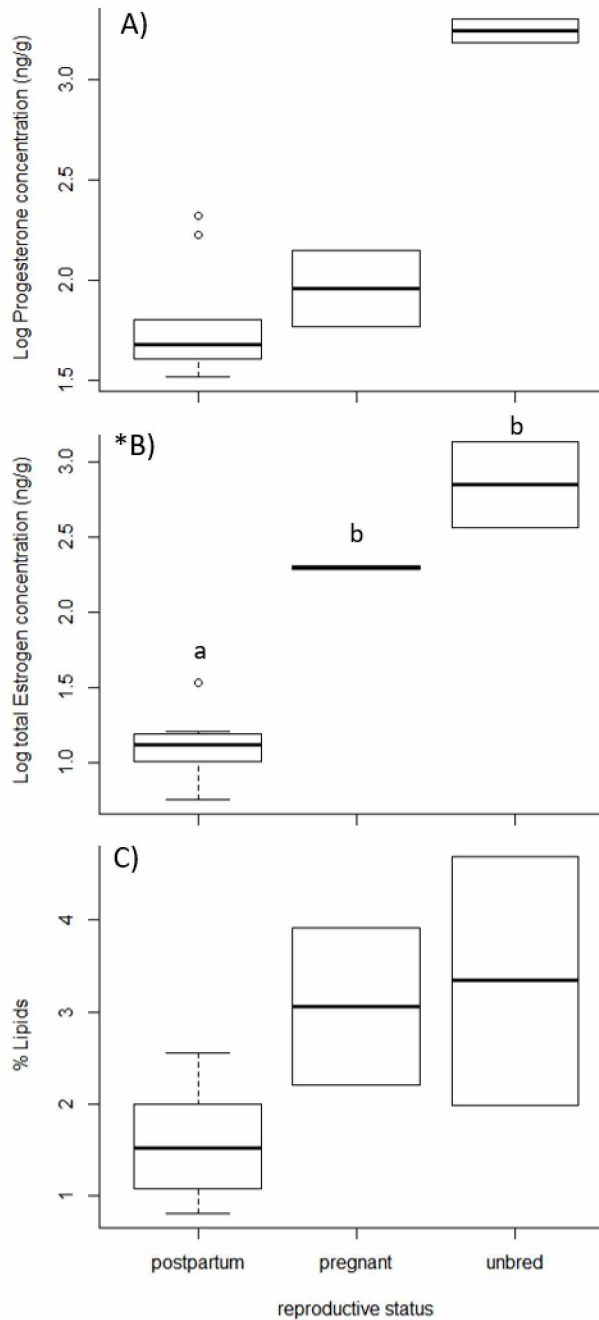


Figure 2.2. Median values of (A) progesterone (ng/g), (B) total estrogens (ng/g) and (C) percent lipids in CL of female Pacific walruses in three reproductive states: postpartum, pregnant and unbred. Animals were hunted by Alaska Native hunters near St. Lawrence Island during May 2011, 2015 and 2016. Note that hormone profiles are presented in a log scale (\log_{10}). Statistically significant differences were found between the total estrogens of pregnant and postpartum females as well as unbred and postpartum females as noted by the *B ($P < 0.05$). Different letters in plot (B) indicate significant differences, whereas the same letters indicate no significant difference. Box plots show medians, quartiles, minima and maxima of hormone profiles/% lipids. Circles indicate outliers.

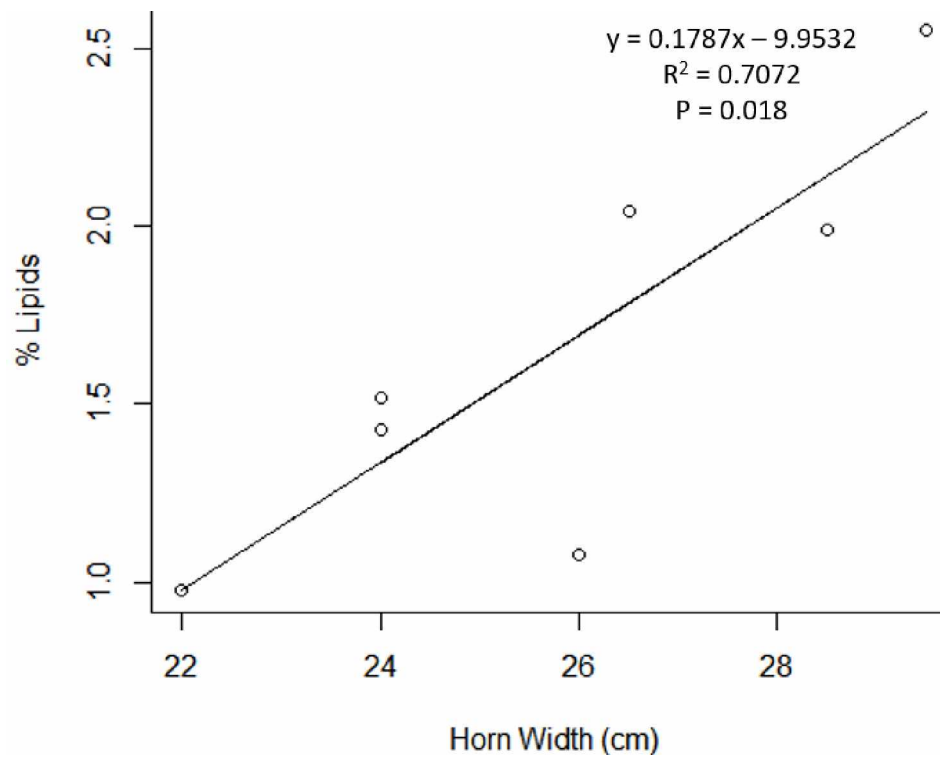


Figure 2.3. The positive, linear relationship between percent lipids and uterine horn width in postpartum female walrus hunted by Alaska Native hunters near St. Lawrence Island, Alaska, in May 2011.

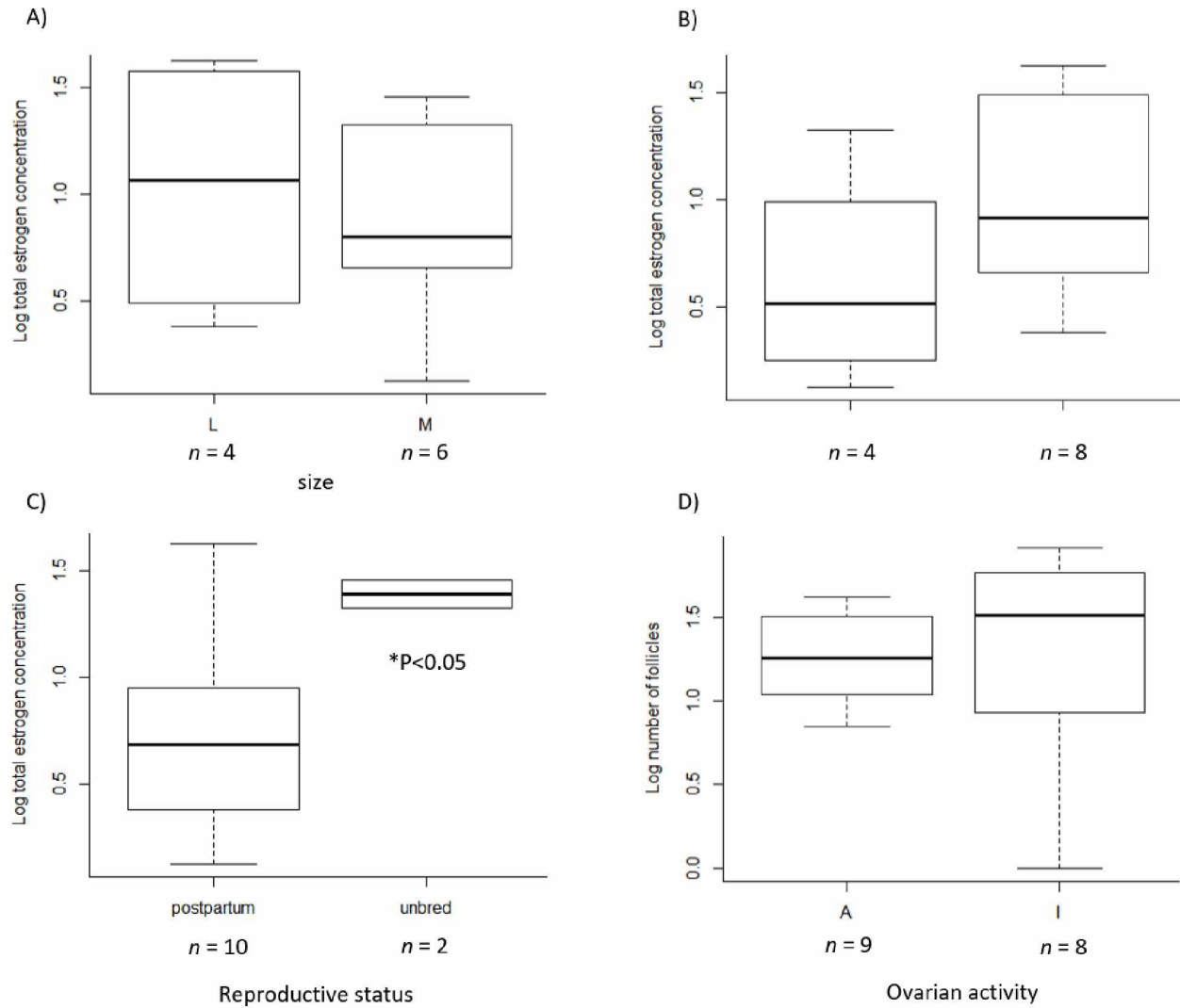


Figure 2.4. Follicular activity in Pacific walrus hunted by Alaska Native hunters near St. Lawrence Island during May 2011, 2015 and 2016. (A) Total estrogen concentrations in follicles for large (L) and medium (M) follicles, (B) total estrogen concentrations in follicles in active (A) versus inactive (I) ovaries, (C) total estrogen concentrations in follicles of postpartum and unbred females, and (D) the number of follicles in active versus inactive ovaries. All values are presented in a log scale (log₁₀). Unbred females had significantly higher levels of estrogen than postpartum females ($P = 0.005$). No significant differences were found between other groups ($P > 0.05$). Follicle sizes were determined as small (S; ≤ 1 mm), medium (M; 2-4 mm) and large (L; >4 mm). Ovarian activity was determined by presence or absence of corpora lutea. Box plots show medians, quartiles, minima and maxima of hormone profiles/% lipids.

2.9 Tables

Table 2.1. Life history data on female Pacific walrus used in this study.

Animal ID	Age	Lactating	Harvest date	Offspring present	Tract present	Reproductive status	Total estrogens (ng/g)		
							Progesterone (ng/g)	CL	OT
G160013	13	Y	5/13/2016	calf and yearling	Y	postpartum	63.24	15.98	n/a
G160048	n/a	Y	5/14/2016	calf	N	postpartum	59.33	10.56	11.05
S110004	7	Y	5/21/2011	calf	Y	postpartum	32.99	9.85	4.63
G110035	10	Y	5/8/2011	calf	Y	postpartum	40.73	14.93	1.85
S110021	11	Y	5/16/2011	calf	Y	postpartum	40.15	14.68	n/a
G110286	16	unknown	unknown	unknown	Y	postpartum	167.29	11.82	4.02
S110014	n/a	Y	5/15/2011	calf	Y	postpartum	n/a	n/a	n/a
S110002	16	Y	5/16/2011	calf	Y	postpartum	36.12	5.71	n/a
G110044	20	Y	5/8/2011	calf	Y	postpartum	47.72	34.09	3.31
G110001	22	Y	5/20/2011	calf	Y	postpartum	n/a	n/a	n/a
S110008	n/a	Y	5/20/2011	calf	Y	postpartum	209.83	n/a	n/a
G160053	n/a	Y	5/13/2016	fetus	N	full term pregnant	58.53	193.7	5.09
G160045	13	unknown	unknown	unknown	Y	pregnant diapause	140.3	205.44	16.37
G150005	19	Y	5/6/2015	yearling	Y	unbred	1533.01	368.92	43.77
G110290	16	unknown	unknown	unknown	Y	unbred	2007.18	1368.82	5.31

Chapter 3. Life without ice: perceptions of environmental impacts on marine resources and subsistence users of St. Lawrence Island³

3.1 Abstract

The Bering Strait region is undergoing rapid environmental change linked to loss of sea ice. The goal of the present study was to assess the impacts that environmental change and loss of sea ice has had on marine subsistence resources and resource users in the communities of St. Lawrence Island (SLI). A total of 24 stakeholders from SLI participated in ethnographic discussions during three weeks in May 2019. Five main themes were assessed: 1) key marine resources for SLI communities, 2) changes in key resources, 3) community responses to change, 4) community attitudes towards oil and gas development in the region, and 5) perceptions about the future of the subsistence way of life in these communities. Discussions were transcribed and uploaded into MAXQDA software, used for coding of themes. The four most discussed marine resources were Pacific walrus (*Odobenus rosmarus divergens*), seals, crabs and fish. The largest changes observed in these resources included: decreased walrus harvests and limited access with increased hunting effort; an increase in the abundance of Hanasaki crab (*Paralithodes brevipes*), also known as the spiny king crab, and limited crab harvests due to lack of shorefast ice; and increasing abundances of walleye pollock (*Gadus chalcogrammus*) and Pacific cod (*Gadus macrocephalus*). Oil/gas development was the most concerning human activity in the region. Two-thirds of stakeholders stated that they would not support oil/gas development near SLI due to the impacts it would have on marine animals either via disturbance or contamination. SLI residents utilize all edible marine flora and fauna and at times eat less preferred prey items. Lastly, the majority of stakeholders who addressed the future of subsistence felt that future generations will be harvesting and eating less subsistence resources. Due to environmental changes and most specifically loss of sea ice, SLI

³ Larsen Tempel J, Wise S, Osborne T, Sparks K, Atkinson SA. Life without ice: perceptions of environmental impacts on marine resources and subsistence users of St. Lawrence Island. Prepared for submission to Ocean and Coastal Management.

communities may have to diversify the species that they hunt and consume to continue their maritime subsistence practices, or stakeholders may turn to consuming more terrestrial wild and domestic resources such as reindeer (*Rangifer tarandus*).

3.2 Introduction

The eastern Bering Sea supports some of the greatest benthic biomass densities in the world (Grebmeier et al. 2006), underpinning some of the world's largest commercial fisheries (FAO 2018) and providing habitat for nearly 30 marine mammal species residing in the Bering Sea year-round or seasonally (Ray et al. 2014), as well as approximately 33 crab species (Byersdorfer & Watson 2010), and 34 avifaunal bird species that nest along the Bering Strait coastline and islands (Drury 1980). In 2019-2024 the Bureau of Ocean and Energy Management (BOEM) proposed waters in the Bering, Chukchi and Beaufort Seas as oil and gas leasing program areas (boem.gov). Lying directly between these two areas of interest is the largest island in the Bering Sea, St. Lawrence Island (SLI). Here, a qualitative study was conducted in 2019 to determine changes in marine resources and to document the perspectives of SLI stakeholders regarding oil and gas development in this region.

SLI is home to two of the largest Alaska Native villages in the Bering Strait region: Gambell and Savoonga, each holding a population of approximately 700 people (2010 US Census). These two villages are primarily Alaska Native as 95.6% and 94.5% of Gambell and Savoonga residents, respectively, are reported as being Alaska Native decent (as of the 2010 US Census). Inhabitants of SLI are Siberian Yupik (hereafter Yupik) and they rely heavily on marine resources as part of their daily dietary needs (Ahmasuk et al. 2008), cultural identity and social cohesion. Alaska Native communities in the Bering Strait, like other northern and Arctic inhabitants, rely on a mixed subsistence economy (Krupnik 1993, Robards 2008). On SLI, marine mammals, terrestrial plants, reindeer, store-bought food and federal aid make up the main components of this economy (Robards 2008), as jobs are scarce in the villages.

Rapid reductions in sea ice and increases in air and seawater temperatures have been well documented in the Arctic in recent decades (Stroeve et al. 2007, 2012, Polyak et al. 2010, Steele et al. 2008, Comiso 2012, Stroeve & Notz 2015, Hamilton & Stroeve 2016). While the effects of climate change are variable across species and regions, research in the Bering Sea has documented the following changes in association with environmental changes: marked shifts in the zooplankton community

structure (Coyle 2008, Coyle et al. 2011), shifts in run timing of salmon (Myers et al. 2010), changes in the diets of walleye pollock (*Gadus chalcogrammus*), as well as pink (*Oncorhynchus gorbuscha*), chum (*Oncorhynchus keta*) and sockeye salmon (*Oncorhynchus nerka*, Coyle et al. 2011), observations of baleen whales overwintering further north than previously recorded (Moore et al. 2006, Moore & Huntington 2008) and alterations in movements of marine mammals in response to changes in sea ice extent (Huntington et al. 2016). For the first time on record, data from a recent National Oceanic and Atmospheric Administration (NOAA) trawl survey in the southeastern Bering Sea showed a lack of the presence of the “cold pool,” a thermal barrier that isolates many fish and some invertebrate species from transiting between the northern and southern Bering Sea (Stabeno et al. 2019). This may have widespread impacts on productivity (Coyle et al. 2011) and predators feeding on benthic prey such as Pacific walrus (*Odobenus rosmarus divergens*), bearded seals (*Erignathus barbatus*) and grey whales (*Eschrichtius robustus*), as well as top trophic level predators such as polar bears (*Ursus maritimus*) and killer whales (*Orcinus orca*), some of which make up key components of protein in the diets of SLI residents (Ahmasuk et al. 2008). While ecosystem changes have been documented, there is a paucity of research on the effects of ecosystem changes on subsistence-based communities in this region.

The total number of walrus harvested across Bering Sea communities has plummeted in recent years (Krupnik & Benter 2016) and SLI hunters have reported earlier and quicker ice-break ups in the spring (Metcalf & Krupnik 2003). NOAA declared an Unusual Mortality Event (UME) during 2011-2016 for Alaska pinnipeds in the Bering and Chukchi Seas that were exhibiting lesions, hair loss and were stranding both live and dead; more than 600 seals and walrus were reported infected (www.fisheries.noaa.gov, a). Another Alaska ice seal UME was declared in the Bering Sea as of June 2018 and is ongoing (www.fisheries.noaa.gov, b). Additional concerns have included high levels of domoic acid and saxitoxin in Alaskan marine mammals due to harmful algal blooms, of which walrus stomach contents were reported to have the highest concentrations (Lefebvre et al. 2016). This has created concerns about consuming bivalves in this region as well, as SLI residents consume bivalves from walrus stomachs. These toxins may affect the health and behavior of walrus as well. These recent changes have

led to concerns about food security and safety, as well as overall environmental changes in the Bering Strait region.

The major aim of this research was to document *local* truths about marine harvested subsistence foods and attitudes towards gas and oil development. In response to identified research gaps, a qualitative research approach was conducted in 2019 on SLI to determine the following objectives: 1) the key marine resources for SLI communities, 2) changes over time in key marine resource harvests and use, 3) community responses to ecosystem change, 4) perceptions about the future of the subsistence way of life in these communities, and 5) community opinions about oil and gas development in the region.

3.3 Methods

3.3.1 *Survey methodology and approval*

The field work for this project was carried out in the communities of Gambell and Savoonga on St. Lawrence Island (Figure 3.1) during three weeks in April and May 2019 using the techniques of Rapid Qualitative Inquiry (RQI) (Beebe 2014). RQI is a technique that is defined by three basic concepts: 1) it is focused on obtaining the insider's or inhabitant's perspectives, 2) data collection involves multiple sources of data and is conducted in a team of two or more researchers, and 3) data analysis is an iterative process also conducted by a team (Beebe 2014). This type of research is appropriate for identifying variables that cannot be easily measured or numerically quantified and is useful to "hear silenced voices" (Creswell 2013) at a local scale.

Approval for this study was granted under Expedited Review by the University of Alaska Fairbanks Institutional Review Board (protocol number 1321844-3; a copy of the approval letter is provided in Appendix 3.1). The Native Villages of Gambell and Savoonga were included in the project development and gave permission for this research to be conducted in their communities (copies of letters of support are located in Appendix 3.2). Several other Alaska Native Organizations in the region were informed of the project including: the Eskimo Walrus Commission, the Alaska Eskimo Whaling

Commission, the Bering Sea Elders Group and Kawerak Inc (a Bering Strait regional non-profit corporation).

3.3.2 Stakeholder selection and study design

Stakeholders were purposefully selected (Creswell 2013) as the informants that could best address the thematic discussion topics (themes were framed as overarching research questions and are included in Appendix 3.3). In order to document information from the communities as a whole, two approaches were used for stakeholder selection. Key informants were identified as expert marine resource users and were comprised of elders, whaling and walrus boat captains and crew members, fishermen and family food preparers because of their enduring experience with hunting, harvesting and/or preparing of indigenous foods. Secondly, stakeholders that had more generalized knowledge of marine subsistence resources contributed information on the household level. To incorporate both stakeholder groups, purposive sampling was used for stakeholder selection. A balanced design was sought based on geography (physical location of the stakeholder's residence), age and gender. Physical location of the stakeholder's residence was plotted using a map and overlaying grid created using Google Maps imagery. Physical location was important for obtaining a sample representative of the population as housing locations vary by income and age. For example, in Gambell the Old Village site is closest to the ocean and typically houses elders or older individuals, whereas newer homes are being built closer to the school.

Ethnographic discussions (hereafter discussions) were conducted by a team of two researchers, one of whom had an established rapport with community members and the other who is from the Bering Strait region, of Alaska Native heritage, and contributed knowledge of indigenous food preparation, harvesting and preferences that engaged stakeholders. Discussions were modified with each stakeholder to explore the informants' expertise. Discussions were initially conducted by both researchers to standardize the protocol and later they were conducted separately. Although St. Lawrence Island Yupik (hereafter Yupik) is the primary language among adults aged 40 and over, all ages fluently speak and write English, therefore all discussions were conducted in English.

Although results were comprised of both expert key informants and household community members, during the data analysis phase all stakeholders were considered to be “stakeholders” due to the fact that they were long-term residents of SLI, and all individuals stated that they participated in subsistence and ate indigenous foods on a weekly or daily basis. “Participation” was defined as hunting animals, collecting plants or beach cast “seafoods”, preparing indigenous foods, and consuming indigenous foods. “Subsistence” has been a term associated with the state of Alaska’s subsistence surveys; the state of Alaska defines subsistence as “customary and traditional uses of fish and wildlife (www.adfg.alaska.gov).” However, some stakeholders expressed dislike for the term and preferred the use of “indigenous foods”, for this reason, here the two terms are used interchangeably.

If stakeholders granted permission, audio was recorded using a Zoom H4nPro Handy Recorder (2016 Zoom Corporation) and the discussion was later transcribed fully. In cases where stakeholders were not comfortable being recorded, notes were taken following the format of the survey instrument.

3.3.3 Data analysis

After compiling field notes, images and audio files, all surveys were transcribed into Microsoft Word. When audio recordings were available the transcription followed the exact flow of the conversation. When audio recordings were not available, notes and photos were compiled and organized into the survey format in a Word document. Transcriptions were then imported into the survey software MAXQDA 2020 Analytics Pro (VERBI software GmbH 2020) for coding of themes. MAXQDA was used to create frequency charts and assess connections between themes.

3.4 Results

3.4.1 Characteristics of participants

A total of 23 discussions were conducted with 24 stakeholders (in one instance two stakeholders conducted the discussion together) and participant ages and genders are presented in Table 3.1. The larger

number of stakeholders in Gambell was due to the fact that more time had been spent in Gambell during previous research, and therefore, more personal connections existed in this community.

Of the 24 stakeholders that participated in the study, the median number of people per household was 6 and the median number of people employed per household was 1 (employment may be full, part-time or seasonal). Stakeholders were asked to estimate how much they spend on groceries per month. The median amount per person was \$200 and per household was \$1200. Stakeholders were in agreement that the store prices have greatly increased in the last 25 years and most stakeholders said store prices increased within the last 10 years, whereas four said prices had not changed. People felt they had no control over store prices and many said that high store prices led them to rely even more on subsistence foods. Stakeholders said spending fluctuated with income and family size. Several men said that when they have more money, they spend more at the store and when they have less money, they rely more on subsistence harvest. One Savoonga resident articulated:

“... the cost of goods is outrageous, you know, I'd rather eat a steak of walrus than a steak from the store, that's like 30 gallons of fuel right there for one steak. Or close to it, you know? Fifty something dollars for a steak that's quite a bit of fuel I could be using for subsistence foods. A lot of times it comes down to - should I get fuel or should I go to store and buy groceries or should I pay for this bill or that bill, you know? A lot times we will chose not to go to the store because of lack of income.” (Savoonga, age 54).

3.4.2 Key marine resources

The importance of specific marine resources was determined by quantifying the number of times stakeholders referenced a particular species or group of animals in proportion to the number of times other species were referenced (Figure 3.2). Another way to interpret this is to say that this is what stakeholders most wanted to talk about and therefore these resources were considered most culturally important, if not the most consumed. All species listed in Figure 3.2 are consumed at times, except for killer whales which are considered sacred to SLI inhabitants. The four most discussed resources, in

descending order were: walruses, seals, fish, and crabs. On the other end of the spectrum, the four least discussed, in descending order were shrimp, porpoises, loons and killer whales. Because the main goal in this study was to determine *key* marine resources and the changes in those resources, the following section documenting changes will focus solely on the top four most important resources: crabs, fish, seals and walruses, as they are also undergoing the most change according to stakeholder knowledge. Though seals appeared second among the most discussed resources, it is because four species of ice-seals that are harvested on SLI were included: ringed (*Phoca hispida*), ribbon (*Phoca fasciata*), bearded and spotted seals (*Phoca largha*). Furthermore, the changes discussed in relation to ice seals were similar to that of walrus and they are therefore addressed together.

3.4.3 Changes in key resources

3.4.3.1 Walruses and seals

As stated by one stakeholder:

“I would say that our most powerful ally in our survival has been the walrus... the walrus provided for us—their tusks for harpoon hits, tools, sewing needles, and the skin for skin boats, and also the skin for summer homes... the summer houses are logs and covered with walrus skin... without the walrus, I think we would have had a very hard time. So the walrus gave us everything that we need, needed for hunting equipment, tools, building homes.” (Savoonga, age 60).

Walruses were the most mentioned resource in all surveys conducted (Figure 3.2), as stated in the quote above the relationship that SLI residents have with walruses goes beyond food for the table, it has been their lifeline to survival. The main changes stakeholders reported were reduced access and overall catch of walrus, increased hunting effort, and concerns about their reproductive success without ice. Decreasing sea ice, climate change and bad weather were attributed as the drivers limiting access to walrus herds. As one stakeholder put it: *“... because the ice re-routing somewhere, our hunting season has been real bad, like we haven’t seen or caught any baby walrus nor female walruses because of climate change.” (Gambell, age 63).* Most hunters witnessed this change in the course of the last two

decades. When asked about recent catch, one stakeholder stated: *“It has dramatically dropped... we’re harvesting probably less than half [of what] we used to harvest 10 years ago.”* (Savoonga, age 60). Seals were also mentioned in all surveys and several hunters reported that like walrus, seals are becoming harder to access. In particular, bearded seals were reported as being difficult to access due to declining sea ice.

“Bad weather” characterized by high winds has occurred more often than in the past and has reduced the number of days hunters can go out for walrus, seals and whales. When calm conditions allow, hunters are traveling further by boat than in previous decades. Several stakeholders reported going 80-100 miles away in search of walrus. The youngest stakeholder in the study also noticed a large change over her lifetime: *“... [referring to a hunting spot] its 83 miles away from this island and that’s the farthest I’ve had to go out before to harvest walrus. And a long time ago when I first started, we used to have to go 5-10 miles out. So that’s a huge difference from there.”* (Savoonga, age 20). Hunters reported that without ice cover, boats are prone to taking on larger swells. Additionally, hunters and nonhunters alike reported a shrinking hunting window. Historically the walrus harvest would last from April through June, now it has been condensed into the month of May. Consequently, a shorter hunting window and more bad weather days have made planning hunting trips near impossible. With changing conditions and small sections of ice moving by quickly, the hunting style has become more “opportunistic.” As one stakeholder explained:

“It’s more of an opportunistic hunting now... we’re starting to push the envelope of safety a little bit more because of open water. And lack of sea ice, you know, that’s factoring into the way we’re hunting. It’s more of an opportunistic technique we’re doing now... Combat hunting. We go out real fast and come back... Like spur of the moment hunting... In the past, we used to plan it out carefully, you know? Make sure we had everything. But now... everything is getting faster. Opportunistic hunting style, yeah.” (Savoonga, age 54).

In addition to low harvests, access to reproductively active female walruses was a concern. Females with calves have long been targeted by SLI residents. A particular delicacy includes fermented baby walrus and fermented lactating mammary glands of females. Some of the older stakeholders were

concerned as low catches of females and calves has meant that their grandchildren have not yet tasted these delicacies and there is a fear that they will not develop “a taste for them” if they are not eating this food from a young age. These dishes were historically aged in underground food caches. While food caches are no longer used to ferment baby walrus, “qasiqaq” (the term for this delicacy in Yupik), is usually eaten on the 4th of July and at birthday celebrations. Some stakeholders reported being worried that this unique part of their culture will be lost.

Additionally, community members reported that they are making less dried meat from bearded, ringed and spotted seals as seal harvests have been lower in recent years. Stakeholders did not all agree on the exact timeframe of declining harvests. One person noted decreasing seal harvests over the past ten years, another person said just in the last two years. Hunters also addressed the health of seal species in reference to the 2011-2016 Alaska Pinniped UME. Several stakeholders noted that seals are healthier now than they were several years ago when animals were reported having “balding skin” and “being skinny.” When asked when she noticed the change in health of harvested seals, an elder and food preparer responded: *“Maybe for the past 7 years, but this year is the first year I seen healthy ones, ‘cause I always cut up seals when my son brings them up.”* (Gambell, age 63). However, stakeholders did not report a change in the stomach contents of seals, providing evidence that dietary changes are not occurring, making it less likely that diet was a major factor related to the UME.

Another concern voiced by hunters regarded the reproductive success of female walruses and ice seals. In reference to the 2019 spring harvest season, one hunter said:

“Hardly any female with calves...they’ve caught none. And whether [they’ve] already gone past us, or [what] we don’t know. That’s one of the things that we get concerned [about], if they gave birth in the water, and there’s no means to feed their calves, there must be a high mortality rate for the females with their calves, and that’s one of the big concerns we have for the female [walrus].” (Gambell, age 68).

Furthermore, lack of sea ice will take away the only platform hunters have to harvest walrus and ice seals on, “... ‘cause if there’s no ice, there’s no means for them to get on ice to give birth to their young, or to

harvest them. [We] harvest the walrus on ice and bearded seal (Gambell, age 68).” Shooting is done while the animal is on the ice so the animal does not sink, and butchering is also conducted on the ice when stable.

Lastly, regarding seals, several stakeholders remarked that young bearded seals have been washing up dead for the past several years. One stakeholder attributed the deaths to the pups being crushed by fragmented sea ice. He estimated female bearded seals are currently losing a quarter of their young to this mortality. Bearded seals are valued for their meat and hunters said, as with walrus, lack of sea ice has made them harder to find in recent years. Other stakeholders remarked that shore ice is important pupping habitat for ringed and spotted seals, that spotted seals are showing up later in the year and that there were few to no ribbon seals in 2018.

3.4.3.2 Fish

Fish were talked about often during discussions. The species consumed included: Pacific halibut (*Hippoglossus stenolepis*), Pacific cod (*Gadus macrocephalus*), walleye pollock, all five salmon species (*Oncorhynchus sp.*) dolly varden (*Salvelinus malma*), sculpin species (*Cottidae sp.*), Bering wolffish (*Anarhichas orientalis*), polar eelpout (*Lycodes turneri*), Pacific herring (*Clupea pallasii*) and herring eggs, Pacific sand lance (*Ammodytes hexapterus*) and salmon sharks (*Lamna ditropis*). Though fish ranked high in the number of times discussed, in part this is attributed to discussions around commercial fishing, as Savoonga has a commercial halibut longline fishery. Subsistence fishing is done for halibut as well, but, in general, fish were not as valued as a food resource as marine mammal meat, including bowhead whales (*Balaena mysticetus*) though they were discussed less frequently. While fish are consumed frequently, it is likely that they outranked whale species because the survey focused on discussing changes seen in subsistence resources.

Stakeholders reported seeing large increases in both walleye pollock and Pacific cod. In Savoonga there is a commercial halibut longline fishery and when asked if Pacific cod are ever caught, one fishermen replied: “[We catch] too much cod... They were biting all our halibut hooks. Maybe 80% of our catch was cod fish. Catch too much cod we have to pull our lines and move ‘em somewhere else.”

(Savoonga, age 54). The perceptions on why fish stocks have moved was variable. While many attributed the shift due to sea ice loss, others who do not participate in the commercial fishery in Savoonga, have also witnessed changes in fish stocks, as evidence by more outside commercial fishing presence in the area. One Gambell resident stated:

“... they’re experiencing less pink salmon in Norton Sound, whereas out here, we see more fish coming around St. Lawrence Island, around Gambell – cause we have no commercial fishing here. And it seems that commercial fisheries, fishermen are pushing the fish further this way because we have no commercial fishing except for Savoonga that does commercial halibut fishing.” (Gambell, age 68).

This person’s view was not that that commercial fish stocks were necessarily moving due environmental changes, but that due to less resource availability in neighboring areas, fishermen are starting to fish the island’s untapped resources.

The increase in Pacific cod was not viewed with optimism by stakeholders. Though some stakeholders do consume the fish, it is not considered a preferred food item and not all stakeholders eat it. One individual considered them “too mushy” and he simply releases Pacific cod he catches when jigging.

Further, the fish do not currently provide economic benefits to fishermen. As one fisherman explained:

“... we have to get a separate license to get cod to sell, but they’re not as expensive as the halibut is. They’re only like 50 cents a pound. And we have to gut ‘em and it’s just not worth it.” (Savoonga, age 54).

Walleye pollock similarly were documented as being consumed by some, but most stakeholders did not mention eating them, again making their increase in abundance not of immediate interest to stakeholders. What was of great concern to stakeholders was that these increases in commercial fish stocks would also bring increases in visiting commercial fishing fleets.

3.4.3.3 Crabs

Stakeholders noted two major changes regarding crabs, namely that 1) the spiny king crab (*Paralithodes brevipes*), called the “Hanasaki crab” by stakeholders, is becoming much more abundant, and 2) crabs are far less accessible in recent years due to sea ice loss. As one individual stated: *“The*

Hanasaki crab from Japan is taking over this 5 miles east of us, we're getting the Hanasaki crab by the hundreds.” (Savoonga, age 60). This comment was echoed by many other stakeholders. Both blue king crabs (*Paralithodes platypus*) and the Hanasaki crabs are harvested primarily in winter under the sea ice using weighted lines. Unfortunately, for several years people have been unable to access these valuable resources due to lack of sea ice: “*But we have no more shore ice, we don't go handline crabbing anymore because there's no ice, for the past 7 years or so I guess it's been like that.*” (Gambell, age 63). one stakeholder reported. The stakeholders we spoke with enjoy harvesting and consuming this new species, however their growing abundance and strange spiny carapace, brought them much attention during discussions.

3.4.4 Community responses to change

One stakeholder stated:

“*[Our] people are here today because we use our own resources and common sense.*” (Gambell, elder age 81).

Community responses to change were classified in two ways: previously documented responses, and undocumented responses. Previously documented responses to ecosystem changes include consuming less preferred food items and utilizing all edible resources (Bockstoce 1995). In our study, stakeholders identified sea ducks (*Histrionicus histrionicus*, *Clangula hyemalis* and *Somateria sp.*), ribbon seals and belugas (*Delphinapterus leucas*) as less preferred food items. Sea duck meat is less desirable than marine mammal meat, however, as one stakeholder said, when he is at Whaling Camp (a place name for the whaling location Savoonga hunters launch from outside of the village) “it is better than nothing.” Stakeholders reported that ribbon seals are present and abundant when the last of the sea ice passes by the island, at the very end of the walrus harvest. Hunters acknowledged that these seals have a dark, bloody meat, but if they are unsuccessful in harvesting walruses they may take a young ribbon seal back to feed their family. Additionally, hunters reported spending more time harvesting bearded seals during other times of the year if they do not fill their freezers with walruses and bowhead. Belugas are considered a

delicacy by mainland Alaska Native communities; however, stakeholders on SLI showed aversion to the idea of consuming that “oily, fishy” meat. In addition to their poor tasting meat, when asked why belugas are not targeted hunters often said because their ancestors did not hunt them and that they are more focused on the opportunity to harvest a bowhead during this time. As one stakeholder said: “... *we don't generally go after them like the mainland hunters. We're hunting bowhead whales instead of belugas.*” (Savoonga, age 54). Food is strongly coupled with cultural identity and such views are common across cultures, especially among Alaska Native cultures (Borré 1991). However, stakeholders did note that belugas are an abundant resource and can be found year-round. On the other hand, bowheads are not successfully hunted each year in either SLI community.

Collecting beachcast seafoods is a community activity and has likely increased in years with decreasing sea ice extent as stakeholders reported more stormy weather due to the lack of protection from sea ice. Beachcast seafoods are edible animals that are washed up on shore and consumed. These include shrimp, sea stars, tunicates, sculpin and sea weeds. Stakeholders reported more storms, erosion and greater wave action along the shorelines due to climate change as diminished shorefast ice no longer protects these coastal communities from surf in winter. While storm surges bring more opportunities for harvesting beachcast seafoods, some residents reported that these seafoods are in poorer condition and are not always fresh enough to eat in comparison to a decade ago.

Through the present study, two previously undocumented responses to ecosystem changes were reported. These responses were not practiced historically, or changes in regulations have altered the way in which stakeholders have been able to harvest indigenous foods. In Savoonga, one resident that had just returned from a nighttime hunting trip explained:

“...this is the first time we went out at night, there was a[n] abundance of walrus with calves out there, so we couldn't miss that opportunity... We have to do a lot of things that are uncomfortable... unfavorable weather. Especially when there's females with calves out there, we went out just before dark, which we would never do in the past. But looking at the weather forecast, there's

going to be north winds coming and this could be our only chance to possibly harvest. So, that was something different.” (Savoonga, age 54).

In his 54 years of life he had always hunted walrus during the day. He found the experience uncomfortable due to the fact that the animals behaved much more aggressively in the dark. Walrus got off of the ice floes and the whole herd approached the boats in the water. The boats had to retreat. However, night hunting was not a new phenomenon for all of the stakeholders that participated. The youngest stakeholder in the study reported: *“We did it because the ice was passing by. My uncle wanted to go and other crews were going out and they were saying there were moms and babies so we went to go harvest them... there’s no reason why we gotta time when to go in or when to go out.” (Savoonga, age 20).* A discussion with an elder brought some clarity to these conflicting reports, stating that growing up they would always hunt walrus in the day time. This suggests that night hunting is likely a new response which is being practiced to allow for access to female and calf pairs of walrus. The second undocumented response reported was that stakeholders utilize the meat, blubber and skin from beachcast cetaceans that regulations imposed by the International Whaling Commission (IWC) prevent them from hunting. These cetacean species include minke (*Balaenoptera acutorostrata*), gray (*Eschrichtius robustus*) and humpback whales (*Megaptera novaengliae*).

3.4.5 Community attitudes towards oil and gas development in the region

Stakeholders were asked about the impacts human activities might have on subsistence practices in the region. The greatest concerns in increasing order were contaminants, commercial fishing, increased shipping and vessel traffic, and oil and gas development (Figure 3.3). Two thirds of stakeholders were concerned about the impacts oil/gas development would have on important subsistence resources and consequently said they would be unsupportive of its development in the region (Figure 3.4). A quarter of stakeholders were unsure if they would support oil/gas development and two stakeholders (8%) said they would be “supportive if...” giving a condition which would need to be met to gain their support. The reasons for the lack of support of oil/gas development were fear of displacing game or contaminating

game in the event of an oil spill. In the words of one individual: “... if those things happen to develop here, our game would just go further out somewhere. Or even we would be exhausted of mammals we hunt around our island. Big impact our diet.” (Gambell 72). To another stakeholder “the region” for consideration was not the immediate vicinity of the island or even the Bering Sea but extended into the Chukchi Sea: “[I’m] kinda worried about the Chukchi Sea being opened up to oil and gas development because we are ... so close to where all the marine animals live and if there should be a disaster, oil spill, it’s gonna kill a lot of what we harvest for food and I’m against it.” (Gambell, age 63). Residents expressed concerns that the community has no facility or training to deal with the response required to clean up an oil spill.

The two individuals that gave conditional support both made clear that the oil/gas company would have to benefit SLI residents and communities. One simply stated: “[I would] support [it], if [it] benefits our community, but un-support if it just benefits those companies.” (Gambell, age 68). The other stakeholder provided an example of a balance that he viewed worked well for the Alaska Native communities in the North Slope Borough:

“... before that happens I’m sure we’ll start seeing... [like what the] Alaska Eskimo Whaling Commission (AEWC) has, their own agreement with [the] oil company, the ships that pass by Arctic Slope, Chukchi, and Beaufort Sea. They have an agreement. They’re out whaling or harvesting walrus or anything that they have to harvest, they (the oil company) got to put a stop to that and after they’re (the hunters) done with that seasonal harvesting, they could start moving again... That agreement, if I remember correctly is a Conflict Avoidance Agreement, CAA... Which is pretty effective, especially to their hunters, providers... I’m sure we’ll start seeing those... we’ll need some sort of agreement in place to protect our lifestyle out here, indigenous lifestyle. Especially to feed our family, friends and relatives.” (Savoonga, age 52).

In brief, CAAs include time and area closures which allow hunters to harvest and provide for their communities while industry takes a break from their operations (Lefevre 2013).

3.4.6 Perceptions about the future of subsistence

Not all stakeholders commented on whether future generations would witness changes in the number of animals harvested or the amount of indigenous foods consumed. However, 14 individuals did address these questions and the majority (64.3%, 9 of 14 stakeholders) predicted that the next generations will see decreasing subsistence harvests, particularly for marine mammals, consume less subsistence foods, and may witness changes in the ways that animals behave. As one retired whaling captain explained:

“I’m very positive and sure that they will. Whether we will continue to have ice, if we don’t there will be no walrus and bearded seals on ice. They’ll be in the water... especially the polar bear, we hunt polar bear too. But it’s the ice that we’re most concerned [about], that will make the biggest change in our hunting practices of marine mammals. The walrus, the bowhead, the bearded seal – cause if there’s no ice, there’s no means for them to get on ice to give birth to their young, or to harvest them.” (Gambell, age 68).

While the anticipated decline in indigenous food consumption was most often attributed to loss of sea ice, it was also attributed to other factors including increasing grocery deliveries from mainland Alaska and economic hardships. When asked if younger generations will experience changes in the amount of subsistence foods that they eat in their lives, one stakeholder said: *“Yup, that’s already impacting us with three or four flights a day that are bringing groceries, but a lot of times when you’re unemployed, you know, you have no choice but to go out. I’d rather get subsistence food right now, compared to the price of groceries from the store.” (Savoonga, age 54).* Another stakeholder said: *“We’re harvesting less than half of what we used to harvest 10 years ago, 20 years ago and as the ice loss continues, that is going to decline and our people... we are really in desperate need of – coming up with our own self-generating, self-sustaining economies.” (Savoonga, age 60).*

Of the remaining five stakeholders that addressed the future of subsistence, two expected to see no change and three were unsure or hopeful that there would be no changes. Here is the perspective of

one individual that expects that younger generations will not see changes in the number of animals that they're able to see or harvest:

"I doubt it... with the help of science nowadays, from my understanding – both the walrus and the bowhead whale, they're pretty much healthy now in population. And that's about the only way I'm hoping it will just grow... [that] they don't drastically get commercially hunted again. That's what kind of depleted them back in the later part of 1880s. From there up until now, population from my understanding, is getting healthy – healthier." (Savoonga, age 52).

3.5 Discussion

This study builds on research documenting how climate change is impacting the ability of coastal Alaska Native communities to utilize subsistence harvested foods from the Bering Sea (Ahmasuk et al. 2008, Huntington et al. 2013, 2016, Krupnik & Benter 2016).

3.5.1 Key marine resources

Walruses, seals, fish and crabs were the top four marine resources discussed by Gambell and Savoonga community members. It was not surprising that walruses were the most discussed species, as approximately 84% of the US harvest of Pacific walrus occurs in the waters around SLI (MacCracken et al. 2017) and historically walrus were valued not just as a human food source but for feeding dog teams, making skin boats, ropes, drums and summer homes. What was surprising was that bowhead whales did not rank higher. We anticipated bowhead whales to rank second among all species. Likely this lower ranking is due to the fact that not all residents have access to harvest bowheads due to social rank, family relations, or the expenses of owning a boat, motor, equipment or the time commitment for those that work full time jobs. Fishing, crabbing and even seal hunting can be done without the expensive equipment, family connections and time commitment required by whaling captains and crew. This information therefore reflects marine resources that are more widely harvested and consumed by the communities of Gambell and Savoonga as a whole, yet still includes the voices of captains, crew and expert harvesters. Ahmasuk et al. (2008) conducted household subsistence surveys in Alaska Native villages in the Bering

Strait and documented harvest and consumption of marine mammals during 2005-2006. Based on the reported total number of pounds of meat harvested, both Gambell and Savoonga reported greatest harvests of walrus followed by bearded, spotted, ringed and ribbon seals and polar bear (in descending order). No harvested cetaceans were reported including bowhead whales. Though not quantified, the present study supports the notion that these targeted subsistence species have not changed in the past 15 years.

3.5.2 Changes in key resources

3.5.2.1 Walruses and seals

The primary changes regarding walruses and seals were 1) limited access to walrus herds during hunting, 2) increased hunting effort for both walruses and seals, and 3) concerns about the reproductive capacity of female walruses and seals. Loss of sea ice was attributed to causing all three changes.

Stakeholders reported declining walrus harvests over the past two decades. This information is supported by US Fish and Wildlife Service (USFWS) harvest reports which show a steady decline in both US and Russian harvests since 2000 (MacCracken et al. 2017). In 2013, Gambell harvested approximately one fifth of their annual mean catch (Krupnik & Benter 2016). Literature attributes hunting success mainly to effort, which in turn is determined by weather (Huntington et al. 2013, Krupnik & Benter 2016).

Huntington et al. (2013) found that one-quarter to one-third of the variability in the number of hunting trips that were made were explained by wind and ice conditions. The vessels used for whaling and walrus/seal hunting are typically 16-foot aluminum skiffs. These increasing distances in open ocean also put hunters in greater danger. Hunters reported that without the protection of sea ice, boats are exposed to larger swells.

Hunters also reported concerns about the health and survival of walruses and seals, particularly with regard to birthing. Walruses are considered an ice-obligate species as they use sea ice as a platform during mating, birthing, molting and resting (Moore & Huntington 2008). Scientists and managers have reported concerns about reproduction and calf survival of walrus. Since 2007 increasingly large herds

have been observed utilizing terrestrial haulouts (Fischbach et al. 2009, Fischbach et al. 2016). Of concern is that stampedes on land, have resulted in disproportionately large deaths of calves and juveniles (Fischbach et al. 2016). Ray et al. (2016) predicts that changing sea ice structure may also have detrimental impacts to winter reproductive habitat and may impact breeding success. However, literature is lacking information regarding birthing behavior, underscoring the value in documenting traditional and indigenous knowledge about the importance of walrus and seals giving birth on sea ice platforms. Also of importance is the tracking of ice seal strandings in Alaska. In 2011-2016 the state declared an ice seal UME, which involved both live and dead stranded ringed, ribbon and spotted seals in northern and western Alaska (www.fisheries.noaa.gov). The minimum estimated number of infected animals was 233 dead seals, 179 subsistence hunted seals and 245 live stranded seals, totaling 657 impacted seals. It was determined that an abnormal molt caused the lesions and symptoms presented by stranded seals, however the underlying cause for the abnormal molt and deaths was not determined. This UME closed in 2016. As of 2019, another ice seal UME involving the same species is currently ongoing in Alaska and has been declared for the Bering and Chukchi Seas. However, animals from this UME are not presenting lesions as in the 2011-2016 event. Additionally, none of our stakeholders reported seeing “balding” or lesions in the seals washed up as they had during the previous UME. Stranded animals in the current UME have represented all age classes and the total as of February 2020 was 277 effected seals (www.fisheries.noaa.gov). All of this information is timely as federal managers prepare to create a proposal for critical habitat for Arctic ringed seals and the Beringia bearded seal distinct population segment (Federal Register 2014).

3.5.2.2 Fish

Stakeholders reported increasing abundances of walleye pollock and Pacific cod, which is supported by recent NOAA trawl surveys. NOAA conducted northern Bering Sea trawl surveys in 2010, 2017 and 2019. The 2017 survey found that walleye pollock biomass and Pacific cod biomass increased by 1.3 million tons and 254 thousand tons respectively (Lauth et al. 2019). Results from the 2019

northern Bering Sea trawl survey found an approximate 5000% increase in walleye pollock biomass and a 1000% increase in Pacific cod biomass since their 2010 survey (personal communication Lyle Britt (AFSC)). Data from this recent trawl survey are still being analyzed during the time of this publication. While stakeholders agreed that the increases in walleye pollock and Pacific cod happened within the last 10 years, as asked in the survey instrument, it was noted that the change has been more abrupt. One SLI expert in fishing recognized the increase in walleye pollock to have occurred 3-4 years ago. Pinpointing this shift in fish distribution is of importance as NOAA trawl surveys suffered a 7-year gap in their data collection and with only three years of effort (2010, 2017, and 2019), it is possible that the survey in 2010 simply missed the walleye pollock population that was already present. However, this knowledge from local users affirms that this species is a recent arrival in the northern Bering Sea and that its abundance is increasing.

Of concern to stakeholders was potential interactions with commercial vessels utilizing these shifting fish stocks. This was concerning because previous interactions with commercial fishing and crabbing vessels have had negative impacts on marine mammal hunting around the island (Magdanz & Olanna 1985). Further, stakeholders were fearful that fishermen would overfish other marine subsistence resources that they harvest and rely upon. One of the advantages to using RQI research is to document “silent voices” (Creswell 2013) during times of rapid change. Bringing this concern to the attention of managers prior to conflict will allow for better relationships between subsistence and commercial users and managers.

3.5.2.3 Crabs

Historically, the blue king crab has been the dominant shellfish harvested in the nearshore waters of SLI. In fact, early Alaska Department of Fish and Game (ADF&G) reports have documented that blue king crabs were a commodity that SLI residents traded and sold to visitors (Lean 2005) and today SLI fishermen continue to barter and trade this resource with other villages on the mainland (Menard et al. 2018). On SLI, people simply call them “blue crabs.” Little information is known about the abundance of

red king crab (*Paralithodes camtschaticus*) around the island. Regional biologists consider red king crabs to be absent or in very low abundance in the nearshore waters of SLI due to the rocky substrate which is preferred by blue king crab (personal communication, Dawn Wehde, Crab Biologist Norton Sound Economic Development Corporation (NSDEC)). However, SLI community members have separate Yupik names for the two subspecies and a comprehensive subsistence report conducted by Kawarek in 2009 found that residents caught both red and blue king crabs, although catches of blue king crab were three times that of red during that year (Tahbone & Trigg 2010). Since 1984, waters within 10 miles of SLI have been closed to commercial fishermen to protect stocks harvested by SLI subsistence fishermen and to limit the impacts of commercial vessels on marine mammal presence in the area, and, unlike neighboring Norton Sound, the Bering Strait waters surrounding SLI have not been consistently surveyed by ADF&G (Menard et al. 2018), making data on crab abundance and catch scarce and undermining the importance of this information coming from SLI residents today. Reasons for discontinuing surveys include the closing of the Division of Subsistence office in Nome due to sudden declines in oil prices in the spring of 1986 (personal communication Jim Magdanz), and establishment of the Northern Bering Sea Research Area and SLI Habitat Conservation Area by the North Pacific Fishery Management Council in 2008 (<https://www.npfmc.org>). Trawl fishing is banned in the Northern Bering Sea Research Area, however permits allow for some research trawling to occur.

The Hanasaki crab was first documented in 2003 when a single crab was harvested at Little Diomedes Island (personal communication, Jim Menard, ADF&G). In 2012-2013 several specimens were caught during the winter commercial fishery in Nome and entire subsistence catches were comprised of Hanasaki crabs according to reports from Gambell in summer of 2013, including gravid females. NSDEC crab biologists speculate that the Hanasaki crab is establishing a population near Norton Sound with an “epicenter of abundance” occurring near SLI (personal communication, Jim Menard, ADF&G). To date, the most recent ADF&G trawl survey report published was from 2014 and encompassed only Norton Sound (Soong & Hamazaki 2015), lacking any abundance estimates of crabs near SLI. Neither *P. brevipes* catch nor was biomass recorded in the more recent NOAA northern Bering Sea trawl survey

reports, as only results for US commercial crab species have been published (Lang et al. 2018, 2019, Zacher et al. 2020). The Hanasaki crab are commercially harvested in Japan, though no commercial fishery has yet been established for them in Alaska. In any case, it is clear that data on the distribution and abundance of Hanasaki crab is lacking and requires further attention by the scientific community.

3.5.3 Food security

The overarching theme we discovered is that lack of sea ice has led to food insecurity. Decreases in walrus catch were attributed as a major factor leading to food scarcity. A woman in Gambell stated: “... *Four or five walruses... our meat racks would be full. Our freezers would be full. Right now, our meat racks are empty and our freezer barely has any.*” (Gambell, age unknown). It does not take many of these large marine mammals to feed one family; this point illustrates that even harvesting a handful of walruses has become a challenge. While the walrus harvest has historically been largely variable (Krupnik & Benter 2016), this is in great contrast within harvest numbers from the last 5-10 years. Between 2010-2014 the average harvests from Gambell and Savoonga were 821 walruses per year (MacCracken et al. 2017). From 2015-2019 catches from Gambell averaged 277 walruses per year (range 193-402) and in Savoonga 391 walruses per year (range 242-507) (personal communication Brad Benter (USFWS)).

None of the stakeholders stated directly that they experienced food scarcity due to a lack of crabs, making this connection less strong than that of walruses. However, historical subsistence surveys conducted on SLI document blue king crab as an important and regular dietary resource for some households (Magdanz & Olanna 1985). Historical subsistence reports regarding crabbing on SLI were conducted in 1983 and 1984 and addressed the harvest years of 1979-1984 (Magdanz & Olanna 1984, 1985). In 1984, a total of 15 households were surveyed and nine reported crabbing, catching a total of 733 blue king crabs, putting the annual household average at 81.4 crabs. In Savoonga, crabbing is done further from the village. Of the 23 households that participated in the study, three reported crabbing at Whaling Camp and six at an area five miles east of town. Catch was low at Whaling Camp, but the annual catch at

the other site was 165 crab with an annual household average of 23.5 crabs in 1984 (Magdanz & Olanna 1985).

Because subsistence harvest surveys have not been continually carried out in these communities, it is impossible to say if household consumption of blue king crab has varied much since the 1980s; however stakeholders made clear that access has been cut off from this resource due to sea ice loss, greatly reducing harvest opportunity. Participants reported that crab pots cannot be used here due to the strong currents that have caused them to be washed offshore in the past. Based on this decreased access and the percentage of times crabs were mentioned across all discussions, we suspect that blue king crabs and now Hanasaki crabs are key marine resources consumed by a sizeable part of the population in both Gambell and Savoonga when ice conditions allow, and that this resource may be largely overlooked by managers due to lack of data.

3.5.4 Community responses to change

The communities of Gambell and Savoonga remain among the most isolated Alaska Native villages in the state. This is evidenced by the fluency with which the adult population still speaks in their native language. Closer to mainland Russia than mainland Alaska, cut off from roads, and at times service from planes and vessels, the survival of SLI inhabitants has been tightly coupled with the sea since time immemorial. Yet, in these recent times of rapid change it seems that the ability of these communities to be resilient is being constrained in new ways. Resilience in social-ecological systems has been defined as the capacity of a system to maintain its fundamental properties in spite of large perturbations (Brinkman et al. 2007), whereas adaptability is the capacity that a system's *stakeholders* have to influence its resilience (Walker et al. 2004). Together, resilience and adaptability, allow subsistence-based communities to persist in today's economic driven society.

Results from this study indicated that SLI residents are responding to environmental changes in a variety of ways. Like any other culture, SLI residents have cultural food preferences, but in times of need these preferences are replaced with whatever foods are available; this adaptive strategy has been practiced

historically and has allowed for survival when marine mammals were scarce. One such example is the harvesting of sea ducks, which are consumed in times of need though they are less preferred than marine mammal meat. The people of SLI have survived in this isolated and remote region because as a Gambell elder stated, “we use our own resources.” This has meant consuming all that is edible including sea stars, tunicates, sculpin, sea weeds and even the hides of walruses during times of great famine (Bockstoece 1995).

Other responses to environmental changes included hunting walruses at night and harvesting beachcast cetaceans that are otherwise prohibited from harvesting, such as humpback, gray and minke whales. Stakeholders stated that historically gray and minke whales were hunted by SLI people and gray whale meat is considered a delicacy that rivals even bowhead. Regulations imposed by the International Whaling Commission currently prevent any take on these species and communities abide by these rules by consuming beachcast whales, whenever they are fresh enough to be consumed.

3.5.5 Community attitudes towards oil and gas development in the region

The majority of stakeholders were against the development of oil and gas near SLI due fear of contamination of game that is consumed and fear of disturbance that would cause game to leave the area. A quarter of stakeholders were unsure if they would be supportive/unsupportive oil and gas development. Likely this large proportion of uncertainty was in part attributed to communication challenges as researchers noted that several times when asked about oil and gas development stakeholders interpreted the question as though the researchers were asking about either engine or heating fuel and responded that “prices were too high.” Words like “exploration” and “development”, which were originally used in the survey, were not clear to stakeholders and researchers used the term “drilling” to provide clarity to stakeholders if the original question was misunderstood. In addition, stakeholders frequently stated that their corporation deals with these issues as SLI residents opted for land ownership of SLI under the Alaska Native Claims Settlement Act (43 USC 1601-1624) in 1971 and SLI is considered a “sovereign

nation.” This may have also increased the number of individuals that could not determine if they would be supportive or unsupportive of oil and gas development.

3.5.6 Perceptions about the future of subsistence

Many stakeholders felt that younger generations will eat less subsistence harvested foods and turn toward “less healthy” store foods, yet most families find them unaffordable. Stakeholders were interested in adaptive solutions for solving issues of both food security and economic hardships. One new avenue for generating a self-sustaining economy on SLI that was discussed was the potential to start producing SLI reindeer (*Rangifer tarandus*) meat as a commercial product. This would provide jobs and new sources of income, yet may reduce the number of people participating in and eating subsistence foods harvested from the Bering Sea and turn them towards more reliance on reindeer consumption.

3.5.7 Conclusions

SLI community members lack control over environmental (sea ice loss and a shifting Bering Sea ecosystem) and extrinsic (food prices and the job market) drivers that are impacting food security and cultural values. In the past, residents have filed for economic disaster relief in years of poor walrus harvests (Krupnik & Benter 2016). Today it is unlikely that any policy actions will return harvest rates to their historical levels.

Cultural preferences may inhibit flexibility of inhabitants to utilize other more abundant resources, as is the case with harvesting belugas on SLI, which are more abundant and more easily harvested than bowhead whales. There are examples in which communities have altered targeted game species based on access and availability. One such example is on Prince of Wales Island in Southeast Alaska, in which extensive logging created easier access to deer. Inhabitants switched hunting practices from primarily maritime resources to Sitka black-tail deer (*Odocoileus hemionus sitkensis*) in one generation (Brinkman et al. 2007). Yet, it is unlikely that such a drastic change will arise in the future on SLI if this adaptation to easier prey did not occur in times of declared disasters. More likely SLI residents will desire to hunt other abundant cetacean species that have historically provided for their ancestors, as mentioned

previously, including grey and minke whales. In the future, regulators may expect these species to be petitioned for hunting quotas in replacement of ice-obligate and ice-associated species such as walrus, bowhead, ice seals and polar bears. Further, community members discussed their interest in commercially producing the island's local reindeer meat. This would require an increase in reindeer herd size and establishing a meat processing plant, which is also being discussed. It is possible that local protein consumption may shift towards more domestic, terrestrial resources within the next decade.

To address community concerns about loss of culture, educational programs are already underway. Fall of 2019 was the first semester in 25 years in which the Savoonga school began teaching the Yupik language again (Bering Strait School District Bilingual Curriculum). This change was brought about by demands from community members during formal community meetings. This is also an avenue for teaching the values and indigenous knowledge embedded within the Yupik language. Most adults age 40 and older are fluent in the language but there is a large generational gap in which younger adults and youth cannot speak or understand it. Another deliverable from this research is to bring subsistence knowledge into the schools. High school is the highest level of education offered on the island and currently there are no units that discuss the Marine Mammal Protection Act or co-management. Youth lack access to understand how they can manage their communities' resources. This may be the greatest inhibitor to community resilience, a lack of knowledge that there are laws in place for indigenous management of resources. By incorporating these lessons in the schools, communities are empowered and empowered communities are not trapped communities, they are resilient ones.

In summary major findings from this study reveal that:

- SLI hunters are concerned about calf/pup mortalities of walrus and seals due to sea ice loss causing drowning mortalities, and fragmentation of sea ice crushing seal pups.
- Some hunters are traveling farther (80-100 miles) to access walrus. This is resulting in increased risks to human safety.

- Hunters are adapting to change by employing a more opportunistic hunting style, including hunting walrus as night.
- Community members are increasing their food security by consuming deceased, beach cast cetaceans whose take is prohibited due to regulations. This is the first documentation of this harvesting strategy on SLI.
- Blue king crab and Hanasaki crab are undervalued, key resources that require further scientific study to document species abundance and distribution around SLI.
- Changes in commercial fish stocks may lead to increased commercial fishing pressure around SLI. Residents expressed concerns about the impacts this may have on local subsistence fish, crabs and marine mammal abundances.
- Most stakeholders were not supportive of oil and gas development near SLI/ in the Bering Strait due to concerns about oils spill contaminating food resources and operations causing disturbance and dispersal of hunted marine mammals.

This information highlights concerns about the perceived impacts of climate change on the communities of Gambell and Savoonga. A proactive approach with clear and open communication between resource managers, SLI residents and commercial fishing vessels as well as oil/gas companies is recommended to ensure SLI inhabitants are able to maintain their indigenous, subsistence-based lifestyle.

3.6 Acknowledgements

We would like to thank and acknowledge all of the SLI stakeholders that contributed their time and knowledge to this study. We also thank the Native Villages of Gambell and Savoonga and their respective IRA Councils that participated in shaping this study and invited us to attend and hold community meetings and discussions. In particular we are grateful to Delbert Pungowiyi, Michael James and Gloria. We thank committee member Gordon Kruse for providing feedback that improved this manuscript. This publication is the result in part of research sponsored by the Cooperative Institute for Alaska Research with funds from the National Oceanic and Atmospheric Administration under

cooperative agreement NA13OAR4320056 with the University of Alaska. Additional support was provided by the Coastal Marine Institute/Bureau of Ocean Energy Management and the Marine Mammal Commission (Grant #: MMC19-172).

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3.8 Figures

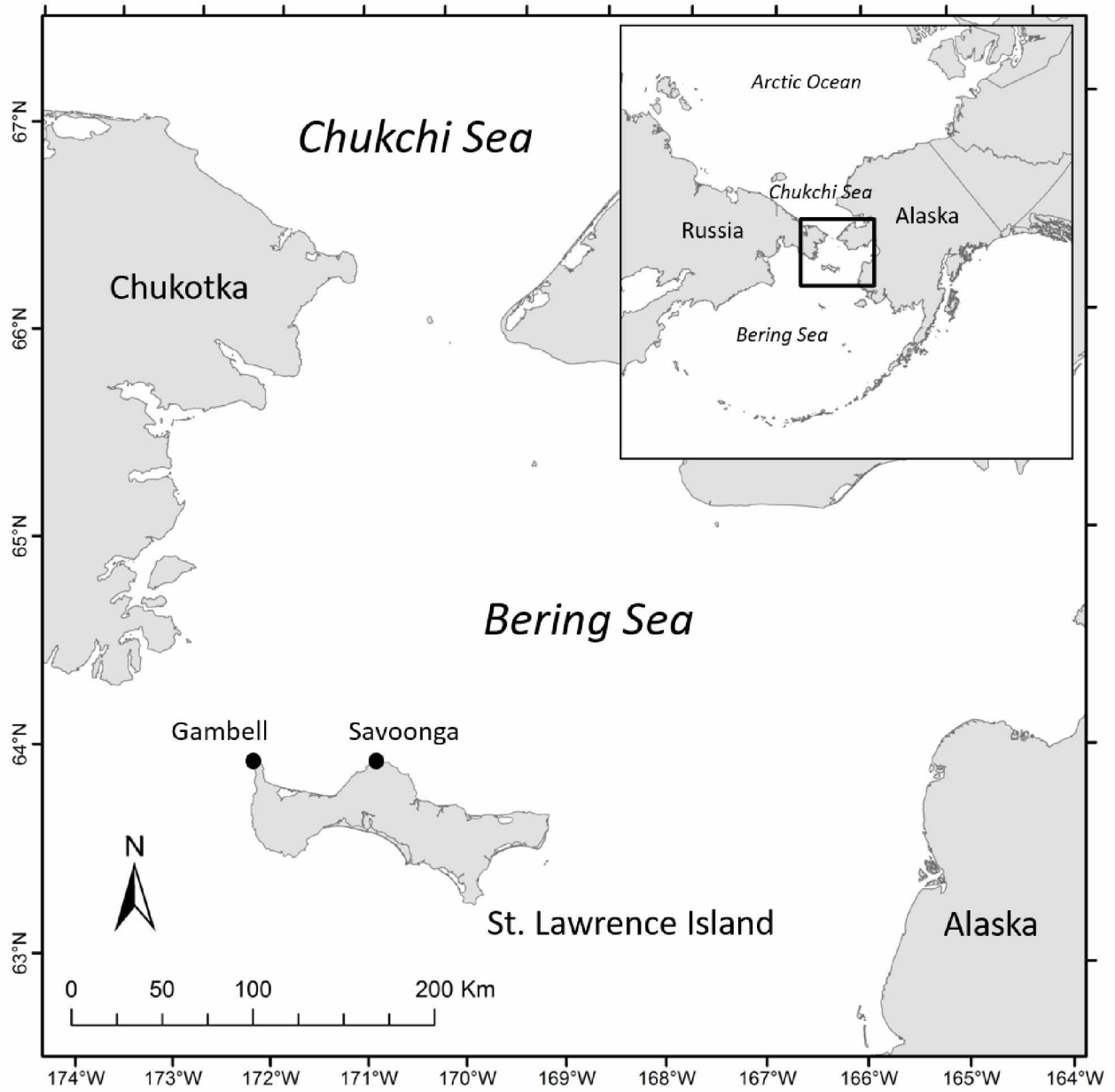


Figure 3.1 Study sites on St. Lawrence Island, Alaska.

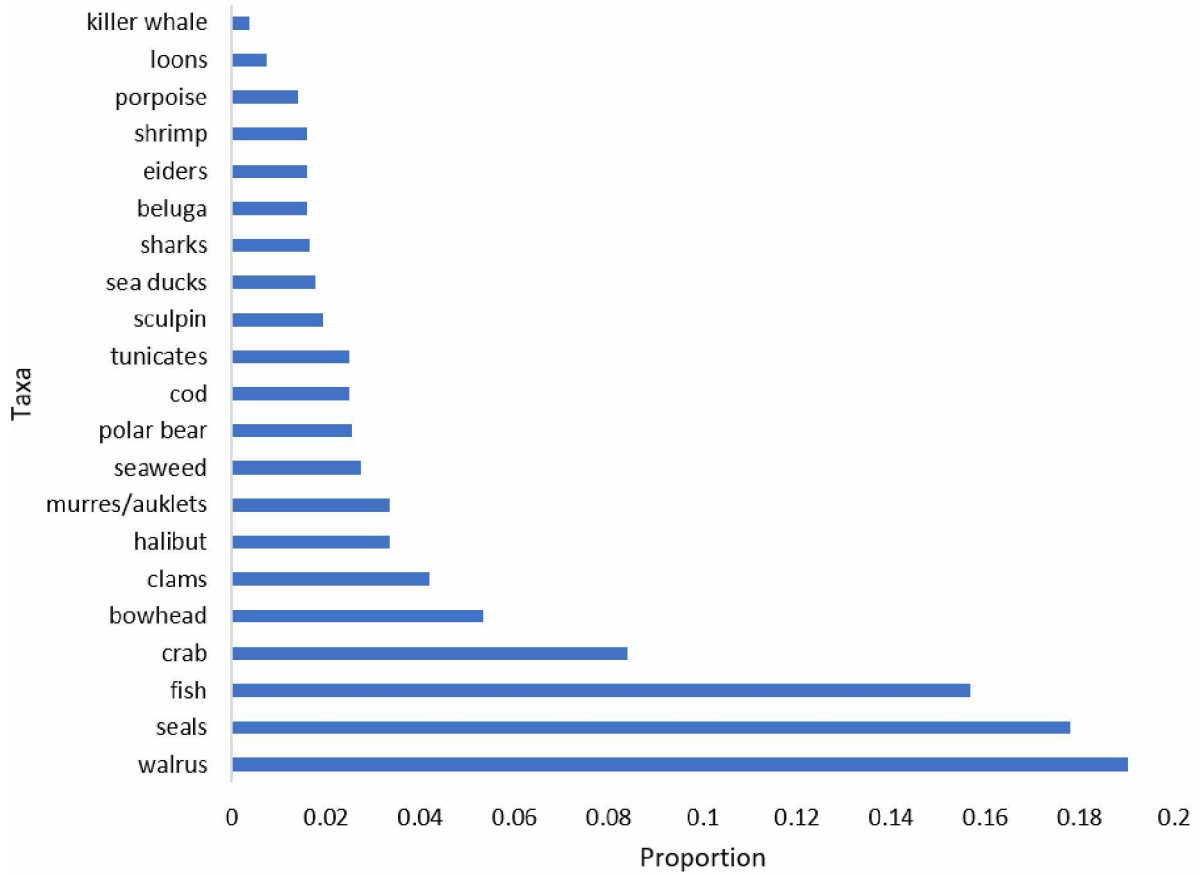


Figure 3.2 The proportion of times resources were referenced across all discussions. Resources at the bottom of the chart were discussed most often referencing cultural and dietary importance, as well as changes regarding the harvest, health or abundance of the resource.

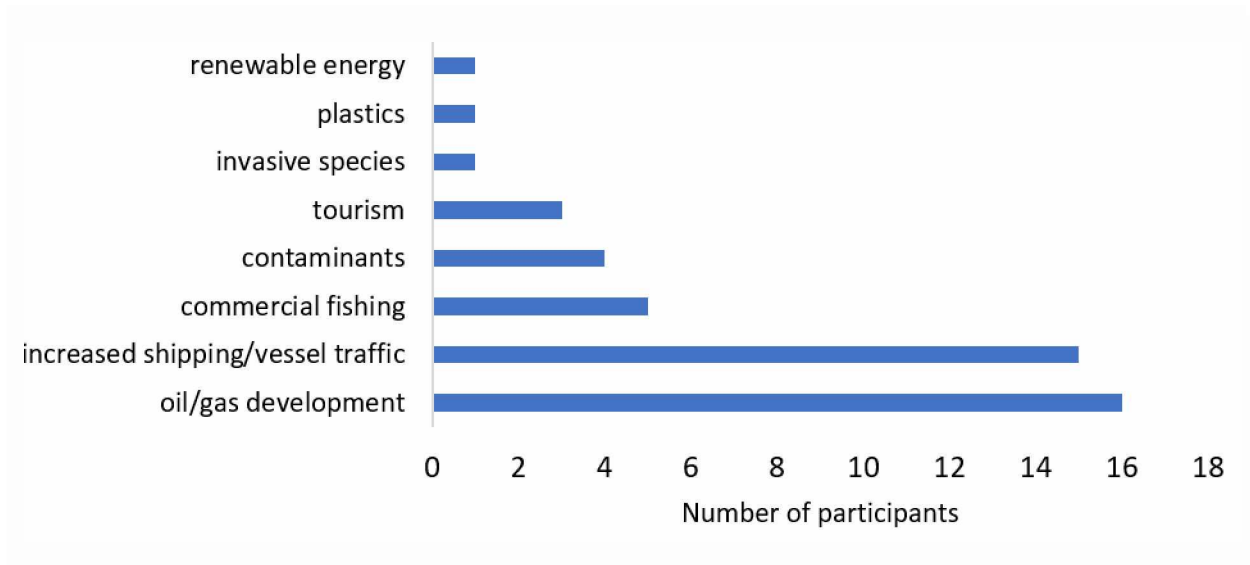


Figure 3.3 The number of participants listing various human activities as a concern due to impacts they may have on subsistence harvested foods.

■ unsupportive ■ conditionally supportive ■ unsure

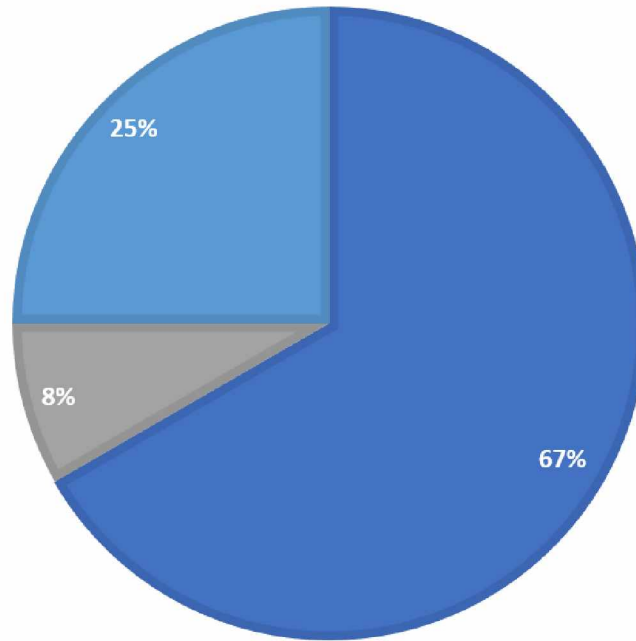


Figure 3.4 The proportion of participants (n = 24) that were unsupportive, conditionally supportive and unsure if they would be supportive of oil/gas development near St. Lawrence Island.

3.9 Tables

Table 3.1 Number of participating stakeholders by (a) age and (b) gender. A total of 24 stakeholders participated and 23 discussions were held.

a)

Ages 20-39	Ages 40-59	Ages 60-79	Ages 79+	Age unknown
5	8	9	1	1

b)

Gambell		Savoonga	
Female	Male	Female	Male
10	6	2	6

3.10 Appendix A. IRB approval.



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Institutional Review Board

909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

June 12, 2019

To: Shannon Atkinson, Ph.D.
Principal Investigator

From: University of Alaska Fairbanks IRB

Re: [1321844-3] Change and resiliency in the Bering Sea ecosystem: assessing changes in abundance and seasonality of marine resources and effects on subsistence communities

Thank you for submitting the Amendment/Modification referenced below. The submission was handled by Expedited Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title:	Change and resiliency in the Bering Sea ecosystem: assessing changes in abundance and seasonality of marine resources and effects on subsistence communities
Received:	June 5, 2019
Expedited Category:	7
Action:	APPROVED
Effective Date:	June 12, 2019
Expiration Date:	February 25, 2020

This action is included on the July 3, 2019 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.

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909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

February 12, 2020

To: Shannon Atkinson, Ph.D.
Principal Investigator
From: University of Alaska Fairbanks IRB
Re: [1321844-4] Change and resiliency in the Bering Sea ecosystem: assessing changes in abundance and seasonality of marine resources and effects on subsistence communities

Thank you for submitting the Continuing Review/Progress Report referenced below. The submission was handled by Expedited Review under the requirements of 45 CFR 46.110, which identifies the categories of research eligible for expedited review.

Title: Change and resiliency in the Bering Sea ecosystem: assessing changes in abundance and seasonality of marine resources and effects on subsistence communities
Received: February 5, 2020
Expedited Category: 7
Action: APPROVED
Effective Date: February 12, 2020
Expiration Date: February 25, 2021

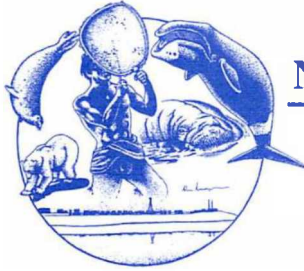
This action is included on the February 5, 2020 IRB Agenda.

No changes may be made to this project without the prior review and approval of the IRB. This includes, but is not limited to, changes in research scope, research tools, consent documents, personnel, or record storage location.

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Appendix B. Letters of support from the Native Village of Gambell and Savoonga.



NATIVE VILLAGE OF GAMBELL

P.O. BOX 90 • Gambell, Alaska 99742
Telephone: (907) 985-5346 • FAX: (907) 985-5014

December 18, 2018

Jenell Larsen and Shannon Atkinson, Ph.D.
University of Alaska Fairbanks, Juneau Campus
College of Fisheries and Ocean Sciences
17101 Point Lena Loop Rd
Juneau, AK 99801

Dear Ms. Jenell Larsen and Dr. Shannon Atkinson,

The Gambell IRA Council strongly supports your proposed research by the University of Alaska Ph.D. Candidate Jenell Larsen and her advisor Dr. Shannon Atkinson, studying "change and resiliency in the Bering Sea ecosystem: assessing changes in marine resources and subsistence use." Ms. Larsen has worked in our community in the past for her previous Ph.D. research on walrus. We understand that this research will include interviews with community members and key informants that are considered experts in subsistence harvesting.

In support of this research, the Gambell IRA Council will assist the project leaders in identifying these knowledgeable experts for interviews on the abundance, seasonality and overall health of our marine animals and plants. We also understand and are in support of the project including a local resident and UAF undergraduate student as a research assistant.

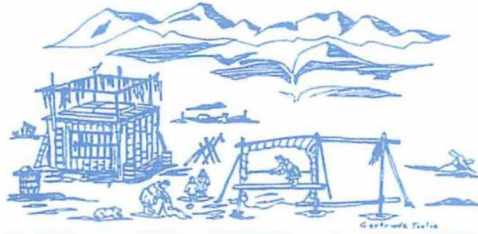
Ms. Larsen and Dr. Atkinson have already shared their project goals and a draft interview questionnaire with our tribal council and we are interested in this work and their results. We understand that this research will take place during the year 2019 and that results will be presented in person to our community by 2020. The Gambell IRA Council strongly supports this research and looks forward to working with Ms. Larsen and Dr. Atkinson to determine how our ecosystem is changing and the effects this is having on our community.

Sincerely,

Brandon Boolowon

President, Native Village of Gambell

"Established in 1934, dedicated to serving the members and preserving the culture."



NATIVE VILLAGE OF SAVOONGA • P.O. BOX 120, SAVOONGA, AK 99769 • PHONE 984-6414 • FAX 984-6027

Jenell Larsen and Shannon Atkinson, PhD
University of Alaska Fairbanks, Juneau Campus
College of Fisheries and Ocean Sciences
17101 Point Lena Loop Rd
Juneau, AK 99801

Dear Ms. Jenell Larsen and Dr. Shannon Atkinson,

The Savoonga IRA Council strongly supports your proposed research by the University of Alaska Ph.D. Candidate Jenell Larsen and her advisor Dr. Shannon Atkinson, studying "change and resiliency in the Bering Sea ecosystem: assessing changes in marine resources and subsistence use." We understand that this research will include interviews with community members and key informants that are considered experts in subsistence harvesting.

In support of this research, the Savoonga IRA Council will assist the project leaders in identifying these knowledgeable experts for interviews on the abundance, seasonality and overall health of our marine animals and plants. We also understand and are in support of the project including a local resident and UAF undergraduate student as a research assistant.

Ms. Larsen and Dr. Atkinson have already shared their project goals and a draft interview questionnaire with our tribal council and we are interested in this work and their results. We understand that this research will take place during the year 2019 and that results will be presented in person to our community by 2020. The Savoonga IRA Council strongly supports this research and looks forward to working with Ms. Larsen and Dr. Atkinson to determine how our ecosystem is changing and the effects this is having on our community.

Sincerely,


Fritz Waghiyi, Vice President

Native Village of Savoonga

Appendix C. Expert subsistence survey questions used in interviews. Community member surveys differed only in that sections B and C were excluded.

Name of interviewee:

Gender:

Village:

Interviews at length with key informants

Preliminary questions:

1. Do you participate in subsistence harvesting? Y/N
2. How often do you eat subsistence harvested foods?
Everyday every other day every week every month less than every month

Community level practices [using resource identification guide]:

1. Which of these do you eat? Will you point to the ones you eat?
2. For each picture can you:
 - a. Say and/or write the Yupik and/or common name you call it?
 - b. Tell me when and how you harvest it?

A) Overarching research question: in what ways have these species changed?

1. In what ways have the species you harvest changed:
in the past 10 years? _____ 25 years? _____ Over your lifetime? _____
 - a. Do you see more, less or the same amount now?
 - b. Do you find them in the same location now as you did in the past
10 years? _____ 25 years? _____ Over your lifetime? _____
 - c. Do you always find them during the same time of year? When?
 - d. Have you noticed changes in the health of these animals? Y/N
 - e. Are they as fat and healthy as they were in the past
10 years? _____ 25 years? _____ Over your lifetime? _____
 - f. Have you noticed any new animals? Y/N
 - i. If yes, what species?
 - ii. When did you first see them?
 - iii. How frequently do you see them?
 - iv. Where do you see them?
2. Why do you think these changes are happening?
3. Do you use these animals and/or their parts for other things besides food? Y/N
 - a. Can you show me which animals and tell me about their other uses.
 - b. Have these uses changed in the past
10 years? _____ 25 years? _____ Over your lifetime? _____

B) Overarching research question: how are individuals and families responding to these changes?

[Looking for physical responses]

1. Have you changed the way you harvest subsistence foods, hunt or fish from in the past:
10 years? _____ 25 years? _____ Over your lifetime? _____
 - a. If yes, how?
 - b. If yes, why?

Name of interviewee:

Gender:

Village:

2. Do you harvest subsistence species in the same areas as in the past? Y/N
 - a. If no, can you describe the changes?
3. Do you spend more (M)/less (L)/or the same (S) amount of time doing subsistence activities now as you did:
10 years ago? S/M/L 25 years ago? S/M/L Over your lifetime? S/M/L
 - a. Why do you think?
4. Would you say that you are you eating the same/more/less amount of subsistence foods as 10 years ago? S/M/L 25 years ago? S/M/L Over your lifetime? S/M/L
 - a. If no, why not?
 - b. In the past, how regularly did you eat subsistence foods:
10 years ago:
Everyday every other day every week every month less than every month

25 years ago:
Everyday every other day every week every month less than every month

C) What does the future of subsistence harvest look like to users?

1. Are you teaching your children (and/or grandchildren) the same subsistence harvesting ways you know? Y/N
 - a. Can you give me an example of what you teach them?
2. Do you think the next generation will see any changes in...
 - a. The numbers of animals they will see or be able to harvest? Y/N
 - b. The amount of subsistence foods they will eat in their lives? Y/N
 - c. How animals behave? Y/N
 - d. Why/why not?
 - e. Are you concerned about any changes in the environment here? Y/N
 - i. If yes, what changes?
 - f. Are you concerned about changes in subsistence foods? Y/N
 - i. If yes, what are you concerned about?

D) How does subsistence contribute to well-being?

1. How important is subsistence harvesting to you?
2. What does the term "well-being" mean to you?
3. Would you consider subsistence harvesting to contribute to your well-being? Y/N
 - i. If yes, in what ways is your well-being dependent on subsistence harvesting?

E) How do community members perceive whether and how changes in human activities may impact subsistence practices?

Name of interviewee:

Gender:

Village:

With a changing environment may come increased human activities in the Bering Straits and near St. Lawrence Island including:

- oil and gas development
- renewable energy exploration
- increased shipping and vessel traffic
- commercial fishing
- tourism

1. Have you heard about any development of these activities in the area?
 - a. If yes, what have you heard?
2. Do you think that any of these activities could impact subsistence harvests? Y/N
 - a. If yes, which ones?
 - b. If yes, in what ways?
3. Do you have any concerns about these activities? Y/N
 - a. If yes, what are your concerns?
4. What do you think would benefit your community?
5. Would you be supportive or unsupportive of these human activities?

Demographic questions:

1. When were you born/how old are you?
2. Where were you born?
3. Where do you live now?
 - a. How long have you lived there?
 - b. Are you a year-round resident?
4. How many people live in your household? [Can ask about ages]
 - a. How many are employed?
5. Are you employed? Y/N
 - a. Can you list your job/s?
 - b. Are they:
Full-time _____ part time _____ seasonal _____
6. How much do you think you spend on food each month to feed your household?
7. Has that amount stayed the same, increased or decreased in the past:
10 years? S/I/D
25 years? S/I/D
Over your lifetime? S/I/D

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General Conclusions

The Pacific walrus has been a vital resource for Alaska Native communities in the Bering Strait region, but none so much as the inhabitants of SLI. Other work has addressed the relationship between this resource and resource user (Metcalf & Robards 2008). Like other Alaskan marine mammals, the Pacific walrus has a history of commercial exploitation due to commercial harvesting by Yankee whalers and Soviet sealing fleets (Fay et al. 1989). Both SLI inhabitants and the Pacific walrus have suffered from the effects of these past exploitations. For SLI residents the result was extreme food insecurity and a famine during the winter of 1878-79, in which it is estimated between two-thirds and 90% of the population on SLI died due to starvation (Bockstoce 1995, Mudar & Speaker 2003). After this, villages that were once scattered throughout the island formed one central community that is now known as Gambell. The walrus population however continued to experience several “boom and bust” population cycles (Fay et al. 1989) as management of the population switched hands from state and federal programs (Dickerson 1998) and a variety of management strategies were used.

Today the MMPA prohibits the commercial harvest of marine mammal species with the exemption of harvests by coastal Alaska Natives for subsistence purposes (16 U.S.C. 1361), yet the walrus population and SLI residents face new challenges associated with environmental changes that may have large impacts on both human and animal populations. In this dissertation, I assessed the reproductive capacity and plasticity of female Pacific walruses to provide insights on how this population may reproductively respond to climate change. I also examined the perceptions that SLI residents have about the impacts of climate change to marine subsistence resources, including walruses.

In Chapter 1 I developed a suite of measures to assess reproductive capacity. Female walrus ovaries were examined during three distinct time frames, known to have differing population levels with regard to carrying capacity. Our findings demonstrate that the reproductive capacity of female walruses was limited when the population was thought to be near carrying capacity (samples from 1975), and increased when harvest levels were high and the population was no longer near its carrying capacity

(samples from 1994-99). Interestingly, females from 2008-10 were as reproductively limited as those sampled in 1975. We are limited in our understanding of what drove this reduction in reproductive capacity. The current carrying capacity of the ecosystem for Pacific walrus may have declined and the population may be near this new, lower carrying capacity. Unfortunately, data are not available to assess changes in carrying capacity for walrus over time. Additionally, environmental stressors, including extremely low sea ice extent in 2007, may have influenced the reproductive success of the females sampled in 2008-2010. Unfortunately, poor harvests in 2015-16, and consequently a small sample size, prohibited the assessment of reproductive capacity during this more recent time frame. This research highlights that this novel method for measuring reproductive capacity is useful for assessing historical reproductive capacity, but also highlights the need for continued reproductive monitoring of the population.

The reproductive physiology of this species is poorly understood. Reproductive events for mammals living at the poles are tightly coupled with environmental conditions to allow offspring the best chance of survival (Atkinson 1997, Villegas-Amtmann et al. 2009). In Chapter 2 we investigated whether female walrus exhibited plasticity in the time of year that they give birth to examine whether walrus were reproductively capable of adapting to sea ice loss by giving birth when sea ice platforms might be larger and more stable. We also analyzed if reproductive status can be determined using endocrine profiles. We found that endocrine profiles are useful for determining reproductive status of postpartum versus pregnant and unbred animals; however, unbred animals could not be distinguished from pregnant animals using hormone concentrations alone. Unexpectedly, unbred females exhibited CL actively producing progesterone at high levels, similar to their pregnant counter-parts. This study documents the first physiological evidence of pseudopregnancy in Pacific walrus.

Currently USFWS lacks data on reproductive rates of walrus and therefore they are not included in population models. Results from this research may aid in developing methods to assess reproductive rates of this species. Our findings indicate that however convenient, utilizing the spring walrus harvest is an inappropriate time of year to use an endocrine approach to assess reproductive status

due to the fact that at least some portion of unbred females will exhibit pseudopregnancy, yielding endocrine profiles indicative of pregnancy. Additionally, the harvest on SLI is skewed toward the most reproductively active portion of the female population, making this sampling location also inappropriate for gathering a random sample of the female population. These recommendations have already been communicated to researchers and managers at USGS and USFWS and may assist in future studies aimed at quantifying reproductive rates of this poorly understood pinniped.

Over the course of the last two decades, walrus harvest levels have declined both in the US and in Russia (MacCracken et al. 2017). The literature attributes decreasing harvest success to inclement weather which has increased with climate change (Huntington et al. 2013, Krupnik & Benter 2016). During my fieldwork working with walrus hunters on SLI in 2015-2017, I perceived that loss of sea ice was affecting more than walrus harvest levels. I sought permission from the Native Villages of Gambell and Savoonga and funding sources to conduct a qualitative study aimed at documenting how these communities perceived loss of sea ice was impacting marine harvested subsistence resources. Aside from a few publications on the impacts of climate change on marine mammal hunting (Huntington et al. 2013, Huntington et al. 2016, Krupnik & Benter 2016), no published literature addresses how SLI resource users' subsistence way of life is being impacted by sea ice loss. I sought to fill this research gap.

Results from Chapter 3 highlighted several major findings:

- 1) Walrus continue to be the most valued marine harvested resource in these communities. Loss of sea ice has limited access to harvest walrus and ice seals and because hunters are traveling further distances which is resulting in increased risks to human safety.
- 2) Hunters expressed concerns about calf and pup mortality of walrus and ice seals. These mortalities could have population level effects and continued monitoring of stranded animals is needed on SLI as the distribution of these animals makes them difficult to monitor for mainland Alaska communities.
- 3) Blue king crab, red king crab and Hanasaki crab are key resources for some SLI inhabitants, and residents reported decreasing crab harvests in recent years.

4) Commercial fish species including Pacific cod and walleye pollock have increased in abundance around SLI and residents are fearful of the impacts that potential commercial fishing vessels may have on their local subsistence resources.

5) Most participants were unsupportive of the idea of oil/gas development in the region and for some participants “the region” extended into the Chukchi Sea. Participants noted oil spills could contaminate subsistence foods and disturbance could cause hunted animals to alter their migration paths.

In summary, this dissertation contributes to the understanding of the reproductive capacity and physiology of the female Pacific walrus. We recommend applying an endocrine approach and broadening the sampled tissue types, location and time of year of the WHMP in order to obtain information useful for calculating reproductive rates of the population. Future studies should include the male segment of the population to further understand the breeding cycle of this species. This dissertation also provides insights about the impacts of sea ice loss to marine resources and resource users of SLI for the first time. More data are needed on the abundance/distribution of blue king crab and Hanasaki crab in the nearshore waters of SLI. We recommend that future NSDEC and NOAA trawl surveys include the nearshore waters of SLI to understand if and how these subsistence resources are changing. These results should be shared with SLI residents promptly. Finally, we urge managers to take a proactive approach to mitigate impacts that potential commercial fishing vessels and oil/gas development may have on subsistence activities in the Bering Strait region, as food security, well-being and livelihoods on SLI are strongly reliant upon their local marine resources.

References: General Introduction and General Conclusion

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