Title

Applying conservation genomic methods to understand spatial and temporal variation of four aquatic mammals

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APPLYING CONSERVATION GENOMIC METHODS TO UNDERSTAND SPATIAL AND TEMPORAL VARIATION OF FOUR AQUATIC MAMMALS

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D. Nevé Baker

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Abstract

Applying conservation genomic methods to understand spatial and temporal variation of four aquatic mammals

D. Nevé Baker

The degree of genomic diversity of a species, how that diversity is partitioned over space, and how it has changed over time are critical aspects that inform the continued viability of a species in a changing environment. Once restricted to humans and model species, decreased costs of next generation sequencing and improved analytical methods have enabled genomic studies of threatened and endangered non-model species, contributing to more effective conservation and management. In this dissertation I generate new genomic data and provide insights into four aquatic mammals, each of which have unique natural histories and conservation needs.

In chapter one, I used spatially dense spatial genomic sampling to understand the distribution of diversity and inbreeding in southern sea otters. Consistent with other studies, I found evidence of a genomic bottleneck that pre-dates the fur trade, likely due to indigenous hunting. I showed that southern sea otters are less diverse than their northern sister subspecies across all measures, likely a legacy of their long term isolation at the southern end of the sea otter range, sequential bottlenecks, their reduction to a single small population by the maritime fur trade, and their current geographic restriction. My results indicate that although southern sea otters have little spatial variation in

neutral genomic diversity, rates of inbreeding and genetic load are significantly higher in the northern part of their small range. These results highlight the vulnerability of southern sea otters - as they are currently a single population and cannot expand their range naturally - and underscore the importance of a metapopulation structure in maintaining and improving the genetic diversity of the species. Translocations of southern sea otters to northern California and Oregon are likely necessary to restore a metapopulation structure. Furthermore, given the ecological importance of sea otters, improving the outlook for southern sea otters is critical to maintaining the viability of coastal kelp forest ecosystems at their more southerly range as the climate continues to change.

In chapter two, I assembled a highly contiguous reference genome for the dugong. While a single genome is insufficient to represent the full diversity of this wide-ranging species, it provides initial insights into the demographic history and diversity of a centrally-located population and will serve as an important resource for future studies. I showed that dugongs have relatively high genome-wide heterozygosity compared to other Vulnerable mammals and that they have a dynamic demographic history that likely reflects Pleistocene glacial cycles and resulting sea level change. Future whole genome resequencing studies will provide useful insights into more recent dugong demographic history, as well as how neutral and adaptive variation are partitioned across their large, but discontinuous geographic range, allowing for more targeted management strategies.

In chapter three, I use whole genome sequencing from museum samples of historic Alaskan and Russian polar bears to investigate two main questions: 1. How do polar bears from understudied Russian subpopulations fit in the range-wide diversity of the species? And 2. How has Alaskan polar bear diversity changed over the past 150 years in response to human hunting and climate change? For question 1. I found that despite broad geographic sampling across

four management units, polar bears from across Russia are closely related to each other and to historic Alaskan bears. This result highlights earlier findings, which indicate that the scale of polar bear population structure is highly variable and does not correspond to management unit boundaries. For question 2. I found that Alaskan polar bear genomic diversity has declined significantly over the past 150 years, with the majority of diversity loss occurring in the second half of the 20th century, likely due to heavy sport hunting. There is also evidence of a potential population replacement in Alaska occurring sometime after 1970, potentially also due to abundance declines from sport hunting.

In chapter four, I expand beyond a single species focus to a more holistic paleoecosystem approach by using sedaDNA techniques to investigate the arrival and persistence of beavers in Grand Teton National Park over the last 10 ka and their interactions with the local climate and vegetation. My findings show that beavers arrived surprisingly late to this region following Pleistocene deglaciation, but thereafter persisted at the watershed scale for the last 5 ka, despite periods of environmental change and regional drought. Their arrival coincided with a regional mid-Holocene neoglacial advance, likely due to increased water availability. Beaver arrival was also associated with a shift from a more coniferous vegetation regime to increased riparian vegetation and higher vegetative diversity. Determining the relative contribution of beavers versus climate in structuring the local plant community will require further study. These results suggest that under certain conditions, the positive effects of beaver engineering on local ecosystems may persist over millennia despite drought and other environmental changes, an encouraging finding that suggests that beaver restoration may be an effective long term solution for conserving ecosystems and mitigating the effects of climate change.

These chapters provide novel insights into the genomic diversity of these four species, and improved understanding of their spatial and temporal vari-

ation, particularly the effects of human exploitation and past and present climate change. Additionally, I have generated high-quality genomic resources which will be made publicly available and will contribute to future studies.

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Tetons and the big world of paleoclimatology. Huge thanks to Emily for her beaver knowledge and totally infectious enthusiasm, for connecting me with opportunities and funding, for being responsive and helpful with absolutely everything, and for championing this beaver sedaDNA work. Every time I talk to Emily I walk away more excited about this research than ever and I'm super grateful to get to continue this work with her in Minnesota.

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Introduction

0.1 Conservation genomics

Conservation geneticists seek to understand and conserve the genetic diversity of imperiled species in order to inform conservation practices and preserve biodiversity (Soulé 1985, Frankham 1995, Allendorf et al. 2012). Conservation genetics sits at the intersection of ecology and evolutionary biology, drawing on principles and methods from both disciplines to address questions related to the genetic diversity, population structure, and evolutionary processes of endangered and at risk species (Allendorf et al. 2012, Fenster et al. 2018, Willi et al. 2022). On the ecological side, conservation genetics examines how genetic factors influence the ecological dynamics of populations and communities and how ecological interactions structure diversity (Brussard 1991, Haig 1998, Moran 2002, Waits and Paetkau 2005). It considers how genetic diversity within populations affects their ability to adapt to changing environmental conditions, respond to disturbances, and interact with other species (Allendorf et al. 2012, DeWoody et al. 2021). From an evolutionary perspective, conservation genetics explores the genetic processes that shape the long-term viability and adaptability of populations (Latta 2008, Höglund 2009). This includes factors such as natural selection, genetic drift, gene flow, and the potential for inbreeding. Understanding these processes is crucial for developing effective conservation strategies that maintain the evolutionary potential of species

(Shefferson et al. 2018).

Genomic data are becoming increasingly applied to conservation biology as sequencing and analytical techniques have improved and costs have declined (Primmer 2009, Ellegren 2014, Benestan et al. 2016, Cammen et al. 2016, Fuentes-Pardo and Ruzzante 2017, Supple and Shapiro 2018, Morin et al. 2021). Neutral genetic markers such as mitochondrial haplotypes and nuclear microsatellite loci have been the foundation of "traditional" conservation genetics, and insights into phylogenetics, population structure, demography, and diversity gained from these markers have been used to inform conservation and management of many non-model organisms (Baker et al. 1998, Chemnick et al. 2000, Pimm et al. 2006, Koskela et al. 2013, Gese et al. 2015, Dufresnes et al. 2019, Jensen et al. 2021). With a higher density of marker loci, genome data provides higher resolution and greater statistical power than traditional markers to analyze neutral variation, while also providing opportunities to investigate non-neutral and structural variation, which were previously challenging to study in non-model organisms (Luikart et al. 2003, Väli et al. 2008, Funk et al. 2012, Schoville et al. 2012, Hoffmann et al. 2015). Genomics is also more robust for analyzing degraded DNA, making it useful for studies of ancient and museum samples as well as low quality modern samples such as non-invasively collected scat and hair, which are particularly valuable for species of conservation concern that may be challenging to sample directly (Nichols et al. 2012, Nussberger et al. 2014, Snyder-Mackler et al. 2016, Murray et al. 2017, Andrews et al. 2018, Gaunitz et al. 2018, van der Valk et al. 2021). Importantly, genomic data are highly reproducible and less subject to potential biases introduced by PCR and restriction enzyme digest (Axelsson et al. 2008, Arnold et al. 2013, Nichols et al. 2018, Loos and Nijland 2021). High-quality genomic data are likely to remain forward compatible, serving as a resource for future studies as analytical techniques will no doubt continue to

improve (Primmer 2009, Supple and Shapiro 2018).

Genomic studies are by and large focused on model organisms, so species of conservation concern typically have few genomic resources available (Hogg et al. 2022). Assembling high-quality reference genomes is a critical first step in applying genomics to species of conservation concern, providing initial insights into diversity, adaptive potential, genetic basis of phenotypic traits, and demographic history, all of which are important factors when considering how a species will persist into the future (Brandies et al. 2019, Rhie et al. 2021). Although much can be learned from reference genomes, a single genome from an individual organism cannot represent the full extent of genomic variation found within species or populations (Des Roches et al. 2017, Wright et al. 2020, Schweizer et al. 2021). Population-scale whole genome resequencing (sequencing novel individuals and mapping to the reference genome) or reduced representation sequencing (i.e. SNP genotyping) can be used to investigate intraspecific variation and test hypotheses about how environmental conditions and historical events have structured current patterns of diversity within a species (Des Roches et al. 2017, Fuentes-Pardo and Ruzzante 2017, Wright et al. 2020). These are critical considerations when developing management plans for threatened and endangered species in order to promote gene flow while preserving unique, potentially locally adapted, variation (Garner et al. 2015, Fernandez-Fournier et al. 2021).

0.2 Diversity through space and time

Understanding how diversity varies over space and time within populations and species is a critical concern of conservation biology.

Spatial variation includes both neutral and adaptive variation, both of which are influenced by landscapes and environmental conditions. Neutral

variation arises primarily through genetic drift. In the absence of barriers to gene flow or assortative mating, genetic variation typically follows a pattern of isolation by distance, in which genetic differentiation increases linearly with increased geographic distance (Wright 1943, Slatkin 1993, Hutchison and Templeton 1999). Barriers to gene flow will lead to genetic isolation and population structure, in which genetic variation is collected in semi-discrete units (Bohonak 1999). Isolation by distance and population structure are not mutually exclusive and often coexist to varying degrees (Meirmans 2012, Perez et al. 2018). Complete genetic isolation (eg. due to barriers such oceans between terrestrial species) will eventually lead to speciation, but incomplete isolation will allow some degree of gene flow to continue, preserving population structure within a species (Nei and others 1975, Hartl et al. 1997, Hendry et al. 2009). Classic examples of barriers to gene flow include mountain ranges, waterways, and ecotones; anthropogenic structures such as roads, settlements and border walls can also prevent or limit gene flow between populations (Nei and others 1975, Su et al. 2003, Riley et al. 2006, Miles et al. 2019, Schmidt et al. 2020). Barriers may also be somewhat cryptic (Irwin 2002). Examples of cryptic barriers to gene flow include: currents and thermoclines in marine environments, breaks in prey or habitat distributions, landscapes of fear due to predation or human presence, phenological variation, or cultural inheritance of movement and mating patterns (Baker et al. 1994, Willis and Anderson 2003, Kocher 2004, Hellberg 2009, Quintero et al. 2014, Berger-Tal and Saltz 2019, McGowan et al. 2023). Population structure may also reflect the existence of past barriers; for example many terrestrial vertebrates in the northern hemisphere exhibit population structure that reflects past patterns of Pleistocene glaciation (Vershinina et al. 2021, Zver et al. 2021, Salis et al. 2022). Spatial genetic sampling can show the extent to which past and current features prevent or promote gene flow and is particularly useful for revealing

cryptic barriers which are otherwise difficult to observe (Cammen et al. 2016, Micheletti and Storfer 2017). The effect of human structures on gene flow is particularly important for the spatial management of threatened species (Miles et al. 2019, Schmidt et al. 2020, Frère et al. 2023).

Adaptive spatial variation arises due to differential selection. Within a species, this is known as local adaptation (Forester et al. 2016). Local adaptation arises due to a heterogeneous environment which selects for different alleles in different locations (Kawecki and Ebert 2004). Environmental variation may include abiotic factors such as climate, or biotic factors such as predator, prey and pathogen distribution (Briscoe Runquist et al. 2020). The scale and relative importance of local adaptation can be highly variable, and identifying it is typically more challenging than identifying neutral variation, requiring high quality genome annotations, dense spatial genomic sampling, an understanding of the underlying neutral population structure, and high resolution environmental data (Hoban et al. 2016, Flanagan et al. 2017).

Effective conservation management requires knowledge and consideration of spatial variation in both neutral variation and local adaptation so that gene flow and diversity can be promoted while preserving uniquely adapted populations (Supple and Shapiro 2018). Furthermore, changing environments due to climate change and other processes can cause population isolation and/or genetic-environmental mismatches which may justify intensive management solutions such as translocations or facilitated gene flow (Schwartz and Martin 2013, Butt et al. 2021).

Temporal changes in genetic variation reveal how past evolutionary processes have contributed to current patterns of diversity and can help us predict how diversity may change in the future (Jensen et al. 2022). Temporal variation reflects how species have changed in response to changing environments and habitats, human exploitation, and neutral processes. Whereas understand-

ing spatial variation requires spatial data, temporal variation can be inferred from contemporary genomic data (Beichman et al. 2018). The current genetic variation within a species reflects its history, so models based on evolutionary principles can be used to reconstruct demographic histories and infer past evolutionary processes (Rosenberg and Nordborg 2002, Mather et al. 2020). Demographic modeling can provide valuable insights into species' histories, but often relies on assumptions such as lack of population structure and selection which may not be realistic (Loog 2020). Furthermore, high resolution reconstruction of demographic histories can be computationally intensive and typically requires high coverage genomes and accurate estimates of mutation and/or recombination rates, which can be difficult to obtain for non-model species (Beichman et al. 2018).

Datasets from long-term monitoring are another source for understanding genetic change over time within a species, but high quality long term datasets are relatively rare (Magurran et al. 2010). Evolutionary processes also proceed at a slower rate than demographic changes, so in long-lived species genetic changes may not be detectable in even multi-decadal datasets.

The most straightforward way to study temporal genetic change over evolutionarily-significant time periods is with samples from the time period of interest, which can be analyzed with ancient DNA methods. Ancient DNA is a relatively recent field of study, concerned with isolating genetic material from ancient and historic samples such as bones, hides, and other tissues preserved either *in situ* or in museums and archives (Pääbo et al. 2004). Ancient DNA is typically highly fragmented and low in quantity, making it difficult to isolate and analyze with standard molecular methods (Dabney et al. 2013). It is easily contaminated by higher quality modern DNA (Cooper 2000). Overcoming these challenges has led to the development and improvement of specialized laboratory and computation techniques over the past ∼30 years which have

facilitated countless discoveries that would have been impossible with contemporary samples alone (Willerslev and Cooper 2004, Hofreiter and Shapiro 2012, Orlando and Cooper 2014, Verry et al. 2024). Freeze-thaw, UV light, heat, moisture, and microbial activity all contribute to DNA degradation, so most ancient DNA research has focused on samples from certain environments with high DNA preservation potential such as permafrost and caves (Dabney et al. 2013, Hofreiter et al. 2015). As the field has matured, the maximum age of recoverable DNA has increased; it is now possible to sequence samples >1 million years old under certain preservation conditions, a feat once thought impossible (Orlando and Cooper 2014, van der Valk et al. 2021, Kjær et al. 2022, Dalén et al. 2023). Ancient DNA methods enable the direct study of past diversity providing unique insights into evolutionary processes and providing baselines against which to measure recent change (Leonard 2008, Orlando and Cooper 2014, Jensen et al. 2022). It can reveal past diversity that has been lost through population or species extinction, or from genetic bottlenecks, including those caused by human exploitation (Barnes et al. 2002, Graham et al. 2016, Murray et al. 2017, Sánchez Barreiro et al. 2020, Le Duc et al. 2022, Sremba et al. 2023). It can provide insights in past population dynamics, including local population extinctions, replacements, and admixture events (Shapiro et al. 2004, Kuhn et al. 2010, Vershinina et al. 2021, Salis et al. 2022, Wang et al. 2022). It can also show how species adapted to past climate and environmental change; by studying extinct species we can investigate why species failed to adapt (Graham et al. 2016, Galetti et al. 2017, Murray et al. 2017).

One of the more recently developed areas of study within ancient DNA research is sedimentary ancient DNA (sedaDNA), in which environmental DNA is isolated from ancient sediments (Capo et al. 2021, Crump 2021). SedaDNA is a promising emerging tool, as each small sediment sample can

yield a broad snapshot of biotic diversity from microbes to vertebrates, enabling full ecosystem reconstructions to understand environmental change over deep time (Williams et al. 2023). Thus sedaDNA provides information on both spatial and temporal variation simultaneously, and provides the opportunity to understand how paleoecosystems were structured and how they evolved in response to changing climates and species composition.

Together, spatial and temporal genomic datasets can tell us about how populations are structured and adapted today, what they looked like in the past, what processes link past to present, and how they may look in the future. These insights are critical to managing threatened and endangered species to maximize genetic diversity and gene flow while preserving local adaptation and the connection between adaptations and local environments. Where traditional conservation protections such as habitat protection and hunting restrictions are insufficient to maintain genetically healthy populations, genomic data can inform more intensive management actions such as captive breeding, translocations and facilitated gene flow, and even genetic engineering (Angeloni et al. 2012, Piaggio et al. 2017, Willoughby et al. 2017, Corlett 2017, Butt et al. 2021). As human development and climate change continue, implementing intensive management will likely become more necessary and widespread.

0.3 Species of interest

In this dissertation, I apply genomic techniques to understand spatial and temporal variation of four aquatic mammal species: southern sea otters (*Enhydra lutra nereis*), dugongs (*Dugong dugon*), polar bears (*Ursus maritimus*), and North American beavers (*Castor canadensis*). The natural histories of these species vary widely: they occupy a range of habitats from the tropical dugong to the Arctic polar bear; polar bears and sea otters are carnivores while dugongs

and beavers are herbivores; dugongs adapted to the marine environment over 60 million years ago whereas polar bears likely diverged from terrestrial brown bears only 500,000 years ago (Liu et al. 2014, Yuan et al. 2021). They employ a diversity of physiological and behavioral strategies for existing as mammals in the water, from dugongs' thick blubber and fusiform bodies, to sea otters' incredibly dense warm fur, polar bears' sea ice hunting strategy, and beavers' unique dam-building behavior.

What ties these species together is their aquatic lifestyle, their history of human exploitation, and their roles as both ecological and cultural keystone species within their given environments. Beavers are the classic example of an ecological engineer; their herbivory, dam building, and associated behaviors alter riparian ecosystems from the bottom up: increasing biodiversity, contributing to nutrient cycling, and changing the physical and ecological structure of their local environment (Larsen et al. 2021). Dugongs are also bottom-up ecological engineers; intensive dugong grazing changes the species composition of seagrass meadows, increasing diversity and changing the nutrient composition of seagrass, the main producer in the local ecosystem (Preen 1995, Bowen 1997). Sea otters are one of the most famous examples of a keystone predator; sea otter predation on sea urchins and other invertebrates releases kelp from herbivory, leading a trophic cascade that allows kelp forest ecosystems to flourish and support biodiversity (Estes and Palmisano 1974). In the absence of sea otters, kelp forests decline and biologically depauperate 'urchin barrens' can take over. Polar bears' trophic role in the Arctic ecosystem has not been well studied, however as one of the few top predators in the Arctic, polar bears are likely top-down controllers of trophic dynamics (Derocher et al. 2004).

These species also all hold both historical and contemporary cultural importance for native peoples, both symbolically and as traditional sources of food, fur, and other materials (Makeyev et al. 1993, Leong 1998, Erlandson et

al. 2005, Voorhees et al. 2014, Berland 2015, Lincoln et al. 2021, Rosell and Campbell-Palmer 2022). Commercial and recreational exploitation of these species beginning in the 18th century encouraged arrival and settlement of primarily European settlers into regions previously occupied by native peoples, contributing to both ecological and cultural destruction. Given their important ecological roles, the reduction in abundance and range of these species due to human exploitation has likely caused greater environmental effects than are currently understood. Anthropogenic climate change and habitat destruction continue to challenge the persistence of these species. The extent and impact of human hunting and habitat change in the context of past and ongoing climate change on the genetic variation of these species is the overarching focus of this thesis.

0.4 Chapter outline

In chapter one, I generate a geographically dense high coverage genomic dataset of southern sea otters in order to understand how past exploitation and current geographic barriers structure inbreeding and genetic load, and to inform potential translocations.

In chapter two, I assemble a highly contiguous reference genome for the dugong and take a first look at diversity and demographic history. This is the first step in a more spatially comprehensive study of dugong population structure across their broad geographic range. This chapter was originally going to incorporate resequencing of both modern and museum samples of dugongs to understand diversity across their broad geographic range and investigate hypothesized diversity loss. The COVID-19 pandemic and issues with international permitting unfortunately precluded these analyses in the time span of a PhD. However, this reference genome will serve as a resource for future

re-sequencing projects to explore these questions.

In chapter three, I improve both spatial and temporal sampling of the polar bear - a sentinel species for climate change. In this chapter I used museum samples to generate the first genomic resources for Russian subpopulations of polar bears - of which little is known - and investigate diversity loss in Alaskan polar bears over the past 150 years. By analyzing these historic samples alongside previously generated modern genomes from Russia and Alaska, I contribute to the understanding of range-wide population structure of polar bears, and how hunting and climate change have impacted the species in recent history.

In chapter four, I expand beyond a single species into a more holistic investigation of ecosystem change over space and time, and shift from species' of conservation concern to a species that may be a conservation solution. I applied the newest advance in ancient DNA - sedaDNA - to understand the prevalence and ecological impact of beaver engineering in Grand Teton National Park over the last 10,000 years in relation to climatic change.

Chapter 1

Ongoing inbreeding may be contributing to lack of recovery in southern sea otters (*Enhydra lutris nereis*)

1.1 Abstract

Sea otter populations throughout their range were heavily impacted by the fur trade in the 18th and 19th centuries, causing a rapid range-wide decline in abundance. Despite the similar impacts, recovery has varied among sea otter populations. In particular, southern sea otters - currently restricted to central and southern California - initially recovered quickly, but growth in abundance and range expansion has recently slowed due to a variety of factors including disease, shark predation and a linear habitat configuration that limits range expansion. Here, we use 54 high coverage genomes of southern sea otters from throughout their current range to demonstrate ongoing inbreeding over the past 100 years among southern sea otters particularly in the northern part of their

range, along with low diversity and high genetic load. Rates of inbreeding are lower among representatives from three northern sea otter populations, diversity is higher across all measures, and genetic load is lower. Our results indicate that effective population size was already small prior to the effects of the fur trade and that the spatial dynamics that limit southern sea otter demographic growth are also contributing to ongoing reduced genetic health compared to their northern counterparts. Without pedigrees we cannot assess the impacts of inbreeding on fitness, however inbreeding depression may be one of the factors contributing to recovery stagnation among southern sea otters, particularly at the northern end of their range. Proposed reintroductions to northern California and Oregon may help increase the genetic diversity of southern sea otters and boost recovery.

1.2 Introduction

Sea otters are generalist predators of the nearshore environment that eat primarily hard-shelled invertebrates (Estes 2015). Their high metabolism (a cold-water adaptation) requires them to eat approximately 25% of their bodyweight per day (Yeates et al. 2007, Zellmer et al. 2021). This heavy predation on benthic herbivores exerts profound top-down effects that structure the community and increase biodiversity and productivity, an ecological process known as keystone predation (Estes and Palmisano 1974).This process has been famously documented for rocky reefs where otter predation on urchins has allowed kelp forests to flourish, but more recently, similar trophic cascades have also been documented in estuarine seagrass habitats, where otters prey primarily on crabs (Hughes et al. 2013).

Sea otters were historically continuously distributed in the nearshore environment around the North Pacific rim, divided into three subspecies with dis-

tinct geographic distributions and slight morphological differences (Wilson et al. 1991). These subspecies are the Asian sea otter (*Enhydra lutris lutris*) distributed from Hokkaido, Japan to the Kamchatka Peninsula and Kuril Islands in Russia; the northern sea otter (*Enhydra lutris kenyoni*) from the Commander and Aleutian Islands, throughout southern Alaska and British Columbia, and historically down into Oregon (contemporary populations in Washington were translocated from Alaska),; and the southern sea otter (*Enhydra lutris nereis*), historically distributed from southern Oregon down to Baja California, Mexico, but presently restricted to approximately 400km of the central California coast.

Sea otters were heavily hunted for their dense, warm fur in the mid-18th to early 20th centuries (Loshbaugh 2021). The maritime fur rush was initiated by Russian explorers in the eastern north Pacific in the early 1740s; with fur traders moving eastward into the Aleutian Islands and then down the west coast of North America as they serially reduced abundance in each region (Dolin 2010 pp. 140–143). British and American traders joined the fur trade in the later 18th century and as northern populations became depleted, fur traders began hunting southern sea otters along what is now the California coasts in the late 1770s (Ogden 1975). The fur rush in the southern sea otter range was relatively brief; within 50 years otters were already depleted in their southern range and Russian fur hunters abandoned their southern fort in 1841 (Thompson 1896). Hunting by American traders continued at low rates until the ratification of the International Fur Seal Treaty, which protected the remaining remnant sea otter populations (Loshbaugh 2021). By then, sea otters had been reduced from an estimated 150,000-300,000 individuals to a handful of isolated relict populations with a global abundance of likely only 1,000-2,000 at their lowest point (Kenyon 1969, Bodkin 2015). 20th century conservation efforts, including legal protections under the Marine Mammal Act

and Endangered Species Act and multiple translocation programs, have helped sea otters rebound and they now occupy approximately two-thirds of their global historic range (discontinuously) and have returned to pre-exploitation densities in some areas (Davis et al. 2019).

Previous genetic and genomic studies show that all extant sea otter populations have low genetic diversity, indicative of past genetic bottlenecks (Larson et al. 2002b, Aguilar et al. 2008, Gagne et al. 2018, Beichman et al. 2019, 2022). Periods of low effective population size will lead to increased inbreeding and consequently higher homozygosity and lower diversity. Low levels of genetic variation tend to increase genetic load, reducing fitness and putting species at higher risk of extinction (Frankham et al. 2017). Low genetic diversity reduces adaptive potential of a species, meaning they have fewer "tools" in the genetic toolbox to respond to emerging challenges such as new pathogens and climate change (Avise 2012, Larson 2012). While low diversity among sea otters is usually primarily attributed to the impact of the maritime fur trade, previous studies suggest that sea otters have had a dynamic demographic history, and have likely undergone at least one earlier bottleneck (Aguilar et al. 2008, Beichman et al. 2019). Low genome-wide heterozygosity – which is typically driven by more ancient demography – supports this hypothesis of one or more ancient bottlenecks (Beichman et al. 2019, 2022).

Indigenous hunting has been proposed as an explanation for these earlier bottlenecks (Larson et al. 2002b, Braje and Rick 2011, Beichman et al. 2019, 2022). Sea otter remains are abundant in many coastal archaeological assemblages (Szpak et al. 2020) and multiple lines of evidence suggest that hunting by indigenous coastal peoples kept otters below carrying capacity prior to European contact and the maritime fur trade (Simenstad et al. 1978, Porcasi et al. 2000, Erlandson et al. 2005, Braje and Rick 2011, Szpak et al. 2012, Slade et al. 2022). Opinions on the primary purpose of indigenous otter hunt-

ing differ; some studies argue that otter pelts were the main target (Wellman 2022), others suggest that otters were primarily culled to reduce competition for shellfish (Slade et al. 2022). Rates of hunting also suggest that in some areas, marine mammals were exploited for consumption and serially depleted by aboriginal hunters: first pinnipeds, then cetaceans, then smaller and less desirable otters (Porcasi et al. 2000). These different uses were not mutually exclusive and the primary purpose of otter hunting most likely varied over space and time.

Southern sea otters are the most deeply diverged sea otter population; diverging from their northern relatives ∼28 ka, likely due to isolation by ice cover during the last glacial maximum (Beichman et al. 2022). As ice retreated, limited gene flow with populations immediately north may have resumed; ancient DNA evidence suggests that Oregon was likely historically a non-contiguous transition zone between the northern and southern subspecies, with occasional admixture (Valentine et al. 2008, Larson 2012, Wellman et al. 2020). Oregon has no otter populations today, but there is much recent interest in translocating southern sea otters to northern California and Oregon to increase population size and diversity, restore the ecological services provided by otters, provide redundancy in case of mass mortality events, and facilitate connectivity between northern and southern subspecies (U.S. Fish and Wildlife Service 2022, Tinker et al. 2023).

Multiple sea otter translocation programs were undertaken among northern sea otters during the 1960s and 1970s, almost all of which resulted in viable populations that persist today (Bodkin 2015, Davis et al. 2019). Otters descending from translocated individuals today account for over a third of the global population, and translocated populations of northern sea otters retain similar levels of heterozygosity as their source populations, despite founder effects (Larson et al. 2002a, 2021, Bodkin 2015). Only one translocation

program has been initiated among southern sea otters, from the mainland coast of California to San Nicolas Island in the 1980s (Rathbun et al. 2000). The majority of translocated individuals swam back to the mainland and the translocation was deemed a failure (Rathbun et al. 2000). However, a small number of otters remain at San Nicolas Island and their population is growing, indicating that translocations of southern sea otters have the potential for success (Yee et al. 2023). No sea otter translocations have been undertaken recently, although translocations of southern sea otters to Northern California and Oregon are being seriously considered by management agencies and conservation groups (U.S. Fish and Wildlife Service 2022, Tinker et al. 2023).

Sea otters were thought to be extinct south of Alaska until a remnant population of approximately 50 southern sea otters was discovered in Big Sur, California in 1938 (Bolin 1938). Whereas Asian and northern subspecies were reduced to multiple remnant populations, all extant southern sea otters are descendants of this single small relict population. Southern sea otters have since increased their population size and reclaimed part of this history range, but abundance remains at a fraction of pre-exploitation levels; population size estimates have hovered around 3,000 individuals for the past 10 years (U.S. Fish and Wildlife Service 2021), compared to an estimated pre-exploitation abundance of approximately 16,000 individuals within California (Hatfield et al. 2018). Southern sea otters are managed as a single stock (U.S. Fish and Wildlife Service 2021).

Range reclamation has also been slow; despite a century of protection southern sea otters still only occupy a portion of the central California coast, representing approximately 13% of their historic range (U.S. Fish and Wildlife Service 2021). The rate of range expansion among southern sea otter is inherently limited by the linear geographic structure of the California coast and the narrow shelf - otters can essentially only expand two dimensionally, either

north or south (Tinker et al. 2008, Tarjan and Tinker 2016, Tim Tinker et al. 2021). In contrast, northern sea otter habitat primarily encompasses island archipelagos, broad shallow shelves, and convoluted fjordland coastline; this more three-dimensional habitat structure facilitates multidirectional range expansion which is consequently much more rapid (Tinker et al. 2019). The lack of recent range expansion in southern sea otters is primarily due to mortality from white shark bites at their range peripheries (Tinker et al. 2016, Nicholson et al. 2018, Moxley et al. 2019). The extent of shark bite mortality at the range edge is such that these areas are essentially population sinks (Nicholson et al. 2018); southern sea otters have not expanded their range in 20 years and are unlikely to do so without intensive management. At their range core, otters are at or close to carrying capacity, so population growth has stalled recently. Further population growth and eventual de-listing is unlikely to occur without range expansion. Strandings within the range core are primarily due to density-dependent factors such as energetic stress, indicative of high levels of competition for prey (Nicholson et al. 2018).

Despite high genetic load due to genetic bottlenecks, population projections indicate that southern sea otters are unlikely to go extinct due to genetic factors alone (Beichman et al. 2022). However, there are many other threats to southern sea otters including oil spills, toxic algal blooms, climate change, which may reduce their habitat and/or prey, and disease, particularly from domestic animals (e.g. parvovirus and toxoplasmosis) (Kreuder et al. 2003, Davis et al. 2019, Miller et al. 2020). High population density, isolation, and low genetic diversity increase vulnerability to all of these threats.

Inbreeding depression, a decrease in reproductive fitness resulting from inbreeding, could also negatively impact recovery ability. Inbreeding depression has been shown to have a strong environmental component (Bijlsma and Loeschcke 2012, Reed et al. 2012); a meta-analysis showed that the magnitude

of environmental stress explained up to 66% of the variation in inbreeding (Fox and Reed 2011). Physiological stress has been shown to be a major source of mortality, particularly among reproductive females (Chinn et al. 2016, Nicholson et al. 2018). Understanding the extent and spatial structure of inbreeding among southern sea otters - particularly as it relates to habitat quality and population density - is therefore important for determining whether inbreeding depression is a risk factor for southern sea otters.

Although sea otters tend to have small home ranges and limited dispersal (Bodkin 2015, Tarjan and Tinker 2016), previous studies have not identified any spatial structuring of genetic diversity within southern sea otters (Aguilar et al. 2008, Gagne et al. 2018, Beichman et al. 2022). This is likely due to the recent founder effect of the fur trade bottleneck and low overall diversity which may obscure population structure. However, population density and habitat suitability varies considerably throughout the southern sea otter range and strongly correlates with stranding rates, cause of death, and dispersal potential (Nicholson et al 2018). This geographic variation may also influence the spatial distribution of inbreeding and genetic load, but has not been directly investigated. Understanding the geographic structure of inbreeding is important both to management within the existing southern sea otter range, and to choosing source individuals for potential translocations.

For this chapter we investigated the fine-scale geographic structure of genomic diversity and inbreeding within southern sea otters by analyzing a geographically dense genomic dataset of southern sea otters from throughout their range, as well as a small number of northern sea otters for comparison. These high coverage genomes were generated as part of a statewide multispecies landscape genomics initiative known as the California Conservation Genomics Consortium (CCGP), the ultimate goal of which is to perform multi-species landscape genomics analyses to inform conservation planning. we tested the

hypothesis that rates of heterozygosity, inbreeding, and genetic load differ across the southern sea otter range. We also compared diversity with a small sample of northern sea otters to compare how past exploitation affected otters in different regions with different population and habitat structure, and how their diversity has recovered since. We investigated the recent demographic history of southern sea otters, testing the hypothesis that southern sea otters underwent one or more ancient bottlenecks that reduced their diversity and left them more vulnerable to the demographic impact of fur trade exploitation.

1.3 Methods

1.3.1 Data generation and sequence processing

Genomic DNA from southern sea otters (*E. l. nereis*) was extracted from frozen archival samples collected between 2004-2006 from sea otter carcasses recovered through a large- scale stranding network conducted by the California of Fish and Wildlife, the US Geological Survey, the Monterey Bay Aquarium, and The Marine Mammal Center (Kreuder et al. 2003) and archived at the National Marine Fisheries Services office in Santa Cruz CA. Northern sea otter (*E. l. kenyoni*) samples were collected between 1991 and 2004 by USGS. Sample information is available in Table A.1.

We extracted genomic DNA from all samples with a Qiagen Blood and Tissue Kit and prepared libraries for sequencing with the NEbNext Ultra II kit. Sequencing was performed on an Illumina Novaseq platform 150 PE S4 lane.

Sequencing reads were processed according to the CCGP pipeline (`https://github.com/cademirch/ccgp_workflow`). We trimmed sequencing adapters using fastp (Chen et al. 2018), mapped to the southern sea otter reference

genome (GenBank assembly ASM641071v1) (Beichman et al. 2019) using BWAmem (Li 2013), and removed duplicates with sambamba (Tarasov et al. 2015). We called and filtered variants using GATK (McKenna et al. 2010) implemented in Sentieon (Freed et al. 2017). Biallelic SNPs were selected and the following filters applied: QUAL < 30.0, FS > 60.0, MQ < 40.0, MQRankSum < -12.5, ReadPosRankSum < -8.0, SOR > 3.0, QD < 2.0, as recommended according to GATK best practices (McKenna et al. 2010). SNPS were further filtered to remove any sites with missing data, singletons, or sites with a global depth less than 3X per sample (180) or greater than 2X the total coverage of all samples (2295). Analyses were restricted to the 93 largest autosomal scaffolds; putative sex scaffolds were identified by comparison to the Eurasian otter (*Lutra lutra*) sex chromosomes (assembly mLutLut1.2) with nucmer (Marçais et al. 2018). We used the vk phylo function in vcf-kit (Cook and Andersen 2017) to convert the filtered vcf file to a fasta alignment.

1.3.2 Population structure

Principal component analysis (PCA) was performed in PLINK 2.0 (Chang et al. 2015) with the –pca var-wts function. PCA was performed for all samples together, as well as for southern sea otters only and for each sex and age class within sea otters independently.

We constructed a maximum likelihood tree in MEGA11 (Tamura et al. 2021) from the fasta alignment with the Tamura-Nei model (Tamura and Nei 1993) and 100 bootstrap replicates.

We assessed ancestry proportions across individuals with ADMIXTURE (Alexander et al. 2009) for values of K from 1 to 8, with 100 bootstrap replicates each. We used the cross-validation error to determine the best value of K. Following ADMIXTURE recommendations, VCF files were first pruned for

linkage disequilibrium using the –indep-pairwise function of PLINK 1.9 (Purcell et al. 2007) and the following parameters: window size of 50 Kb, step size of 10 variants, and a pairwise r2 threshold of 0.1.

We investigated isolated by distance (IBD) within southern sea otters by performing a Mantel test of correlation (Mantel 1967) between geographic and genetic distance matrices in R with 9,999 permutations. We generated a genetic distance matrix from the fasta alignment with snp-dists (`https://github.com/tseemann/snp-dists`). The geographic distance matrix was calculated from the sample coordinates using the dist function in R.

1.3.3 Diversity

We used VCFtools (Danecek et al. 2011) to estimate sliding window nucleotide diversity (π) over 10 Kb for each population separately. Windows with fewer than 10 SNPs were excluded from the final output.

We estimated relative genome-wide heterozygosity for each individual in PLINK 1.9 with the –het function. We also calculated an adjusted genome-wide heterozygosity rate for each sample to account for significant portions of the genome in ROH with the formula: ROH-adjusted heterozygosity = Heterozygosity/(1 fraction of genome in ROH).

1.3.4 Inbreeding and genetic load

Runs of homozygosity (ROH) in all scaffolds larger than 1 Mb were identified with a window based approach implemented in PLINK 1.9 with the function –homozyg (Meyermans et al. 2020). We allowed for up to three heterozygous sites and 10 missing sites per window, following Foote et al. (2021) and otherwise used default settings. ROHs were limited to a minimum size of 1 Mb with at least 50 SNPs. We correlated the lengths of ROHs with the expected

number of generations since the individual's maternal and paternal lineages shared a common ancestor using an estimated average g = 100/(2rL), where g is the time in generations, r is the recombination rate, and L is the length of the ROH tract in Mb (Thompson 2013, Kardos et al. 2017). We used estimated average recombination rate for domestic cat of 1.100 cM per Mb and for the domestic dog of 1.554 cM per Mb (Dumont and Payseur 2008) to obtain a range of generation times, as no recombination rate is available for the sea otter or any closely-related species. We used an estimated generation time of 8 years (Ralls et al. 1983).

In order to estimate the frequency of deleterious mutations, SnpEff 5.0112 (Cingolani et al. 2012) was used to annotate the functional effects of variants. Genes were located using the southern sea otter reference genome annotation. Variants identified as "loss of function" by SnpEff were considered deleterious mutations. This impact category includes mutations heavily affecting the function of the protein, for example mutations which eliminate start or stop codons, or frameshifting insertions and deletions. The number of variants with different functional consequences was tallied per individual; both for all variants and those that were homozygous for each functional consequence.

We used t-tests implemented in R to test for statistical differences in average heterozygosity, nucleotide diversity, ROH average length, count, and proportion of genome, and genetic load between sea otter subspecies and between northern and southern California regions within the southern sea otter subspecies (Big Sur was used as the geographic breakpoint between north and south). Where there were significant differences between northern and southern California, We also compared Monterey Bay with the remainder of California, as Monterey Bay has the densest population of anywhere in the state, and had a larger sample size than any other region. These groups do not have equal sample sizes, so in order to statistically compare mean values, for each com-

parison we performed 100 bootstrap replicates of randomly downsampling the larger group to equal the smaller group, and compared the bootstrap replicate mean of the larger group with the mean of the smaller group.

1.3.5 Demographic history

We estimated trends in effective population size (N_e) of southern sea otters over the past 100 generations with the linkage disequilibrium (LD) based method of HapNe-1.2 (Fournier et al. 2022). A recombination rate of 1.1 cM/Mb was used to estimate the genetic map. We used the 8-year generation time (Ralls et al. 1983) and scaled generation 1 to the year 2000 to estimate the approximate date of N_e changes.

We used an LD-based method (Waples and Do 2008) implemented in NeEstimator v2 (Do et al. 2014) to estimate the contemporary effective population size of southern sea otters. For NeEstimator we first phased haplotypes for each individual using Beagle v5.4 (Browning and Browning 2016), running first on the 30X samples, then using these high coverage individuals as a reference panel to impute the lower coverage samples before merging both subsets back into a single VCF. To reduce computational load, we randomly downsampled the dataset to 10,000 SNPs before running NeEstimator and ran ten replicates using the singleton minor allele frequency filter.

All statistical analyses and visualizations were performed using R statistical software v4.2.1 (R Core Team 2022).

1.4 Results

We generated whole genome data for 54 southern sea otters, 42 at ∼10X coverage and 12 at ∼30X coverage (Fig. 1.1A; Table 1.1). Two samples each were sequenced for three northern populations: the Commander Islands, the

Aleutian Islands, and Prince William Sound (Fig. 1.1A). For each northern population one individual was sequenced to ∼10X coverage and one to ∼30X (Table A.1). 558,692 SNPs were retained after filtering.

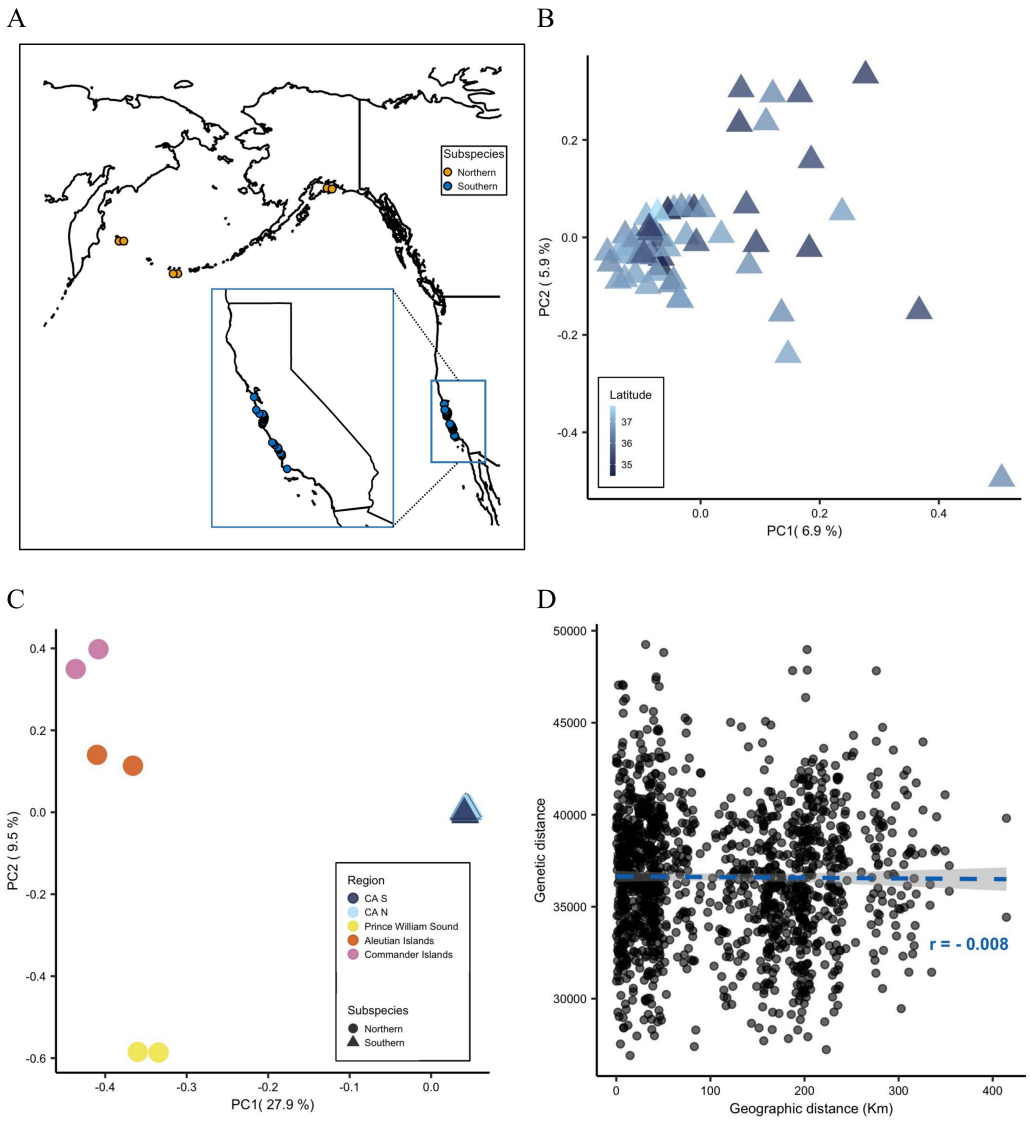

Figure 1.1: Sea otter neutral population structure. A) Map of all samples used in this analysis, colored by subspecies. B) Principal components 1 and 2 for Southern sea otters only, colored by region (north and south). C) Principal components 1 and 2 for both subspecies; colors represent regions, shapes represent subspecies. D) Genetic vs geographic distance for southern sea otters, no correlation indicates a lack of isolation by distance.

1.4.1 Population structure

Population structure analyses of southern sea otter whole genomes from throughout their range indicate panmixia within the subspecies, consistent with the extreme bottleneck and recent founder effect. Principal component analysis showed little variation and no geographic structuring within the subspecies (Fig. 1.1B). When plotted with northern sea otters, 27.9% of the variation was explained by the first principal component, which separated the two subspecies. Principal component two separated the three northern sea otter populations, with southern sea otters falling closest to the Aleutian Islands individuals (Fig. 1.1C). Individual principal component analyses performed for separate sexes and age classes of southern sea otters also did show any geographic structuring, nor did principal components 3 or 4 (Figs. A.1 and A.2). A maximum likelihood tree separated the subspecies and northern sea otter populations (Fig. A.3).

Genomic variation in southern sea otters is not explained by isolation by distance Fig. 1.1D); a Mantel test showed no correlation between genetic and geographic distance (r = -0.008, p = 0.537). ADMIXTURE analyses also showed no geographic structuring and a best K value of K=1 (Fig. A.4).

1.4.2 Diversity

Average 10 Kb sliding window nucleotide diversity among southern sea otters was significantly lower than northern sea otters (Fig. 1.2A).

All individuals had low genome-wide heterozygosity, ranging from 1.4×10^{-4} to 2.07×10^{-4}. Mean heterozygosity was slightly lower among southern sea otters than northern (Fig. 1.2B), and within southern sea otters was lower in northern California than southern (Fig. A.5A). However none of these differ-

Table 1.1: T-test comparison results for diversity metrics. Group 1 mean represents mean of 100 bootstrap replicates of group 1 downsampled to group 2 sample size. *Significant (<0.05) p-values.

Group 1	**Group 2**	**Metric**	**Group 1 mean**	**Group 2 mean**	**P value**
E. l. nereis	*E. l. kenyoni*	Genome-wide heterozygosity	$1.49e^{-4}$	$1.67e^{-4}$	$3.04e^{-1}$
N CA	S CA		$1.47e^{-4}$	$1.53e^{-4}$	$1.92e^{-1}$
E. l. nereis	*E. l. kenyoni*	ROH proportion	20.04%	13.83%	$6.9e^{-3}$*
N CA	S CA		21.36%	17.89%	$3.81e^{-2}$*
CA (excluding Monterey)	Monterey Bay		19.71%	21.03%	$3.86e^{-1}$
E. l. nereis	*E. l. kenyoni*	ROH size	1.60Mb	1.46Mb	$1.19e^{-2}$*
N CA	S CA		1.62Mb	1.54Mb	$1.47e^{-2}$*
CA (excluding Monterey)	Monterey Bay		1.57Mb	1.62Mb	$8.40e^{-2}$
E. l. nereis	*E. l. kenyoni*	LOF/synonymous ratio	$6.00e^{-2}$	$4.90e^{-2}$	$7.92e^{-4}$*
N CA	S CA		$5.90e^{-2}$	$6.10e^{-2}$	$4.50e^{-1}$
E. l. nereis	*E. l. kenyoni*	LOF/synonymous ratio (homozygous)	$2.40e^{-2}$	$1.80e^{-2}$	$7.48e^{-2}$
N CA	S CA		$2.20e^{-2}$	$2.80e^{-2}$	$9.52e^{-2}$

ences were statistically significant (Table 1.1), indicating similarly low levels of historic diversity (genome-wide rates of heterozygosity tends to reflect more ancient demography).

1.4.3 Inbreeding and genetic load

All individuals showed evidence of ROHs in their genomes, indicating past inbreeding; with over a third of the genome in ROHs in some individuals (Fig. 1.2C). Southern sea otters had a significantly larger proportion of their genomes in ROH; within California, northern Californian otters had a significantly larger proportion of their genomes in ROH (Fig. A.5C; Table 1.1). This pattern within California did not seem to be dominated by Monterey Bay; the average proportion of genome in ROH was not significantly different between Monterey Bay and the rest of California (Table 1.1). Average size of ROHs followed the same trends between groups (Fig. A.5C; Table 1.1). 42.5% of

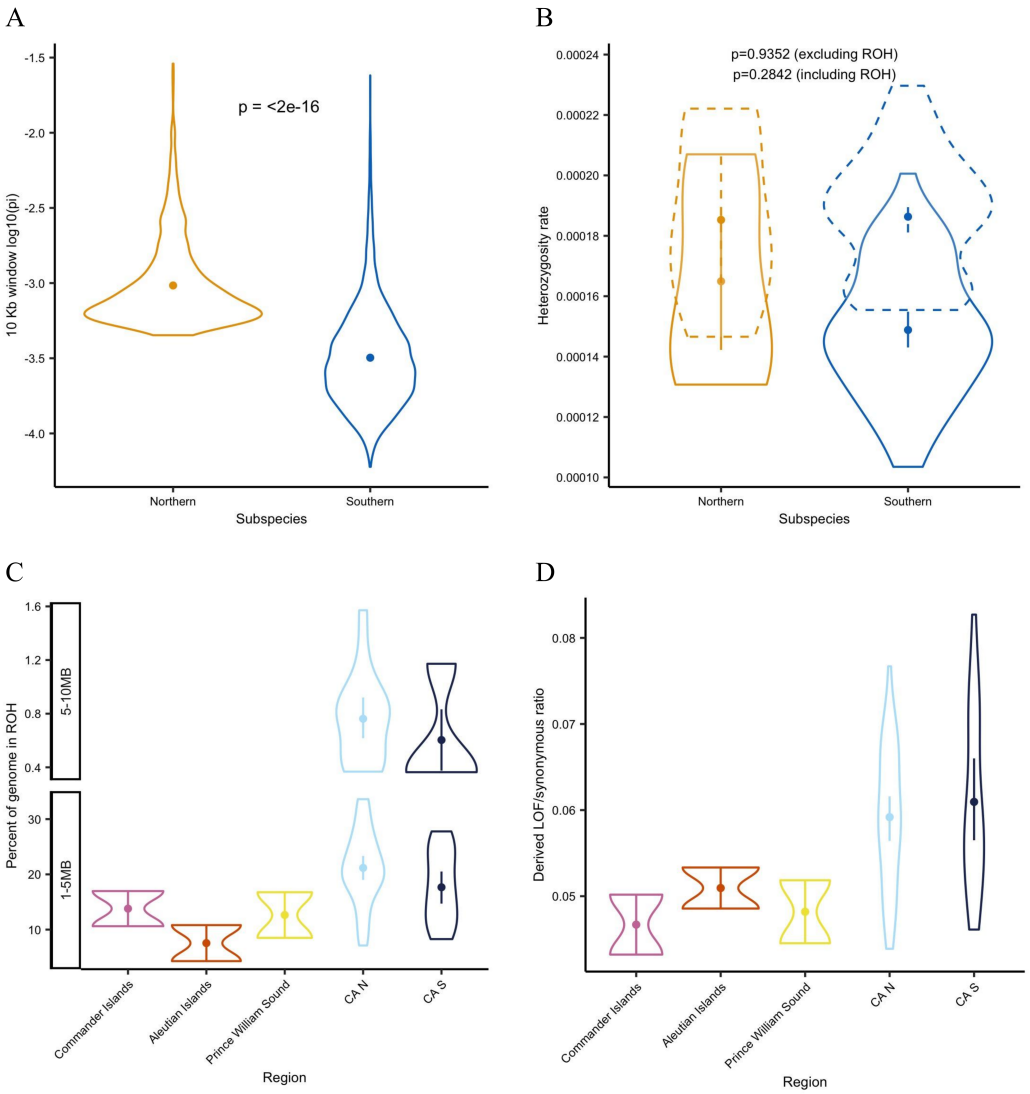

Figure 1.2: Diversity comparisons between northern and southern sea otters. A) log10 transformation of average nucleotide diversity over 10 Kb windows. B) Average genome-wide heterozygosity across all regions (solid lines), and excluding ROH regions (dashed lines). C) Percent of genome in 1-5 Mb (top) and 5-10 Mb (bottom) ROHs. D) Genetic load inferred by ration of loss of function (LOF) to synonymous mutations.

southern sea otters had at least one large ROH (5-10 Mb) indicating inbreeding within the past 10 generations; no northern sea otters had any ROHs larger than 5 Mb.

Southern sea otters had a significantly higher ratio of loss of function (LOF) to synonymous mutations than northern sea otters (Table 1.1; Fig. 1.2D), indicating a higher genetic load of deleterious mutations. Southern California

had a larger but non-significant LOF/synonymous ratio than northern California (Table 1.1; Fig. 1.2D). Differences in homozygous LOF/synonymous ratios followed similar patterns but were non-significant (Table 1.1; Fig. A.5D).

1.4.4 Recent demographic history

LD-based inference of southern sea otter effective population size (N_e) over the last 100 generations indicated that from approximately 1200 c.e., N_e was low ($N_e \approx 500$) but fairly stable for ~250 years, before entering a period of exponential decline beginning approximately 550 years ago (Fig. 1.3). This decline persisted through the period of fur trade exploitation in California. The fur trade did not appear to change the rate of decline (Fig. A.6B), but by this time N_e was already very low ($N \approx 130$). N_e reached its lowest point ($N_e = 108$) in the 1840s, before beginning to increase.

We estimated contemporary $N_e = 355.5$ (95% CI: 298.1 - 390.6) (Fig. 1.3). This is within the confidence interval for generation 1 estimated by HapNe.

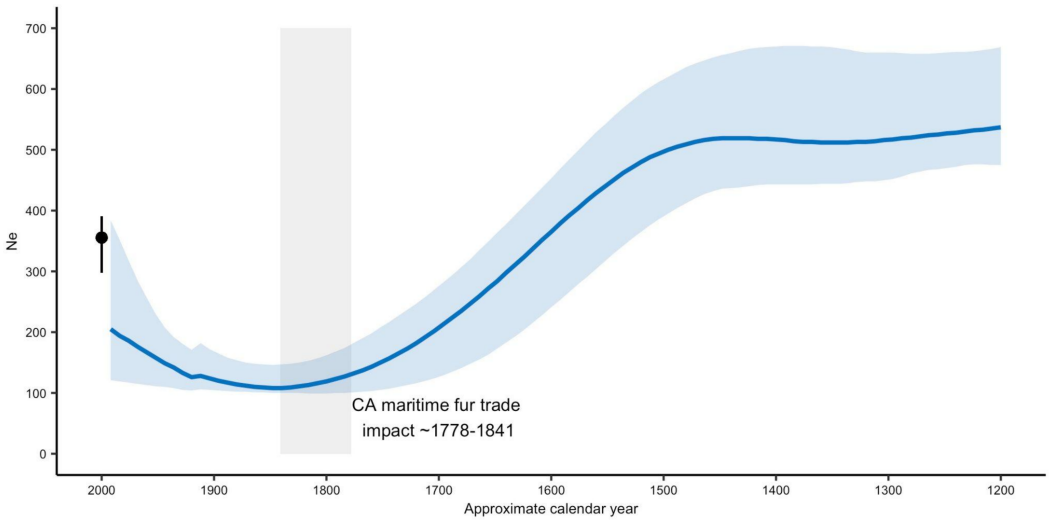

Figure 1.3: Southern sea otter effective population size (N_e) over the past 100 generations (800 years, assuming an 8 year generation time) based on linkage disequilibrium (LD). Lighter band represents 95% confidence interval. Gray rectangle represents approximate time period of fur trade exploitation in California. Black dot at year 2000 (generation 1) indicates contemporary N_e (also inferred with LD) and 95% confidence interval.

1.5 Discussion

Although otters have small home ranges, we found no evidence of population structuring within southern sea otters, despite dense geographic sampling and whole genome data. This is consistent with previous studies using microsatellite genotypes and reduced representation genomic data (Aguilar et al. 2008, Beichman et al. 2021).

We found significantly lower levels of nucleotide diversity and significantly higher levels of inbreeding and genetic load in southern sea otters compared to northern. Samples of northern sea otters were taken from three different geographically distant populations, whereas southern sea otters are all members of one population. This metapopulation structure within northern sea otters has been shown to facilitate dispersal, allowing for faster population growth and outbreeding, which is reflected in our results. Despite differences in inbreeding and genetic load, northern and southern sea otters show similarly low levels of heterozygosity, indicating that these subspecies likely had similarly low historic effective population sizes.

Although there was no spatial structure within southern sea otter neutral diversity, we observed significantly higher levels of inbreeding in northern California as compared to southern. Interestingly, this pattern did not seem to be driven by Monterey Bay, where sea otters are at or near carrying capacity, suggesting that this pattern is not driven purely by population density. All of the sampled southern sea otters have large portions of their genomes in ROH, and large fragments of ROH, indicating significant recent inbreeding within the last 10 generations. Low dispersal ability is likely contributing to high rates of inbreeding. Without pedigrees or life history data from our sampled individuals, it is difficult to determine whether inbreeding depression is occurring within southern sea otters. However, inbreeding depression has a strong

environmental component and there is evidence of high rates of mortality from physiological stress among southern sea otters, particularly in reproductive females (Chinn et al. 2016, Nicholson et al. 2018). It is important to note that the individuals sampled here were all stranded individuals and therefore may not be a representative sample of healthy individuals. If inbreeding depression is contributing to mortality, our results may be skewed towards more inbred individuals. Regardless, it is clear that the components for inbreeding depression are all present among southern sea otters with no available mechanism available to increase outbreeding naturally. Facilitating outbreeding in combination with translocations to new habitat, thereby reducing population density and resource competition, could improve the genetic health and reduce the vulnerability of southern sea otters. Future studies integrating pedigree and sighting history data with genomic sampling will be able to directly test whether inbreeding is affecting reproductive fitness.

As in other studies, we found a decline in southern sea otter effective population size that pre-dates the maritime fur trade. The ∼550 ya start date of this decline is very similar to that detected by Aguilar et al. (2008) with microsatellite data but later that the bottleneck beginning ∼6 ka detected by Beichman et al. (2019). The differing methods used (PSMC in Beichman et al. (2019) vs HapNe here) are more effective for older demographic fluctuations vs younger respectively, providing perspectives on different periods of sea otter history. In our analysis, even N_e is low even prior to the observed decline, slightly more than 500 individuals for ∼250 years. Given this low estimate, it is likely that this is a separate, later bottleneck than that identified by Beichman et al. (2019), perhaps as a series of sequential declines. The multiple bottlenecks identified here and by Beichman et al. (2019) may represent serial depletion by indigenous hunting. Multiple studies have suggested that hunting by indigenous coastal peoples kept otters at low abundance prior to European

contact (Simenstad et al. 1978, Porcasi et al. 2000, Szpak et al. 2012, Slade et al. 2022, Wellman 2022). Human populations along the California coast and the Channel Islands increased during the late Holocene (Kennett and Kennett 2000, Erlandson et al. 2005). This was also a time of increased maritime technological development, intensified trade, and a general shift in subsistence strategies (Kennett and Kennett 2000, Erlandson et al. 2005, Monks 2017). These developments may have increased hunting pressure on otters, leading to the observed bottlenecks. In particular, the start of the bottleneck observed here corresponds roughly to the start of the Little Ice Age, which caused a period of major global cooling (Hodell et al. 2005, Miller et al. 2012) and may have changed the needs and resource use of coastal indigenous peoples. The warm pelts of sea otters may have become a more valuable trade item at this time, leading to more intense hunting pressure and contributed to the decline observed here. This is speculative, however, pelts as a material good among pre-contact indigenous North Americans have rarely been directly studied as hides and fur do not preserve well in the archaeological record (Hallett et al. 2021, Skandfer 2022). Recent re-analyses of cut marks on sea otter bones from late Holocene archaeological sites in Oregon and Southeast Alaska indicate that otters in these locations were skinned for their pelts (Moss 2020, Wellman 2022); but this cut mark analysis has not been widely deployed for sea otter archaeological studies.

The population decline that we observed persisted through the maritime fur trade period (beginning in the late 1770s in California), although by this time effective population size was already very low. The decline in effective population size bottoms out at a little more than 100 individuals, but begins to grow again in the 1840s. This corresponds to historical records: the maritime fur rush in California was intense but effectively quite brief; by the 1830s otters were too depleted to be hunted economically and Russian fur traders

abandoned their fort on the Sonoma coast in 1841 (Thompson 1896, Loshbaugh 2021).

The spatial structure of the California coast and the dangers that otters face at the edges of their range is such that natural range expansion is currently effectively impossible (Tinker et al. 2008, Nicholson et al. 2018). This spatial restriction also constrains population growth, which limits genetic diversification and contributes to inbreeding. High population density due to spatial constraints increases physiological stress and stress-induced mortality and also puts southern sea otters at risk of disease epidemics (Nicholson et al. 2018, Miller et al. 2020). These density-dependent factors may be worsened by low genetic diversity and inbreeding depression. Density-independent factors also pose a high threat to a spatially constrained population; a major oil spill, marine heat wave, or other disaster could have a devastating effect. In essence, a single, closed, panmictic population is highly vulnerable due to a variety of interacting, mutually reinforcing factors.

Translocations are likely necessary to improve the outlook for southern sea otters, as natural range expansion is currently effectively impossible and much of the current range is at or near carrying capacity (Davis et al. 2019, U.S. Fish and Wildlife Service 2022). Introducing a metapopulation structure to southern sea otters through translocations to northern California and Oregon would provide redundancy in case of a mass mortality event and facilitate range expansion and population growth. It also may reduce inbreeding and the environmental stress that may be contributing to inbreeding depression. Because levels of genetic load and and inbreeding are geographically variable within California, careful consideration of the geographic origin of potential source individuals as well as genetic screening could help maximize the success of translocations.

Our results highlight the difficult position that southern sea otters are in,

as compared to their northern relatives. While all otters suffered intense demographic loss from the fur trade, northern sea otters were reduced to multiple remnant populations, whereas southern sea otters were reduced to just one (Estes 2015). This metapopulation structure of northern sea otters appears to have facilitated their genetic recovery, in combination with translocations and the more three-dimensional geography of their habitat (Rathbun et al. 2000, Tinker et al. 2019, Larson et al. 2021, U.S. Fish and Wildlife Service 2023a, 2023b). These differences are reflected in our genomic results, which show lower genetic "health" in southern sea otters across multiple measures. Our results are further evidence that management interventions such as translocations are likely necessary to preserve and increase genetic diversity and outbreeding in southern sea otters, which will increase their resilience to climate change, disease, and other threats. The spatial structure of inbreeding within California also indicates that close monitoring and potential intervention may be helpful for decreasing levels of inbreeding in northern California, and improving the genetic health of southern sea otters as a whole. Furthermore, our results have implications for the management of other species with low diversity, as they show that even in the absence of neutral population structure, inbreeding levels may follow fine-scale patterns of geographic variation. A better understanding of these patterns can improve the conservation and management of threatened and endangered species.

Chapter 2

A chromosome-level genome assembly for the dugong (*Dugong dugon*)

2.1 Abstract

The dugong (*Dugong dugon*) is a marine mammal widely distributed throughout the Indo-Pacific and the Red Sea, with a Vulnerable conservation status, and little is known about many of the more peripheral populations, some of which are thought to be close to extinction. We present a de novo high-quality genome assembly for the dugong, from an individual belonging to the well-monitored Moreton Bay population in Queensland, Australia. Our assembly uses long-read PacBio HiFi sequencing and Omni-C data following the Vertebrate Genome Project pipeline to reach chromosome-level contiguity (24 chromosome-level scaffolds; 3.16 Gbp) and high completeness (97.9% complete BUSCOs). We observed relatively high genome-wide heterozygosity, which likely reflects historical population abundance before the last interglacial period, approximately 125,000 years ago. Demographic inference suggests that

dugong populations began declining as sea levels fell after the last interglacial period, likely a result of population fragmentation and habitat loss due to the exposure of seagrass meadows. We find no evidence for ongoing recent inbreeding in this individual, however runs of homozygosity indicate some past inbreeding. Our draft genome assembly will enable rangewide assessments of genetic diversity and adaptation, facilitate effective management of dugong populations, and allow comparative genomics analyses including with other Sirenians, the oldest marine mammal lineage.

2.2 Introduction

Dugongs (*Dugong dugon*) are marine mammals with a broad but fragmented distribution throughout the Indian and western Pacific Oceans (Husar 1978). Dugongs belong to the order Sirenia along with manatees, and are the only extant representative of the family Dugongidae. They are also the closest relative of the Steller's sea cow, a giant Sirenian that was hunted to extinction in the 18th century. Dugongs prefer shallow coastal waters and are mainly herbivorous, relying on seagrass meadows for both food and habitat (Best 1981). Dugongs are a culturally important species to Torres Strait Islander and many coastal Aboriginal communities for cultural ceremonies, hunting, and in custodianship of Sea Country (Leong 1998, Lincoln et al. 2021). Little is published in the literature about dugong behavior - their shy and elusive nature makes them challenging to study in the wild and, unlike many other small marine mammals, they are difficult to maintain in captivity (Bertram and Bertram 1973, Goto et al. 2004). While some areas, such as northern and eastern Australia, have robust ecological monitoring programs for dugongs and co-management programs with Indigenous communities (Tibbetts et al. 2019, Lincoln et al. 2021, Cleguer et al. 2023), other dugong populations throughout

south Asia and eastern Africa are data deficient (Marsh et al. 2002). The IUCN lists dugongs as Vulnerable, however some populations are thought to be close to extinction due primarily to habitat destruction and fisheries bycatch (Marsh et al. 1995, 2002). Evidence from aerial surveys, habitat mapping, and interviews with local communities suggests that the global range of dugongs has contracted (Marsh et al. 2002), leaving potentially endangered and isolated relict populations – particularly in the western Indian Ocean – and generating concern about loss of genetic diversity (Plön et al. 2019). However, substantial uncertainty remains concerning the global status of dugongs.

Many questions remain relating to dugong demographics, movement, and population structure that can be addressed using whole-genome data. Previous genetic studies have relied primarily on analyzing the distribution of mitochondrial control region haplotypes (Blair et al. 2014, Plön et al. 2019, Srinivas et al. 2020, Garrigue et al. 2022). These studies have shown that dugong mitochondrial haplotypes show significant geographic structure throughout their range and generally high mitochondrial haplotype diversity range-wide (Seddon et al. 2014, Blair et al. 2014, Plön et al. 2019), with lower diversity at the range periphery (Plön et al. 2019, Garrigue et al. 2022), Microsatellite and SNP genotypes also recovered significant geographic structure as well as isolation by distance, reflecting generally low dispersal among dugongs (Seddon et al. 2014, Cope et al. 2015, McGowan et al. 2023). The environmental forces contributing to this structure are not fully understood; however sea level fluctuations associated with Pleistocene glacial cycles may have allowed range expansion and contraction by repeatedly creating and destroying the shallow near-shore seagrass habitat upon which dugongs rely (Woodruff 2010). For example, much of the marine near-shore environment around northern Australia and southeast Asia – the approximate geographic center of present-day dugong range – was not submerged until the end of the last glacial maximum 17,000

years ago (Ludt and Rocha 2015). Cryptic marine barriers (eg. tidal and current patterns) and breaks in seagrass habitat may also play a role (McGowan et al. 2023).

Here, we present a highly contiguous, chromosome level de novo high-quality genome assembly for the dugong, along with initial estimates of genomic diversity and demographic history. Our assembly provides a resource for future genomic studies of dugong population structure, conservation status, and evolutionary history, and will contribute to the larger Vertebrate Genome Project (Rhie et al. 2021). Along with existing draft-quality genome assemblies for manatees and the extinct Steller's sea cow, this assembly will also allow future comparative studies of Sirenians and other marine mammals.

2.3 Methods

2.3.1 Biological Materials

The sample was collected from a wild adult female dugong captured as part of an ongoing research program in Moreton Bay, Queensland, Australia (-27.15148032, 153.0415985) on May 17, 2022. A total volume of 16 mL of whole blood in EDTA was collected nonlethally and immediately flash frozen in liquid nitrogen and stored at -80 until genomic DNA extraction. Samples were collected under Scientific Purposes Permit # WA0019236, Moreton Bay Marine Park permit # MPP18-001119, and UQ Animal Ethics permit # 2021/AE000821.

Table 2.1: Genome assembly pipeline and software used.

	Software and options	**Version**
Assembly		
Filtering PacBio HiFi adapters	cutadapt -j=32 -b ATCTCTCT-CAACAACAACAACGGAGGAG-GAGGAAAAGAGAGAGAT -b ATCTCTCTCTTTTCCTCCTC-CTCCGTTGTTGTTGTTGA-GAGAGAT –output=out1.fq.gz –error-rate=0.1 –times=1 –overlap=3 –action=trim –revcomp –discard-trimmed	4.0+galaxy0
K-mer counting	Meryl (k = 21)	1.3+galaxy4
Estimation of genome size and heterozygosity	GenomeScope	2.0+galaxy1
De novo assembly (contiging)	hifiasm in HiC mode: hifiasm -t 32 -o output -f 37 -l 3 -s 0.75 -O 1 –l-msjoin 500000 –primary	0.16.1+galaxy3
Scaffolding		
Omni-C scaffolding	yahs –no-mem-check	1.2a.2+galaxy0
Omni-C contact map generation		
Short-read alignment	BWA-MEM2	2.2.1+galaxy0
SAM/BAM processing and filtering	Arima mapping pipeline (implemented as bellerophon)	1.0+galaxy0
Contact map visualization	PretextMap	1.0+galaxy0
	PretextSnapshot	0.0.3
Organelle assembly		
Mitogenome assembly	mitohifi.py -f AY075116.1.fasta -g AY075116.1.gb -p 70 -t 32 -o 2	2
Genome quality assessment		
Basic assembly metrics	gfastats	1.3.0+galaxy0
Assembly completeness	"BUSCO (-m geno, -l vertebrata)"	5.3.2+galaxy0
	Merqury	1.3+galaxy2
Contamination screening		
Local alignment tool	Blast+	2.14.0
General contamination screening	BlobToolKit	4.1.7
Comparison to *E. maximus*		
Sequence alignment	nucmer (mummer)	3.9.4alpha
Diversity and demographic history		
Runs of homozygosity detection	ROHan	
Effective population size fluctuations	PSMC -N25 -t15 -r5 -p 4+25*2+4+6	0.6.5-r67

2.3.2 Nucleic acid extraction

We isolated high molecular weight (HMW) genomic DNA (> 40 Kbp) using a Circulomics Nanobind CBB kit (Pacific Biosciences - PacBio, Cat. #102-207-600). Prior to library preparation, the genomic DNA was pre-treated for damage using the NEBNext FFPE DNA Repair Mix (New England Biolabs, MA), according to the manufacturer's instructions.

2.3.3 PacBio HiFi library preparation and sequencing

Two HiFi SMRTbell libraries were constructed using the SMRTbell Express Template Prep Kit v2.0 (PacBio, Cat. #100-938-900) according to the manufacturer's instructions. HMW gDNA was sheared to a target DNA size distribution between 15 and 20 Kbp. The sheared gDNA was concentrated using 0.45× of AMPure PB beads (PacBio, Cat. #100-265-900) for the removal of single-strand overhangs at 37for 15 min, followed by further enzymatic steps of DNA damage repair at 37for 30 min, end repair and A-tailing at 20for 10 min and 65for 30 min, ligation of overhang adapter v3 at 20for 60 min and 65for 10 min to inactivate the ligase, then nuclease treated at 37for 1 h. The SMRTbell library was purified and concentrated with 0.45× Ampure PB beads (PacBio, Cat. #100-265-900) for size selection using the BluePippin/PippinHT system (Sage Science, MA; Cat. #BLF7510/HPE7510) to collect fragments greater than 7 to 9 Kbp. The 15 Kbp average HiFi SMRTbell libraries were sequenced at the Australian Genome Research Facility in the University of Queensland using 3 8M SMRT cells, Sequel II sequencing chemistry 2.0, and 30-h movies each on a PacBio Sequel II sequencer.

2.3.4 Omni-C library preparation and sequencing

The Omni-C library was prepared from 3 mL of frozen blood using Dovetail Omni-C Kit (Dovetail Genomics, CA) according to the manufacturer's Mammalian protocol v1.4 with minor modifications. In brief, cells were isolated from thawed blood and chromatin fixed in place in the nucleus. Fixed chromatin was digested with DNase I then extracted and digestion profiles were assessed using TapeStation D5000 screen tapes (Agilent Technologies, CA). Chromatin ends were repaired and ligated to a biotinylated bridge adapter followed by proximity ligation of adapter containing ends. After proximity ligation, crosslinks were reversed and the DNA purified from proteins. Purified DNA was treated to remove biotin that was not internal to ligated fragments. An NGS library was generated using an NEB Ultra II DNA Library Prep kit (New England Biolabs, MA) with an Illumina compatible y-adaptor. Biotin-containing fragments were then captured using streptavidin beads. The post capture product was split into 2 replicates prior to PCR enrichment to preserve library complexity with each replicate receiving unique dual indices. The libraries were then sequenced at the Ramaciotti Center for Genomics at the University of New South Wales (Sydney, Australia) on an Illumina NextSeq 500 platform to generate approximately 100 million 2 × 150 bp read pairs per Gbp genome size.

2.3.5 Nuclear genome assembly

We assembled the dugong genome following the Vertebrate Genomes Project (VGP) v2.0 Galaxy assembly pipeline (Table 2.1, see Data availability statement for link to all assembly scripts) (Rhie et al. 2021, Larivière et al. 2023). In particular, we removed remnant adapter sequences from the PacBio HiFi dataset using cutadapt (Martin 2011) and used them to generate the initial

phased diploid contigs using HiFiasm in Hi-C mode, with Omni-C used to phase the haplotypes (Cheng et al. 2021). We scaffolded both contig haplotypes using the Omni-C data with YaHS (Zhou et al. 2023). We generated Omni-C contact maps for both assemblies by aligning the Omni-C data against the corresponding assembly with BWA-MEM (Li 2013). We identified ligation junctions, and merged alignments using the Arima mapping pipeline (`https://github.com/ArimaGenomics/mapping_pipeline`) implemented as bellerophon in Galaxy (Kerkvliet et al. 2019). We then performed manual curation on haplotype 1 to correct structural errors, improve contiguity, and name chromosomes following Howe et al. (2021). To do so, we used the PretextSuite (`https://github.com/wtsi-hpag/PretextView`; `https://github.com/wtsi-hpag/PretextMap`; `https://github.com/wtsi-hpag/PretextSnapshot`) to visualize the contact maps and checked for major misassemblies and cut the assemblies at the closest joins where the misassemblies were found. We then checked for contamination using the BlobToolKit Framework (Challis et al. 2020). Finally, we trimmed remnants of sequence adaptors identified during NCBI contamination screening.

To obtain draft chromosome assignments, we aligned our genome (mDugDug1.hap1) to the annotated genome assembly for the Indian elephant (*Elephas maximus indicus*) (Vertebrate Genome Project, GenBank Accession: GCA_024166365.1) using nucmer (Marçais et al. 2018), as this was the closest dugong relative with a chromosome-level assembly available.

2.3.6 Mitochondrial genome assembly

We assembled the mitochondrial genome of the dugong from the PacBio HiFi reads using the reference-guided pipeline MitoHiFi (`https://github.com/`

`marcelauliano/MitoHiFi`) (Uliano-Silva et al. 2023). A previously assembled dugong mitogenome (GenBank Accession: AY075116.1) was used as the starting reference sequence. After completion of the nuclear genome, we searched for matches of the resulting mitochondrial assembly sequence in the nuclear genome assembly using BLAST+ (Camacho et al. 2009) and filtered out contigs and scaffolds from the nuclear genome with a percentage of sequence identity >99% and size smaller than the mitochondrial assembly sequence.

2.3.7 Genome size estimation and quality assessment

We generated k-mer counts from the PacBio HiFi reads using meryl (`https://github.com/marbl/meryl`). We then applied GenomeScope 2.0 (Ranallo-Benavidez et al. 2020) to the k-mer database to estimate genome features including genome size, heterozygosity, and repeat content. To evaluate genome quality and completeness we used BUSCO (Manni et al. 2021) with both the vertebrate ortholog database (vertebrata_odb10) which contains 3,354 genes and the mammalian ortholog database (mammalia_odb10) which contains 9,226 genes. Assessment of base level accuracy (QV) and k-mer completeness was performed using the previously generated meryl database and merqury (Rhie et al. 2021). To obtain general contiguity metrics, we ran gfastats (Gurevich et al. 2013). We further estimated genome assembly accuracy via BUSCO gene set frameshift analysis using the pipeline described in Korlach et al. (2017) with the mammalian database. Measurements of the size of the phased blocks are based on the size of the contigs generated by HiFiasm in HiC mode (initial diploid assembly).

Following the quality metrics nomenclature established by Rhie et al. (2020), we used the derived genome quality notation x·y·P·Q·C, where x = log10[contig NG50]; y = log10[scaffold NG50]; P = log10[phased block NG50]; Q = Phred

base accuracy QV (quality value); C = % genome represented by the first "n" scaffolds, following a karyotype of 2n = 48 inferred from ancestral taxa *Trichechus manatus manatus* (Noronha et al. 2022). Quality metrics for the notation were calculated on the primary assembly.

2.3.8 Diversity and demographic history

We used ROHan (Renaud et al. 2019) on the filtered and aligned Omni-C data to refine estimates of genome-wide heterozygosity and identify runs of homozygosity (ROH), indicative of inbreeding. We applied the pairwise sequentially Markovian coalescent (PSMC) (Li and Durbin 2011) approach to infer historical effective population size of dugongs over time. We generated a diploid consensus sequence using the mpileup function of SAMtools (v0.1.18; with "-C50" option), bcftools to call variants, and available scripts from PSMC package to convert file formats. We required that sequencing depth for each locus was above one-third of average coverage ("-d" option) and less than twice of average coverage ("-D" option), and that consensus base quality was above Q20. We ran PSMC using the recommended parameters (Tabl 2.1) and 100 rounds of bootstrapping. We scaled our estimates using the previously-reported dugong generation time of 27 years (McDonald 2005) and a mutation rate of 6.25e-9 mutations per nucleotide per generation, calculated using the divergence rate between dugongs and Steller's sea cows (Le Duc et al. 2022).

Table 2.2: Sequencing and assembly statistics, and accession numbers.*Assembly quality code x.y.P.Q.C derived notation, from (Rhie et al. 2021). x = log10[contig NG50]; y = log10[scaffold NG50]; P = log10 [phased block NG50]; Q = Phred base accuracy QV (Quality value); C = % genome represented by the first "n" scaffolds, following a karyotype of 2n = 48 inferred from ancestral taxa *Trichechus manatus manatus* (Noronha et al. 2022). **Read coverage and NGx statistics have been calculated based on the estimated genome size of 3.16 Gb

BioProjects and vouchers		
VGP NCBI BioProject	PRJNA489243	
Species NCBI BioProject	PRJNA970804	
NCBI BioSample	SAMN33212336	
NCBI Genome accessions	Primary	Alternate
Assembly accession	GCA_030035585.1	GCA_030020955.1
Genome sequences	JASCZL000000000	JASCZM000000000
Genome sequence		
PacBio HiFi reads	3 PACBIO_SMRT (Sequel II) runs: 6.5 million reads, 102 Gbases	
Omni-C Illumina reads	2 ILLUMINA (Illumina NovaSeq 6000) runs: 457.5 million reads, 138.2Gb	
Assembly identifier (quality code)*	mDugDug1 1(8.8.P8.Q70.C99)	
HiFi read coverage**	32.0X	
Genome Assembly Quality metrics		
	Haplotype 1	**Haplotype 2**
Number of contigs	294	256
Contig N50 (bp)	57,632,671	57,883,746
Contig NG50 (bp)	57,632,671	57,883,746
Longest contigs	162,184,114	209,448,431
Number of scaffolds	198	167
Scaffold N50 (bp)	177,379,183	138,031,769
Scaffold NG50 (bp)	177,379,183	138,031,769
Largest scaffold	267,865,978	230,272,189
Size of final assembly (bp)	3,159,179,246	3,154,861,630
Phased block NG50 (bp)	57,632,671	57,883,746
Gaps per Gbp (# Gaps)	25 (79)	28 (88)
Indel QV (frameshift)	41.52	42.16
Base pair QV	70.4553 Full assembly = 70.3899	70.3254
K-mer completeness	97.9001 Full assembly = 99.7025	97.8847
Organelles	1 complete mitochondrial sequence (pending NCBI accession code)	

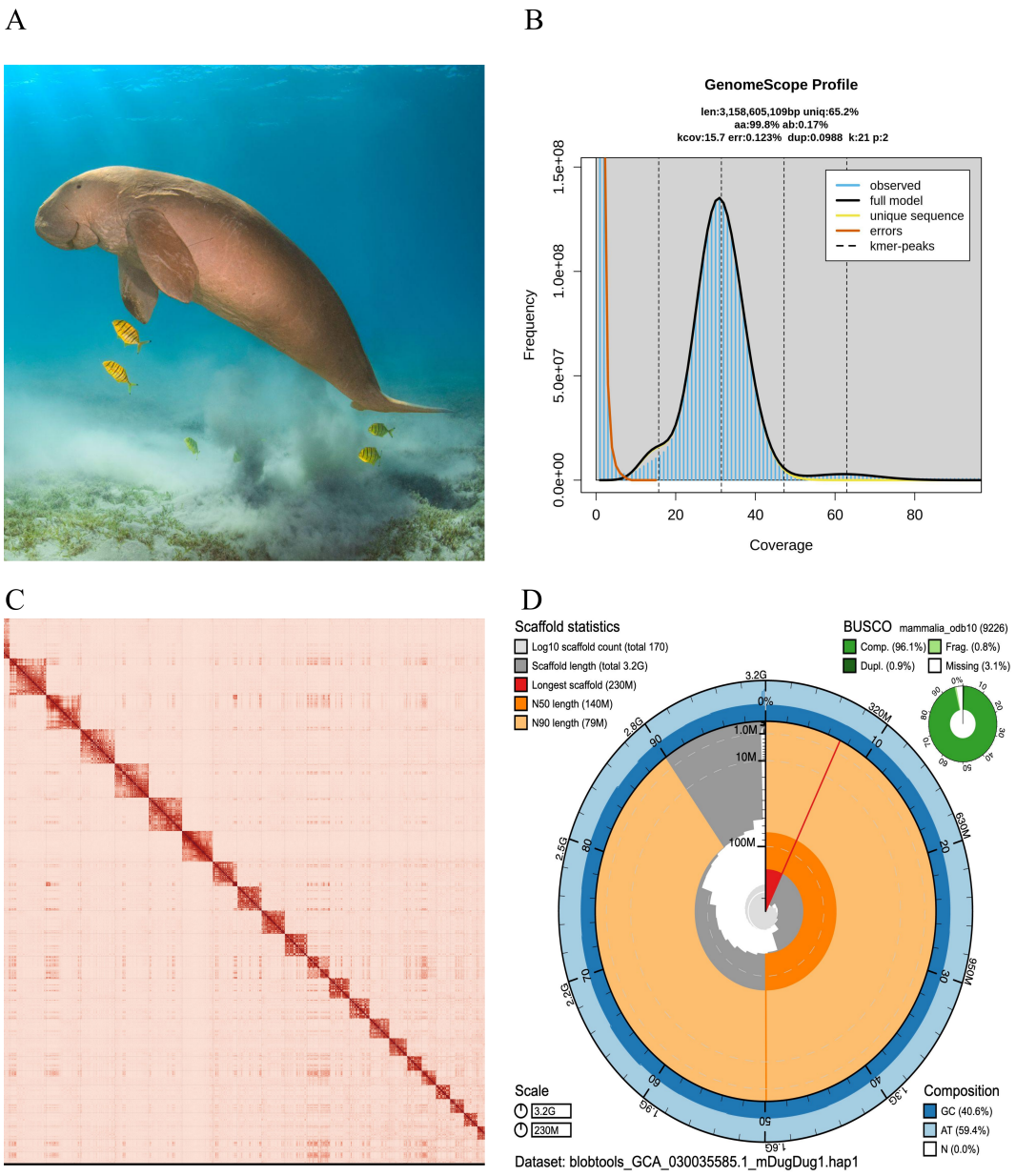

Figure 2.1: Dugong high-quality reference assembly. (A) An adult dugong. Margarita Granovskaya via stock.adobe.com (B) K-mer spectrum output generated from adapter filtered PacBio HiFi data using GenomeScope 2.0. The bimodal pattern observed corresponds to a diploid genome. K-mers covered at lower coverage and lower frequency correspond to differences between haplotypes, and the higher coverage and higher frequency k-mers correspond to the similarities between haplotypes. (C) Omni-C Contact maps for the curated genome assembly of haplotype 1 generated with PretextSnapshot. Omni-C contact maps translate proximity of genomic regions in 3D space to contiguous linear organization. Each cell in the contact map corresponds to sequencing data supporting the linkage (or join) between 2 of such regions. Scaffolds are separated by black lines. (D) BlobToolKit Snail plot showing a graphical representation of the quality metrics presented in Table 2.2 for the *Dugong dugong* assembly for haplotype 1 (mDugdug1.hap1). Full description available in Fig. A.7

2.4 Results

2.4.1 Nuclear genome assembly

The PacBio HiFi and Omni-C sequencing libraries generated 6.5 million read pairs and 457.5 million reads, respectively. The PacBio HiFi reads yielded a mean read length of 15,629 bp and 32-fold coverage based on the GenomeScope 2.0 genome size estimation of 3.16 Gbp. From the same software and HiFi reads we estimated 0.123% sequencing error rate and 0.211% nucleotide heterozygosity rate. The k-mer spectrum based on PacBio HiFi reads shows a slightly bimodal distribution with 2 peaks at 18- and 32-fold coverage (Fig. 2.1B), where peaks correspond to heterozygous and homozygous states of a diploid species.

The final assembly (mDugDug1) consists of two haplotypes (haplotype 1 and haplotype 2), both with genome assembly sizes similar to the estimated value from GenomeScope 2.0 (Fig. 2.1B). Haplotype 1 (mDugDug1.hap1) consists of 198 scaffolds spanning 3.159 Gbp with contig N50 of 57.6 Mbp, scaffold N50 of 140.7 Mbp, longest contig of 162.2 Mbp and largest scaffold of 267.9 Mbp. Haplotype 2 (mDugDug1.hap2) consists of 167 scaffolds, spanning 3.155 Gbp with contig N50 of 57.9 Mbp, scaffold N50 of 138.0 Mbp, largest contig 209.4 Mbp and largest scaffold of 230.2 Mbp. Detailed assembly statistics are reported in Tables 2.2 and 2.3; graphical representation for haplotype 1 in Fig. 2.1D (Fig. A.7B for haplotype 2). Haplotype 1 has a BUSCO completeness score of 97.9% using the Vertebrata gene set, a per-base quality (QV) of 70.5, a k-mer completeness of 97.9, and a frameshift indel QV of 41.52; while haplotype 2 has a BUSCO completeness score of 97.8% using the same gene set, a per-base quality (QV) of 70.3, a k-mer completeness of 97.9, and a frameshift indel QV of 42.16 (Table 2.3).

During manual curation of haplotype 1, we broke six joins made by YaHS,

closed a total of 23 gaps, and removed one mitochondrial haplotig identified as contamination. The Omni-C contact maps show that both assemblies are highly contiguous; with 24 chromosome-level scaffolds, 23 autosomes and an X chromosome (Fig. 2.1C and Fig. A.7A). We have deposited both assemblies on NCBI (see Table 2.2 and Data Availability for details).

Table 2.3: Benchmarking Universal Single-Copy Orthologs (BUSCO) assembly values for Haplotype 1 (H1) and Haplotype 2 (H2): Complete BUSCOs (C), Complete and single-copy BUSCOs (S), Complete and duplicated BUSCOs (D), Fragmented BUSCOs (F), Missing BUSCOs (M).

	C	**S**	**D**	**F**	**M**
Vertebrata n = 3354					
H1	97.9%	95.9%	2.0%	1.0%	1.1%
H2	97.8%	95.7%	2.1%	1.1%	1.1%
Mammalia n = 9226					
H1	96.2%	95.3%	0.9%	0.8%	3.0%
H2	96.1%	95.2%	0.9%	0.8%	3.1%

2.4.2 Mitchondrial genome assembly

Final mitochondrial genome size assembled with MitoHiFi was 16,858 bp. The base composition of the final mitochondria assembly is A = 30.29%, C = 28.60%, G = 14.73%, T = 26.37%, and consists of 22 unique transfer RNAs and 13 protein-coding genes.

2.4.3 Diversity and demographic history

We estimated average genome-wide heterozygosity to be 0.165% (0.129 - 0.211%), relatively high for a species of conservation concern (Fig 2.2A). Approximately 11% of the genome is in ROH, however the majority of these are relatively small (<20 Mbp), indicating that most inbreeding did not occur recently (Fig 2.2B).

PSMC estimates of effective population size over time indicate that dugong abundance was high (∼600,000 individuals) prior to the last interglacial pe-

riod ∼100 ka (thousand years ago) but underwent several fluctuations before declining steeply ∼100 ka (Fig. 2.2C).

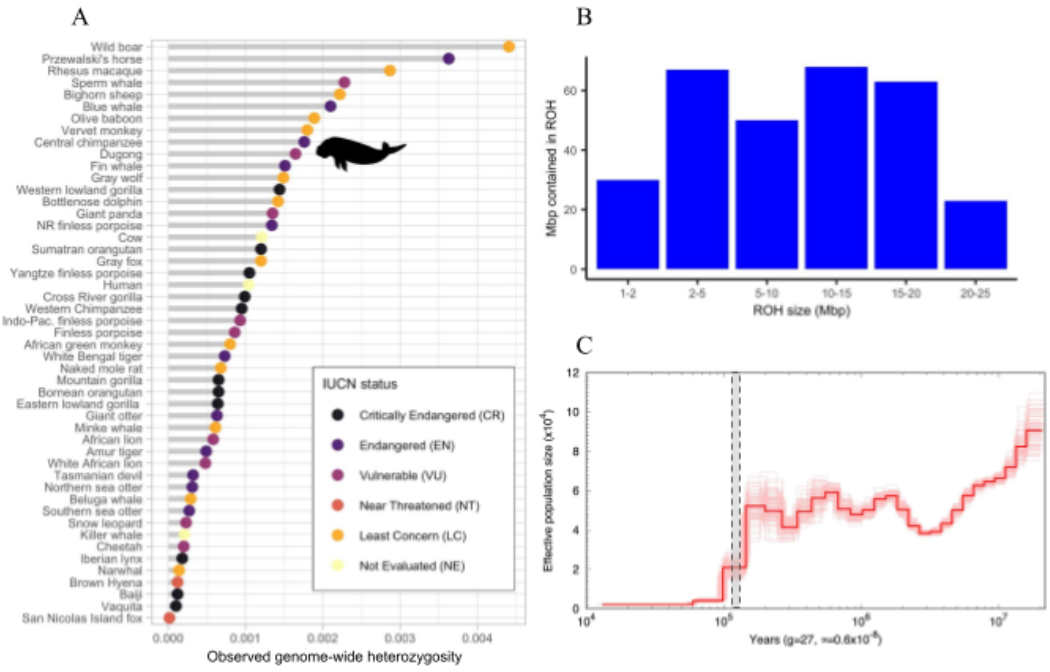

Figure 2.2: Dugong diversity and demographic history. (A) Comparison of genome-wide heterozygosity in dugongs and other mammals drawn from the literature, based on Robinson et al. (2016). Dots are colored by the endangered status according to the International Union for Conservation of Nature (IUCN) Red List for Threatened Species. (B) Count of runs of homozygosity (ROH) ≥1 Mbp across the dugong autosomal chromosomes of this study, binned by size. (C) Effective population size over time, inferred with PSMC and scaled to the dugong generation time and mutation rate. Lighter lines represent bootstrap replicates. Vertical dashed line represents the end of the last interglacial period at approximately 115 ka.

2.5 Discussion

We present a draft genome assembly for the culturally important dugong, assembled using long reads and chromosome-scale sequencing data. Genome assemblies are available on NCBI for two other Sirenians, the Florida subspecies of the West Indian manatee (Trichechus manatus) (GenBank Assem-

blies: GCA_000243295.1 and GCA_030013775.1) and the extinct Steller's sea cow (GenBank assembly: GCA_013391785.1), as well as two previous de novo assemblies for the dugong (GenBank assemblies: GCA_905400935.1 and GCA_905400935.1). No genomic data has been published for the Amazonian (*Trichechus inunguis*) or West African (*Trichechus senegalensis*) manatee species, both of which are listed as Vulnerable by the IUCN. Our assembly is the most contiguous Sirenian genome assembly to date, improving on previous assemblies - all assembled with short read data - by at least an order of magnitude in contigs and scaffold N50s.

Initial estimates of genome-wide heterozygosity based on our new genome assembly are relatively high for a mammal of conservation concern, probably reflecting the previously high abundance of dugongs prior to the last interglacial period (ca. 125,000 years ago). While runs of homozygosity indicate past inbreeding, we find no evidence in the genome of ongoing inbreeding among the Moreton Bay population of dugongs where this reference individual was sourced from. Future analyses of individuals from different populations may show whether these patterns of diversity are replicated in smaller and more isolated populations.

Our demographic inference analysis based on PSMC suggests that dugongs in Eastern Australia were variably abundant from around 1 million years ago (Ma) to 150 ka. This earlier estimate coincides with the mid-Pleistocene transition, during which longer and more intense glacial cycling began. However, more recently fluctuations in dugong abundance do not precisely track the approximately 100 ka glacial cycles that drove changes in global sea level (Yehudai et al. 2021). Dugong abundance declined steeply beginning at ∼100 ka, probably due to population fragmentation (Blair et al. 2014) and habitat loss that occurred as sea levels fell after the last interglacial period and the shallow seagrass meadows in which they lived disappeared.

Our draft genome assembly promises to advance understanding of marine mammal evolution and diversification as well as provide crucial insights into dugong conservation and management. Sirenians are the most ancient lineage of marine mammals, having split from their most recent terrestrial ancestor ∼63.9 Ma (Yuan et al. 2021). Future comparative genomic studies both within Sirenia and between Sirenians and other marine mammal lineages will shed light on the genomic changes that allowed for these lineages to adapt to the marine environment. For example, a more contiguous dugong reference genome will improve reference-guided assembly of the extinct Steller's sea cow, which was notable for both its large size and its adaptation to a subpolar kelp forest environment, unique among the typically warm water dwelling Sirenia. Future generation of genomic data from other dugong populations, many of which are geographically isolated and/or live in quite different environments, will allow evolutionary analyses of adaptations unique to this lineage. The species' large but discontinuous geographic range raises the possibility that some populations are genetically distinct and locally adapted. By identifying isolated populations and better defining subpopulation units, future work will allow development of more targeted management strategies that can support the continued persistence of this unique marine mammal in changing global habitats.

2.6 Funding

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2.7 Acknowledgements

We acknowledge the traditional custodians of the land and water, the Quandamooka people, who care for the yangang (dugong) and the Sea Country where the reference individual was sampled. We pay our respects to their elders past and present and recognize their ongoing connection between culture and Country. Thanks also to E. McLennan for undertaking the genomic DNA extractions. The authors wish to acknowledge the services of the Australian Genome Research Facility and the Ramaciotti Centre for Genomics.

2.8 Data availability

Data generated for this study are available under NCBI BioProject PRJNA970804. Raw PacBio HiFi and Omni-C Illumina sequencing data for NCBI BioSample SAMN33212336 are available at `https://genomeark.s3.amazonaws.com/index.html?prefix=species/Dugong_dugon/mDugDug1/genomic_data/`, pending submission to the the NCBI Short Read Archive (SRA). GenBank accessions for both primary and alternate assemblies are GCA_030035585.1 and GCA_030020955.1. The mitochondrial genome is available at `https://genomeark.s3.amazonaws.com/index.html?prefix=species/Dugong_dugon/mDugDug1/assembly_MT_rockefeller/` pending submission to GenBank. Assembly scripts and other data for the analyses presented can be found at the VGP galaxy project: `https://galaxyproject.org/projects/vgp/`.

Chapter 3

Genomic diversity and population structure of historic Russian and Alaskan polar bears (*Ursus maritimus*)

3.1 Abstract

Sea ice loss and associated habitat changes due climate change pose significant threats to polar bear (*Ursus maritimus*) populations. Some of the 19 polar bear "subpopulations" (management units) were also heavily hunted during the first half of the 20th century. The effects of hunting and climate change on different polar bear populations are not fully understood, nor is the population structure and levels of gene flow between these subpopulations, which are affected differently by climate change. A major impediment to understanding range-wide polar bear diversity, population structure, and climate change response is a lack of data from Russia, which manages a large portion of the polar bear range and four subpopulations. In this chapter we sequence historic polar

bear genomes from Russian and Alaskan museum samples alongside modern genomes to explore two main questions: 1. How are Russian polar bears related to each other and to other polar bear populations range wide and how well do subpopulation boundaries represent genetic structure? 2. How has diversity in Alaskan polar bear subpopulations changed over the last ∼150 years in response to hunting and climae change? Our findings show that historically, Russian and Alaskan populations had high connectivity across subpopulations and low levels of genetic structure despite being sampled from a broad geographic region. Diversity appears to have declined significantly in Alaska over the last century, with an 88% decline in average heterozygosity over the last century, with the majority occurring some time after 1957. Population structure analyses suggest a potential population replacement in Alaska between the 1970s and 2000s, possibly influenced by abundance declines due to hunting and climate-induced sea ice changes. This study underscores the need for geographically diverse studies incorporating historic data, and ongoing monitoring of polar bear populations as their habitat changes.

3.2 Introduction

Polar bears (*Ursus maritimus*) are a sentinel species for wildlife conservation in the era of anthropogenic climate change. A large marine mammal with a circumpolar Arctic distribution, they have many specialized adaptations to their high-latitude habitat. Despite their unique phenotype, polar bears diverged relatively recently from brown bears (∼300-500 ka) and have low genetic diversity, indicative of relatively small effective population sizes over their demographic history (Liu et al. 2014). Polar bears prey primarily on seals and are highly dependent on sea ice for foraging, dispersal, and raising young. Climate change is proceeding quickly in the Arctic, causing spatial decreases of

sea ice as well as temporal decreases in annual sea ice extent. Recent climate projections predict that the first ice-free summer in the Arctic Ocean will arrive before 2050 (Notz and SIMIP 2020). Currently listed as Vulnerable by the IUCN, polar bears's sea ice dependence along with their energetically expensive lifestyle (Pagano et al. 2018), leaves them highly vulnerable to sea ice changes and declines, as well as to other habitat changes that are likely to accompany climate change, including anthropogenic development in the Arctic and altered prey abundance and distribution (Amstrup et al. 2008, Molnár et al. 2020, Notz and SIMIP 2020).

Polar bears have long been hunted by indigenous Arctic peoples for food, fur, and other raw materials. However hunting pressure on polar bears increased as non-indigenous people began to populate the Arctic, bringing mechanized hunting equipment - initially firearms but eventually snowmobiles and aircraft as well. With these efficient methods, annual hunts of polar bears - primarily for trophies - grew throughout the early 20^{th} century until abundance began to measurably decrease, initiating concern about the survival of the species (Prestrud and Stirling 1994). Arctic nations began passing regulations to protect polar bears in the mid 1950s, and in 1973 all five Arctic nations with polar bears (Norway, Canada, Denmark, the Soviet Union, and the United States) signed the International Agreement on the Conservation of Polar Bears, restricting recreational and commercial hunting (IUCN/SSC Polar Bear Specialist Group 1970).

Hunting pressure is no longer the main conservation threat to polar bears, due to both more effective management, as well as the emergence of the greater threat of climate change. The temporal and spatial extent of polar bear habitat has been in decline since the 1970s at higher rates than predicted by climate modeling (Stroeve et al. 2007), with documented negative effects on various aspects of polar bear biology including populations ranges and abundance

(Stirling and Derocher 2012).

Polar bears have an estimated global abundance of approximately 26,000 individuals and are managed as 19 subpopulations distributed among five Arctic nations as well the transnational Arctic basin (Regehr et al. 2016, IUCN/SSC Polar Bear Specialist Group 2021). However, subpopulation boundaries do not necessarily reflect either individual ranges or genetic structure (Viengkone et al. 2018, IUCN/SSC Polar Bear Specialist Group 2021), which is relatively weak in polar bears (Paetkau et al. 1999, Peacock et al. 2015). Polar bears are capable of making large movements, both under their own power and due to sea ice currents, which can move quite rapidly. Polar bear telemetry is largely limited to females (as the large size of males' necks compared to their heads means that telemetry collars easily come off) and the extent and patterns of polar bear movements and consequently gene flow throughout the Arctic is not fully understood. Abundance trends and climate change vulnerability of these 19 subpopulation vary considerably due to physical geography, biological productivity, sea ice dynamics, and other factors (Durner et al. 2018). Some subpopulations are exhibiting demographic declines attributed to climate change (Bromaghin et al. 2015, Lunn et al. 2016), as well as other markers of population stress such as low recruitment (Rode et al. 2010), poor body condition of individuals (Obbard et al. 2016), and high levels of biological pollutants and cortisol (Oskam et al. 2004, Tartu et al. 2017), yet other subpopulations are stable or even increasing ((Durner et al. 2018). These increases may be due to ephemeral positive effects of climate change such as increased prey density of Arctic seals due to decreased haul-out space, and/or migration dynamics between populations (Rode et al. 2012, Cherry et al. 2013, Rode et al. 2021a). Sea ice loss is predicted to cause large declines in polar bear abundance in the next 30-40 years (Regehr et al. 2016).

The United States co-manages two polar bear subpopulations: the South-

ern Beaufort Sea (SB) with Canada, and the Chukchi Sea (CS) with Russia (Fig. 3.1A). In addition to the Chukchi Sea, Russia independently manages the Laptev Sea (LV) and Kara Sea (KS) populations and co-manages the Barents Sea (BS) population with Norway. After overharvesting through the 1960s, the Southern Beaufort Sea population rebounded during the 1980s and 1990s (Amstrup et al. 2001), but appears to have declined in more recent years, with a current abundance estimate of ∼900 individuals (Bromaghin et al. 2015). The Southern Beaufort Sea is considered one of the most vulnerable subpopulations to climate change (Hamilton and Derocher 2018), with increased fasting and time spent on land in response to declining sea ice already apparent (Cherry et al. 2009, Atwood et al. 2016, Rode et al. 2018). The Chukchi Sea population has an estimated abundance of ∼2900 bears and appears to be currently stable, although there is insufficient data to estimate long-term abundance trends (Regehr et al. 2018). The Chukchi Sea subpopulation appears to have moderate resilience to sea ice loss due primarily to high biological productivity in the region (Hamilton and Derocher 2018, IUCN/SSC Polar Bear Specialist Group 2021). From 2016-2020, annual surveys on Wrangel Island provided important demographic monitoring on denning polar bears in the Chukchi Sea (IUCN/SSC Polar Bear Specialist Group 2021), but these surveys have not taken place in the last few years due to the COVID-19 pandemic and the political climate in Russia.

Russia independently manages two subpopulations, Laptev Sea and Kara Sea, neither of which have abundance estimates, and co-manages the Barents Sea (BS) with Norway in addition to the Chukchi Sea with the U.S.. Barents Sea abundance is estimated to be ∼2,650 (Aars et al. 2009), however due to lack of data from Russia, none of the subpopulations from Russia - neither independently or co-managed - have sufficient data for the IUCN Polar Bear Specialist Group to estimate long-term population trends (IUCN/SSC Polar

Bear Specialist Group 2021). No genomic data and little genetic data have been published from Russia (Paetkau et al. 1999, Peacock et al. 2015, Laidre et al. 2022) and exporting biological samples from Russia has been extremely difficult due to: permitting requirements for endangered marine mammals, the COVID-19 pandemic, and the Russian war in Ukraine. The status of polar bears in Russia, including the extent of illegal, unmonitored hunting and the effects of sea ice loss remains a concern (IUCN/SSC Polar Bear Specialist Group 2021, Regehr et al. 2021).

This lack of data from a large portion of the polar bear range leaves a major gap in scientific understanding of global polar bear demography and diversity and presents challenges to management. Although not a replacement for mark-recapture, telemetry, and other direct sources for measuring abundance and population health, genetic data can provide useful information about population connectivity, diversity, and historical demography. Thus, in the absence of more traditional ecological data sources, genetic data can provide useful insights as to population status. Characterizing the Holarctic genetic diversity of polar bears and identifying diverged populations is important to understanding how genetic variation and local adaptation is structured across the polar bear distribution, and how this may change with loss of sea ice and other effects of climate change (Laidre et al. 2015).

The demographic and ecological consequences of climate change have been predicted to cause declines in genetic diversity and connectivity in polar bears (Stirling and Derocher 2012, Regehr et al. 2016, Laidre et al. 2018). Direct assessments of these predictions are sparse; population genetic processes proceed at slower rates than demographic changes, making them difficult to document without long-term datasets spanning multiple generations. However, evidence from a recent study of polar bears in Svalbard suggested a significant loss in genetic diversity and increase in population fragmentation associated with sea

ice loss across 20 years (Maduna et al. 2021), indicating that the population genetic effects of climate change on polar bears may be proceeding faster than expected, and highlighting the importance of long-term genetic datasets for the species. It is not known how widespread this pattern may be, or whether it spans a longer time period than measured. Loss of genetic diversity is of particular concern in polar bears as their standing diversity is already naturally low due to relatively small effective population sizes over their demographic history (Paetkau et al. 1999, Liu et al. 2014, Peacock et al. 2015). Species with low diversity have a reduced potential to genetically adapt to changing environments and are also vulnerable to inbreeding depression (Weber et al. 2013).

Previous studies of circumpolar population structure have relied primarily on microsatellites and grouped polar bears range-wide into three to six genetic clusters, with the majority of population structuring concentrated in the Canadian archipelago (Paetkau et al. 1999, Peacock et al. 2015, Malenfant et al. 2016, Laidre et al. 2022). Sampling in Russian subpopulations has been limited in all previous studies, but Russian subpopulations cluster broadly into an "eastern polar basin" group, with a pattern of isolation by distance running around the polar rim from western Canada to northeast Greenland. Peacock et al. (2015) showed substantial directional gene flow from the Russian Arctic west into Alaska and Canada, although the directionality and extent of this gene flow has been disputed (Malenfant et al. 2016)

Museum collections can be a valuable source of longitudinal data for genetic studies (Andrews et al. 2018, Clark et al. 2023, Benham and Bowie 2023). Museum samples offer a unique opportunity to test directly for change in diversity over recent history, as methods used to infer demographic history from modern genomic data have low statistical power to infer recent change (Beichman et al. 2018). While historic samples may not necessarily be rep-

resentative of modern diversity, they can provide initial insights for regions without modern data, and can provide a baseline against which to measure ongoing and future changes in diversity in response to climate change and other anthropogenic threats (Benham and Bowie 2023) . However, microsatellite loci cannot be reliably amplified from museum samples or other sources of ancient DNA. Ancient DNA tends to be highly fragmented whereas microsatellite loci are generally long and correct interpretation relies on accurate lengths. Fortunately, genomic data are robust for analyzing ancient DNA (Hofreiter et al. 2015, Orlando et al. 2021, Liu et al. 2022).

Previous genomic studies of polar bears have provided insights into admixture, demographic history, adaptation, and fine-scale population structure, including the identification of a unique, isolated populations in Norwegian Bay and Southeast Greenland (Miller et al. 2012, Liu et al. 2014, Cahill et al. 2015, 2018, Viengkone et al. 2016, Laidre et al. 2022, Jensen et al. 2020, Malenfant et al. 2020, Samaniego Castruita et al. 2020, Wang et al. 2022).

Here, we newly sequence historic polar bear genomes from 19^{th} and 20^{th} century museum samples from Russian and Alaska and analyze them alongside previously generated modern genomes to achieve two main aims. The first is to provide an initial picture of Russian polar bear genomic diversity and population structure, putting Russian polar bears in context of global polar bear connectivity and investigating the geographic scale of polar bear population structure range-wide, particularly in relation to subpopulation boundaries. The second is to use a time series of samples from Alaska to investigate change in diversity and population structure over time in response to hunting and climate change. These historic samples can also serve as a baseline against which to compare modern diversity and assess future change. By analyzing these historic polar bear genomes alongside modern and ancient ($\sim$100 ka) genomes from Alaska and Greenland we gain insights into the spatial and temporal

variation in polar bear diversity.

3.3 Methods

3.3.1 Sample selection and sequencing

Samples were selected from a larger dataset (n = 329) of polar bear bone museum samples stored at UCSC Paleogenomics Lab , none of which have been previously published. Samples were selected primarily based on geographic origin and collection year (with an attempt to include a broad sample), and secondarily on completeness of associated metadata, quantity of available tissue, and sample preservation (estimated from low coverage sequencing, detailed below).

We performed all ancient DNA (aDNA) wet lab work in a dedicated clean room according to established aDNA techniques (Fulton and Shapiro 2019). We first powdered bone samples, then extracted DNA from bone powder following an initial low concentration bleach pre-treatment to reduce contaminating DNA (Dabney and Meyer 2019). We measured extract concentration with a Qubit, and prepared sequencing libraries following a single-stranded DNA protocol optimized for ancient DNA (Kapp et al. 2021). We quantified amplified libraries using Qubit to determine concentration and a fragment analyzer to estimate length distribution, pooled libraries in equimolar concentrations, and generated a small number of sequencing reads (0.5 million reads per sample) on an in-house Illumina NextSeq (2 x 75 cycles). We mapped these sequences to the reference genome (GenBank accession: ASM1731132v1) (Laidre et al. 2022) and assessed quality using a custom bioinformatics pipeline to estimate endogenous content (proportion of reads mapping to the target genome), fragment length, mapping statistics, and ancient DNA damage profile. We selected

libraries for deeper sequencing based on these statistics.

Libraries of sufficient quality were then sent for deeper sequencing Illumina NovaSeq platform, using a 2 x 150 paired end S4 kit. We targeted 2-5X coverage for the majority of the samples with a subset sequenced to 20-25X. For high coverage samples, multiple sequencing libraries were made (exact number depends on sample quality and amount of available material but approximately 10-15 libraries from 3-5 extracts), assessed for quality, and pooled before sequencing in order to maximize the number of unique molecules sequenced.

3.3.2 Bioinformatic processing

We trimmed adapter sequences from the raw sequencing reads, filtered for length and quality, and merged overlapping read pairs using SeqPrep2 (`https://github.com/jstjohn/SeqPrep`); mapped reads to the polar bear reference genome assembly using the BWA-aln algorithm, (which performs better for ancient DNA (Li and Durbin 2009)); removed duplicates with SAMtools (Li et al. 2009); and merged different lanes and libraries for the same sample with BWA merge. We visualized ancient DNA damage and rescaled base call quality scores with MapDamage (Ginolhac et al. 2011). All analyses were limited to the 36 largest autosomal scaffolds, assumed to be chromosomes.

3.3.3 Population structure

In order to account for varying depth of coverage and incorporate both ancient and modern samples, population-genomic analyses were performed using a genotype likelihood approach in ANGSD (v1.13) (Korneliussen et al. 2014), Genotype likelihoods were calculated using the GATK genotype model (-GL 2 -doGlf 2 -doMajorMinor 1 -doCounts 1 -doMaf 1) with the following filters: mapping quality for regions with excessive mismatches adjusted (-C 50), re-

moving secondary reads (-remove-bads 1), removing reads with multiple hits (-uniqueOnly 1), minimum base quality and mapping quality scores of 20 (-minQ 20 -minmapq 20), trasition mutations excluded to control for ancient DNA damage (-rmTrans 1), sites covered in 75% of the samples (-minInd 60), a minimum global depth of 1X per sample (-setMinDepth 80), and a maximum global depth of three times the summed average coverage (-setMaxDepth 4000).

PCA was performed on the genotype likelihoods using PCAngsd (Meisner and Albrechtsen 2018) with default parameters and eigenvalues were calculated from the resulting covariance matrix in R (R Core Team 2022). The same covariance matrix was used to construct a neighbor joining tree for all individuals, which was visualized with the ape package in R (Paradis et al. 2004). NGSadmix (Skotte et al. 2013) was used to calculate admixture proportions values of K from 2-10. Admixture proportions were plotted with the pophelper package in R (Francis 2017). Admixture proportions were mapped with the ggOceanMaps package in R (Vihtakari 2023).

We calculated the F_{st} between subpopulations in ANGSD. We first calculated the folded site frequency spectrum (SFS) for each subpopulation with genotype likelihoods estimated with ANGSD's GATK model (-GL 2 -doSaf 1 -fold 1), with transitions and bases with quality or mapping quality scores lower than 30 excluded (-noTrans 1 -minQ 30 -minmapq 30). The polar bear reference genome was used both as reference and as ancestral (- ref and -anc options). We then calculated the two-dimensional SFS for all pairs of subpopulations with the RealSFS utility tool provided in ANGSD and estimated F_{st} with the RealSFS fst stat function.

3.3.4 Diversity

We used ANGSD to estimate the heterozygosity of each individual by calculating the folded site frequency spectrum (SFS) for each individual. Only samples with a minimum coverage of 4X were included and all included samples were first downsampled to 4X control for variation in coverage. We estimated genotype likelihoods for each of the samples independently using ANGSD's GATK model (-GL 2 -doSaf 1 -fold 1), removing transitions (-noTrans 1), bases with quality or mapping quality scores lower than 30 (-minQ 30 -minmapq 30). The polar bear reference genome was used both as reference and as ancestral (- ref and -anc options) and five bootstrap repetitions were performed per sample. The SFS for each individual was estimated using the realSFS utility tool provided in ANGSD and subsequently the final heterozygosity per bootstrap was calculated as the ratio of heterozygous sites/total sites.

We estimated nucleotide diversity (π) for each subpopulation in ANGSD. We first calculated the folded SFS for each subpopulation as above and used realSFS to calculate values of θ for each site from the SFS (saf2theta and thetaStat). We then calculated π at 1 Kb steps over 10 Kb sliding windows by dividing Pattersons's θ by the number of sites per window.

Runs of homozygosity (ROH) were identified in high coverage samples using ROHan (Renaud et al. 2019) using a background mutation rate of 2×10^{-5} and a transition/transversion rate of 4.71 (calculated with VCFtools). Deamination profiles were calculated for ancient samples using ROHan's bam2prof and applied using the –deam5p and –deam3p flags.

All statistical analyses and visualizations were performed using R statistical software v4.2.1 (R Core Team 2022).

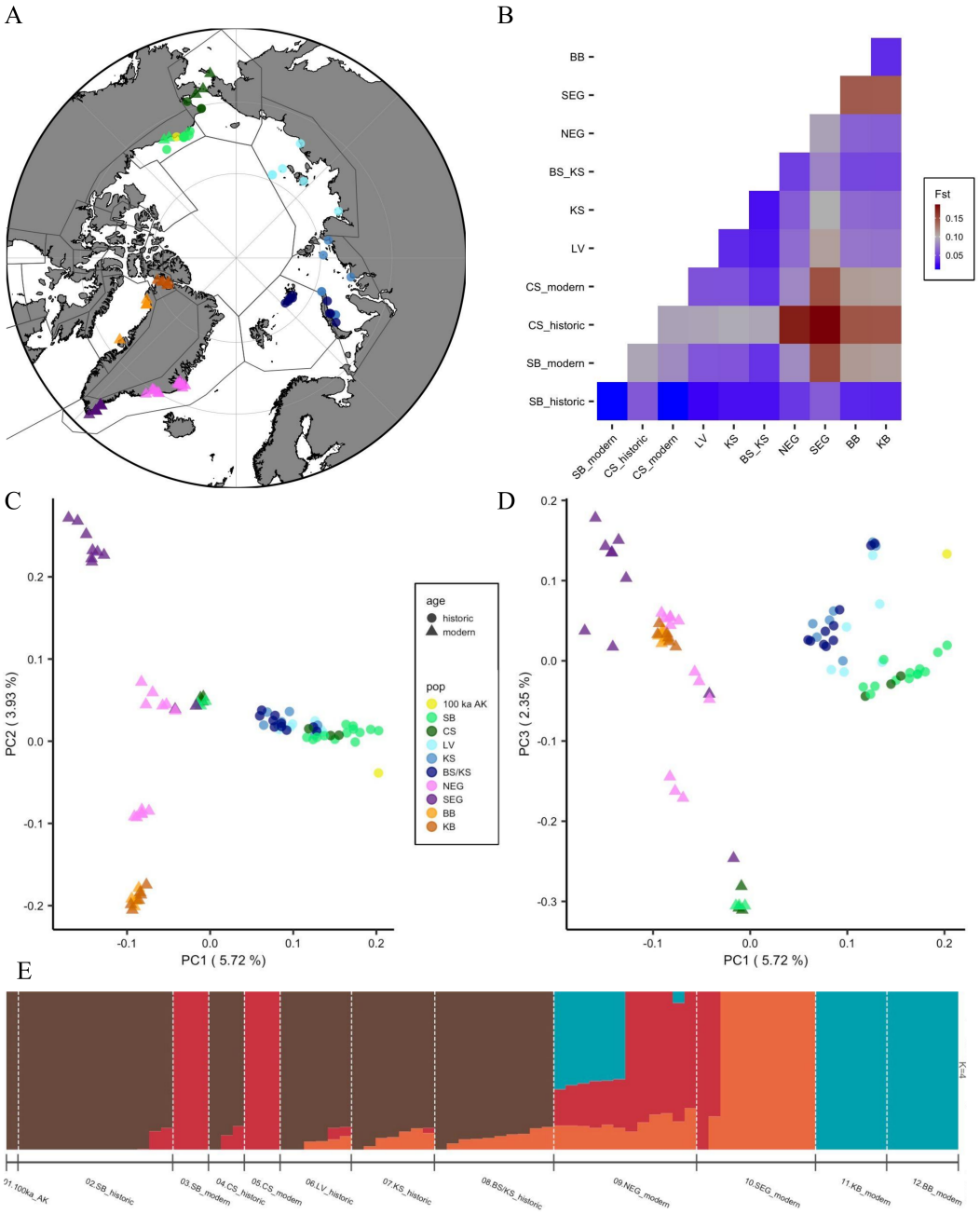

Figure 3.1: Polar bear population structure. A) Polar bear subpopulation boundaries and collection locations for all samples included in analysis. Colors indicate subpopulation as in C), triangles represent modern samples, circles represent historic samples. Note that BS/KS historic samples have the general location "Novaya Zemlya and Franz Josef Land" and are mapped for display purposes. B) F_{st} between all subpopulations; CS and SB subpopulations separated by historic and modern samples. C) Principal components 1 and 2 and D) 1 and 3 for all samples. Population abbreviations are in Table A.2. E) Ancestry groups assuming four clusters. Each column represents one sample.

3.4 Results

Whole genome resequencing data was newly generated for 39 historic polar bear individuals from five subpopulations in the eastern Arctic: Southern Beaufort Sea (SB), Chukchi Sea (CS), Laptev Sea (LV), Kara Sea (KS), and a region on the border of the Kara Sea and Barents Sea units (BS/KS) (Fig. 3.1A). Coverage ranged from <1 to 52X (Table 1). Russian samples (LV, KS, and BS/KS) were primarily collected during the 1930s by scientific hunting expeditions, with a minority collected earlier during the 1880s and 1910s, and subsampled with permission from specimens stored at the Zoological Institute of the Russian Academy of Sciences in St. Petersburg, Russia in 2008. Alaskan samples (SB and CS) spanned a time period from 1880s to 1970s and were subsampled from the Smithsonian Museum of Natural History (Washington D.C.) and the University of Alaska Fairbanks Museum (Fairbanks, Alaska) in 2010. Individual collection years are listed in (Table A.2). These newly-sequenced historic genomes were analyzed alongside 40 previously-published modern polar bear genomes from four subpopulations in Alaska (SB and CS) and Greenland (Kane Basin (KB), Baffin Bay (BB), Northeast Greenland (NEG), and Southeast Greenland (SEG)). Although Northeast Greenland and Southeast Greenland are currently managed as one unit (East Greenland (EG)), recent studies have shown a strong geographic division (Laidre et al. 2022), and we therefore considered them separately. Our dataset also included whole genome data from a bear sampled from Alaska that lived during the last interglacial (approximately 103.5 ka) (Wang et al. 2022). Citations and other sample information are available in Table A.2.

3.4.1 Population structure

Principal component analysis (PCA) showed weak population structure overall, and little separation between historic Alaskan (CS and SB) and Russian (LV, KS, and BS/KS) polar bears (Fig. 3.1C and D and Fig. A.15). Principal component one primarily separated historic and modern samples with some separation between Alaskan and Russian subpopulations, and principal component two was largely dominated by diversity within Greenland and showed a strong latitudinal cline (Fig. A.8). With the exception of Southeast Greenland and Northeast Greenland, neighboring subpopulations plot closely together. Historic Alaskan and Russian populations show further separation on principal component three (Fig. 3.1D), with Laptev Sea individuals falling in between the Alaskan individuals and the other Russian subpopulations, indicating some longitudinal isolation by distance. Historic and modern Alaskan bears group separately on all of the first three principal components, with modern Alaskan bears grouping most closely to Northeast Greenland. A neighbor joining tree showed similar geographic grouping, with historic samples more basal (Fig. A.9).

Admixture analysis largely shows a similar pattern to PCA, with log likelihood supporting four ancestry groups (K = 4)(Fig. A.15E). Historic Alaskan and Russian populations show similar ancestry proportions; but ancestry grouping is quite different between historic and modern Alaskan individuals. In both the PCA and the admixture analysis, a latitudinal division is apparent at approximately the 69^{th} parallel within Northeast Greenland.

In contrast to the PCA and admixture results, F_{st} shows separation between historic Chukchi Sea and Southern Beaufort Sea individuals, although not between modern individuals in these subpopulations (Fig. 3.1B). Historic Chukchi Sea had high average F_{st}, whereas historic Southern Beaufort Sea had

very low average F_{st} (Fig. A.10). Average F_{st} declined between historic and modern individuals in Chukchi Sea, but increased in Southern Beaufort Sea. Historic Russian populations also had low average F_{st}, indicating high historic connectivity.

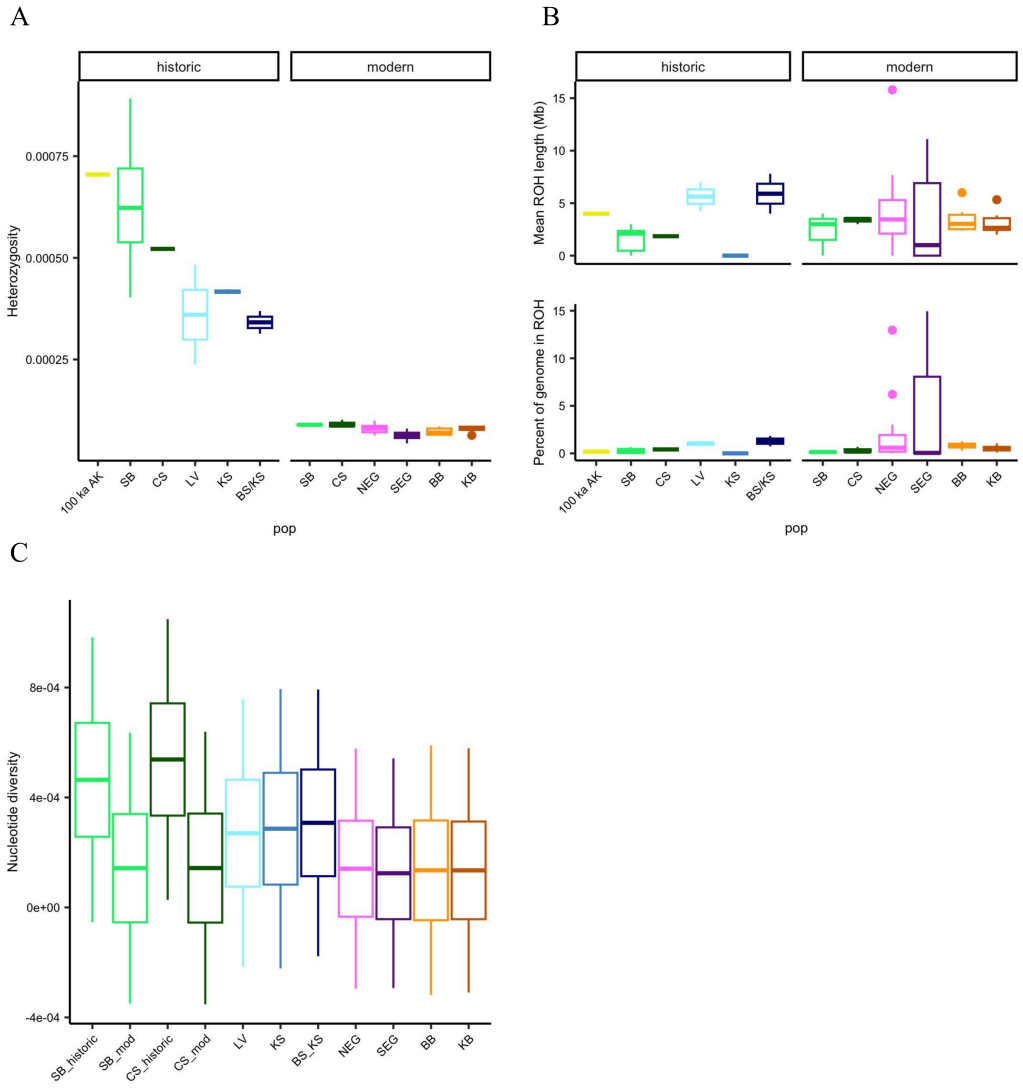

Figure 3.2: Diversity comparisons between historic and modern individuals, grouped by subpopulation. A) Average genome-wide heterozygosity. B) Mean length (top) of ROH and percentage of genome (bottom) in ROH. C) Average 10 Kb sliding window nucleotide diversity. Subpopulations colored as in Figure 1.

3.4.2 Diversity

We observed significantly lower genome-wide heterozygosity in all modern individuals as compared to historic ($p < 0.001$, Fig. 3.2A). Among Alaskan populations, heterozygosity showed a temporal decline (Fig. 3.3A); we observed a 29% decrease in average heterozygosity in Alaskan bears between 1883 and 1959 (the most recent year for which we have historic data of sufficient coverage), and an 83% decrease between 1959 and 2000. This decline was non-significant ($r = –0.52$, $p = 0.23$) when only historic individuals were included, but significant when modern individuals were included ($r = -0.91$, $p < 0.001$) and when grouped as modern vs historic ($p < 0.001$; Fig A.11A). We observed lower nucleotide diversity in all modern populations as compared to historic ($p = 0.017$), a decrease between historic and modern in Alaskan populations was apparent but not significant ($p = 0.066$).

Average percent of each genome in ROH was generally low except for in the east Greenland subpopulations (Fig 3.2B). Two historic Russian populations had long average ROH lengths, indicating somewhat recent inbreeding (Fig. A.12). Average length (Fig A.11C) and proportion (3.3C) of ROH increased slightly over time in Alaska, but comparisons between modern and historic Alaskans as a group were non-significant ($p = 0.13$ for ROH average length; $p = 0.56$ for percent of genome in ROH; Fig. A.11B).

3.4.3 Temporal trends

Alaskan individuals also exhibited a decline in principal component space over time (Fig. 3.3A). Ancestry group proportions differed between historic and modern individuals, but no temporal trend was apparent (Fig. A.13).

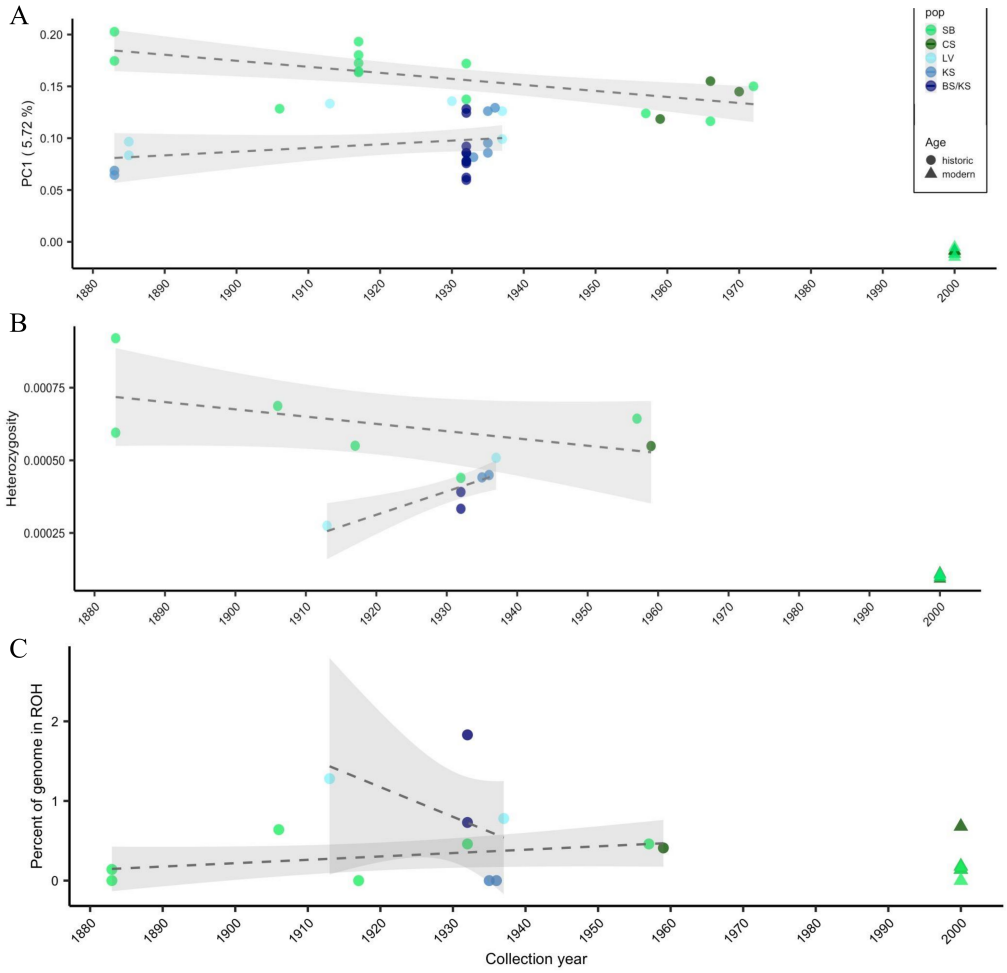

Figure 3.3: Change over time in Alaskan and Russian individuals. A) Principal component 1 vs time B) Mean genome-wide heterozygosity vs time (individuals <4X coverage excluded). C) Proportion of genome in ROH (individuals <4X coverage excluded). Trend lines group Alaskan historic individuals (CS and SB) and Russian historic individuals (LV, KS, BS/KS). Subpopulations colored as in Figures 1 and 2.

3.5 Discussion

Although different analyses provide slightly different results, overall it appears that compared to Greenland, which contains substantial geographic structuring of genomic diversity, late 19th and early 20th century polar bears from Russia and Alaska were closely related with some isolation by distance but little population structure, despite being sampled from a broad geographic region encompassing five subpopulations. This grouping roughly corresponds to

the the divergent ice region of the Arctic. As shown in other studies with both genomic data and microsatellite loci (Peacock et al. 2015, Laidre et al. 2022) the scale of polar bears population structure is highly heterogeneous and does not necessarily correspond to subpopulation boundaries but does have some relation to ice ecotypes. Genetic divergence does not scale with geographic distance, with more divergence between bears sampled less than 800 km in Eastern Greenland than bears sampled up to 2,200 km apart in Russia.

Among our sampled populations, polar bear heterozygosity and nucleotide was higher in all historic populations than modern. Our uneven geographic and temporal sampling makes it difficult to draw conclusions about the geographic extent and/or distribution of diversity loss, but this result raises concerns about the genetic health of contemporary polar bear populations. Among Southern Beaufort Sea polar bears - the population for which we had the best temporal sampling - we observed a significant loss of diversity over time, with average heterozygosity declining by 88% over the ∼130 year timespan investigated. Diversity appears to have already been declining over the first half of the 20^{th} century, however the majority of observed diversity decline occurred sometime between 1957 and 2000. Only one historic Chukchi Sea individual had sufficient coverage to assess heterozygosity, but this individual's heterozygosity fell within the range of values among Southern Beaufort Sea individuals. We also observed a slight but non-significant increase in inbreeding over time among Alaskan bears, which may be contributing to this diversity loss. Intensive sport hunting in the 1950s and 1960s depleted abundance among Alaskan bears and by the 1970s - when hunting became more widely regulated - climate change-induced sea ice declines were becoming apparent in the Arctic. The Southern Beaufort Sea has already experienced significant sea ice loss and subsequent physiological and behavioral changes in polar bears (Cherry et al. 2009, Atwood et al. 2016); our results indicate that these environmental

changes may also be contributing to diversity loss.

All of our population structure analyses showed substantial divergence between historic and modern bears from Chukchi Sea and Southern Beaufort Sea, suggesting that a population replacement may have occurred in Alaska between the 1970s and 2000s. Given the decline in diversity prior to this shift, it is possible that significantly reduced abundance from hunting within Alaska created space for bears to move in from elsewhere. Both abundance estimates and indigenous knowledge indicate that overall polar bear abundance in the Southern Beaufort Sea and Chukchi Sea populations declined during the sport hunting era in the 1950s and 1960s, then increased through the later 1970s and into the 1980s (Amstrup and Road 1986, Stirling 2002, Voorhees et al. 2014, Rode et al. 2021b). These studies also document finer scale temporal and spatial changes, such as an ephemeral population declines due to anomalous ice conditions. It's possible that these abundance changes reflect the population turnover we observed due to immigration from other regions. Sea ice loss and changing ice patterns due to climate change may have also contributed to a population sink effect in Alaska - sea ice loss is not uniform and has been shown to be particularly severe in the Southern Beaufort Sea region. Decreased sea ice could lead to increased isolation, with less opportunity for migration and gene flow. This has been shown to be the mechanism for diversity loss in Svalbard (Maduna et a. 2021). Historic Southern Beaufort Sea bears appear to have low average F_{st} with all other sampled populations, suggesting higher historic connectivity. With the temporal gap in our sampling in the late 20^{th} century, it is difficult to tease out the relative impacts of hunting and sea ice loss, particularly as there is likely a temporal lag between demographic changes and resulting diversity loss. However, evidence for hunting being the primary cause is the observation the Chukchi Sea and Southern Beaufort Sea subpopulations appear to follow similar trends. These subpopulations were

similarly impacted by hunting but responses to climate change differ, with the Southern Beaufort Sea being much more strongly affected.

The source is of this potential population replacement in Alaska is not clear from our results: modern Southern Beaufort Sea and Chukchi Sea bears show affinity with both Northeast Greenland and Russian populations, and the closest population differs between analyses. Much of the global diversity of polar bears is within Canada, where we have no sampling, limiting our ability to assign sources. It is possible that this population replacement is the result of admixture between individuals from multiple populations. At K = 4, modern Chukchi Sea and Southern Beaufort Sea don't appear admixed, but they do at lower values of K (Fig. A.14). Recently admixed populations are likely to have higher diversity and among the sampled modern populations, average heterozygosity and nucleotide was highest in Southern Beaufort Sea and Chukchi Sea, supporting this hypothesis of an admixed population replacement in Alaska.

It is possible that polar bear population structure is quite dynamic, changing frequently over time as polar bears are highly mobile and sea ice is a dynamic habitat. However the ancient Alaskan bear from the last interglacial period does not appear to be significantly different from historic Alaskan bears from the 19$^{\text{th}}$ and 20$^{\text{th}}$ centuries, suggesting that the population structure has been relatively stable for the last 100 ka, only changing in the last ∼50 years.

Historic Russian bears appear to have had high diversity and clustered closely together despite a broad geographic range, with a pattern of isolation by distance and a small degree of divergence with historic Alaskan individuals. These are the first genomic data from Russia and help fill a geographic gap, increasing our understanding of global polar bear population structure. However, the substantial change we see occurring over time in Alaskan bears suggests that even relatively recent historic samples may not be representative

of modern diversity in polar bears, particularly in regions with a history of heavy hunting. These results highlight the urgency and importance of obtaining data from modern Russian polar bears.

Our results suggest that Alaskan polar bears have undergone significant loss of diversity and change in population identity over the 20th century and highlight the need for geographically diverse studies incorporating historic data, and ongoing monitoring. This diversity loss is a concerning finding in a species known to already have low diversity and consequently a lack of adaptive potential. Diversity will likely be of increased importance for polar bears as they face new challenges in a changing Arctic.

Given that we see a degree of diversity loss prior to major effects of climate change, this decline in diversity is likely at least partially due to sport hunting - which caused major abundance declines in Alaska. However, the majority of observed diversity decline occurred in the gap between the end of our historic sampling in 1959 and our modern sampling in 2000. This presumed rate increase in diversity decline during the second half of the 20th century after sport hunting was banned suggests that sea ice decline and other effects of climate change may also play a role. Maduna et al. (2021) also documented a significant loss of diversity and increased genetic divergence in Svalbard between 1995 and 2016, suggesting that this pattern may be widespread and is proceeding rapidly. A more comprehensive study of paired modern and historic samples from multiple regions throughout the Arctic will provide a better understanding of how widespread this pattern of diversity loss is. Sampling from regions where sport hunting was less intense could also shed light on the relative impacts of hunting vs climate change. It could also determine the source of population replacement for Alaska and show whether or not this is a unique event. Understanding the rate and spatial extent of diversity loss and change in population structure will improve management and conservation of

polar bears as the Arctic continues to change.

Chapter 4

Ancient sedimentary DNA shows 5000 years of continuous beaver (*Castor canadensis*) occupancy in Grand Teton National Park

4.1 Abstract

Beaver-based restoration is gaining momentum as a low-cost conservation and climate adaptation solution. However, relatively little is known about how beavers in North America were temporally and spatially distributed prior to their near-extirpation by the European-American fur trade. Similarly, our understanding of how beaver ecosystem engineering alters the local environment on long (beyond decadal) time scales is limited. Here, we apply sedaDNA techniques to investigate the history of beaver occupancy in three lakes in Grand Teton National Park over the last ∼10 ka, as well as their interactions with the

local plant community. Using a species-specific qPCR assay, we documented a dynamic history of beaver presence in the two lower altitude lakes, however no history of beaver occupancy was detected at a higher elevation lake with more marginal habitat. We first detected beavers at 7.2 ka; beavers were continuously detected in Taggart Lake from 5.2 ka, but detection was more variable in the larger Jenny Lake, with detection gaps roughly coinciding with regional droughts. Vegetation metabarcoding revealed a shift in plant community coinciding with beaver establishment in these two low altitude lakes, with a decrease in conifer dominance and an increase in riparian taxa, as well as an increase in overall taxonomic diversity. Beaver establishment and vegetation regime shifts coincide with the beginning of a regional neoglacial advance, which was likely driven by higher winter precipitation and increased regional water balance. These larger-scale changes likely facilitated beaver arrival and contributed to the observed plant community changes. Continuous presence of beavers in Taggart Lake throughout multi-century droughts in the late Holocene suggests that under certain conditions beavers may be able to maintain wetlands through extended periods of climatic stress, providing refugia for plants and animals and buffering the effects of climate change at the local scale. sedaDNA is a powerful novel technique for reconstructing past beaver occupancy dynamics in the absence of other forms of physical evidence.

4.2 Introduction

As climate change intensifies, so does our need to find low-cost, sustainable ecosystem conservation and restoration solutions. Beaver-based restoration, which entails encouraging beaver establishment in low functioning watersheds through reintroductions and beaver mimicry, is one solution that is rapidly gaining momentum.

Beavers (genus *Castor*) are large semiaquatic rodents with a unique behavior of engineering their own environmental niche. There are two extant species, the Eurasian beaver (*Castor fiber*) and the North American beaver (*Castor canadensis*) with slight morphological differences but similar behavior and ecological roles (MacDougall 2004, Rosell et al. 2005). They are generalist herbivores, feeding primarily on aquatic plants, tree bark (with a preference for poplars and willows), and grasses and sedges (Law et al. 2014, Vorel et al. 2015).

Beavers are ecosystem engineers, significantly altering the hydrology, geomorphology, and ecological community of a riparian system (Naiman et al. 1988, Gurnell 1998, Hood and Bayley 2008, Fairfax and Small 2018, Puttock et al. 2021). Beavers construct channel-spanning dams from sediment and woody material on low-order rivers and streams in order to create slow-moving ponds that allow them to forage and avoid predators while remaining submerged (Naiman et al. 1988). Beavers further construct their environment by digging canals and coppicing trees for both food and building material (Grudzinski et al. 2020). Beaver engineering causes cascading changes to the geomorphology, hydrology, geochemistry, and ecology of an environment and the interactions between them (Rosell et al. 2005, Brazier et al. 2021, Larsen et al. 2021). Beaver dams slow water flow velocity and increase overbank flow into floodplains, which raises the water table (Westbrook et al. 2006, Hood and Bayley 2008). Sediment transport is slowed and fine-grained sediment is stored behind dams and deposited in floodplains by overbank flow, reducing channel incision, promoting avulsion, and increasing channel-floodplain connectivity (Westbrook et al. 2006, 2011). Carbon is sequestered and nutrient transport is slowed (Wohl et al. 2012, Puttock et al. 2018). Beaver wetlands promote vegetation diversity and productivity and provide habitat for aquatic and riparian animals (Collen and Gibson 2000, Miranda 2017). Beaver dam systems

are resilient to disturbance events: stored and slowed water reduces the effect of drought, increased moisture makes river systems more resilient to wildfire, and dams attenuate peak flows during flood events (Fairfax and Small 2018, Fairfax and Whittle 2020, Puttock et al. 2021, Wohl et al. 2022). Over time, beavers engage in a cycle of maintaining and abandoning individual dams within a watershed, creating a spatial mosaic of ecological and geomorphic succession and increasing diversity (Johnson-Bice et al. 2022).

However, questions remain as to where beaver reintroduction is appropriate, how beaver engineering affects the local environment at long (beyond decadal) timescales, and where beavers can survive and thrive in the future as land use patterns and local climates continue to change. These questions arise in part from a lack of understanding of the distribution and extent of historic beaver activity. Beavers occupied a wide variety of environments throughout North America for at least seven million years, but extensive trapping for the commercial fur trade caused a severe, range-wide decline of the species and local extirpation in many areas by the 19$^{\text{th}}$ century (Naiman et al. 1988). Beavers have partially recovered due to both natural recolonization and assisted reintroduction, but their current abundance of 9-12 million individuals is a fraction of the estimated pre-exploitation abundance of 60-400 million (Naiman et al. 1988, Castro et al. 2017).

However, this estimate of historic abundance is an extrapolation from small contemporary populations and contains substantial uncertainty; little is known about the historic density and distribution of beavers in North America. Most evidence of the range and distribution of beavers prior to fur trade decline is sociocultural, based largely on Traditional Ecological Knowledge, limited historical records from fur trappers, and indirect information such as place names (Lanman et al. 2012, 2013, Tape et al. 2021, Richmond et al. 2021). Physical evidence of beavers such as fossils, woody debris, and sedimentary proxies

of dam building tends to be sparse and stochastically distributed (Robinson et al. 2007, Persico and Meyer 2009, 2013, Kramer et al. 2012, Mitchell et al. 2016, Davies et al. 2022). This lack of physical historical data hinders efforts to reintroduce beavers to historically occupied regions and also limits scientific understanding of how a major biotic driver influenced ecological and geological processes prior to European colonization of North America (Kramer et al. 2012). Given the ecogeomorphic impact of beaver at the local scale, it is likely that the large number of beavers that occupied North America prior to European settlement had a profound impact on past landscape processes, but estimates of regional-scale beaver influence prior to the fur-trade are difficult to extrapolate from primarily short-term, local-scale contemporary studies (Wohl 2021, Scamardo et al. 2022). The few studies of long-term beaver engineering indicate that beavers are important drivers of sedimentation dynamics and strongly influence long-term processes such as channel planform and valley formation both directly and indirectly through their controlling effect on riparian vegetation (Persico and Meyer 2009, 2013, Kramer et al. 2012, Polvi and Wohl 2012, 2013, Śnieszko et al. 2021). A better understanding of the long-term legacy of beaver damming on ecosystem and river corridor processes has been identified as a critical gap in beaver research (Brazier et al. 2021, Larsen et al. 2021).

Analysis of environmental DNA isolated from ancient sediments (sedaDNA) is a relatively new type of physical evidence used to understand paleoenvironments, facilitated by advances in ancient and degraded DNA methodologies (Rawlence et al. 2014, Capo et al. 2021, Crump 2021). SedaDNA is a promising emerging tool for reconstructing past ecosystems, as each small sediment sample can yield a broad snapshot of biotic diversity from microbes to vertebrates. sedaDNA typically provides greater taxonomic diversity and resolution than macrofossils and is more spatially precise for vegetation than fossil pollen

(Jørgensen et al. 2012, Parducci et al. 2017, Capo et al. 2021). Various sedaDNA analytical techniques offer different advantages - metabarcoding is ideal for evaluating community structure and diversity as it provides a broad overview of taxonomic groups, whereas more targeted techniques such as quantitative PCR (qPCR) assays are a sensitive technique for species-specific detection. SedaDNA has been used to document arrival times and local extinction events of specific taxa, reconstruct local paleoenvironments, and identify broad-scale regime shifts indicative of major climatic and environmental change (Haile et al. 2009, Graham et al. 2016, Crump et al. 2019, Voldstad et al. 2020).

Two critical considerations in application of sedaDNA are preservation and contamination. High elevation lacustrine sediment cores are ideal sample sources for sedaDNA as the cold, dark, anaerobic environments at lake bottoms provide excellent conditions for DNA preservation, slowing the microbial and physical processes that fragment and damage DNA over time (Dabney et al. 2013, Parducci et al. 2017, Capo et al. 2021). Paleoecological reconstructions from lacustrine sedaDNA demonstrate good DNA preservation beyond 10 ka (Epp et al. 2015, Kisand et al. 2018). Decades of ancient DNA validation have yielded strict field and lab procedures to control for and identify contamination in sequencing results including the use of dedicated clean rooms and incorporating experimental controls at all stages of DNA processing (Cooper 2000, Hebsgaard et al. 2005, Thomas et al. 2005, Hofreiter and Shapiro 2012) Validation studies have confirmed that DNA leaching does not occur in lake sediments, ensuring stratigraphically secure results from appropriately treated cores (Haile et al. 2007). When employed alongside other paleosedimentary analyses, sedaDNA is a powerful tool for reconstructing past environments and understanding the interaction between geological and ecological processes over deep timescales (Thomsen and Willerslev 2015, Graham et al. 2016, Parducci

et al. 2017, Crump 2021).

Here, we use sedaDNA from lake sediment cores from three post-glacial lakes to document the historical presence of beavers in Grand Teton National Park (GTNP) in Wyoming, USA over the last 10 ka and investigate beavers' potential impact on the local vegetation community. These lakes contain a well-described sedimentary record of paleoenvironmental change since approximately 15 ka (Larsen et al. 2016, 2020). Furthermore, GTNP and the greater region - including Yellowstone National Park - is one of the few areas in North America where Holocene beaver activity has been reconstructed from sedimentary analyses (Persico and Meyer 2009, 2013), making this an ideal location for testing this novel method. Sedimentary proxies from multiple stream beds in this region indicate sporadic beaver activity in the early Holocene and fairly consistent activity in the later Holocene with notable gaps corresponding to periods of regional drought and climatic anomalies (Persico and Meyer 2009, 2013). Beavers in the greater GTNP region were heavily trapped in the early 1800s but rebounded in the 20$^{\text{th}}$ century. As of 2014, 83 active beaver lodges were documented in GTNP, representing an estimated 400 individuals (Collins 1976, Gribb and Harlow 2014). The purpose of our study is twofold: 1) to demonstrate the utility of sedaDNA for documenting past presence of beavers, and 2) to enrich the current understanding of Holocene ecological dynamics in GTNP by reconstructing the local history of an environmental engineer and its interaction with plant diversity and community structure on a geological time scale.

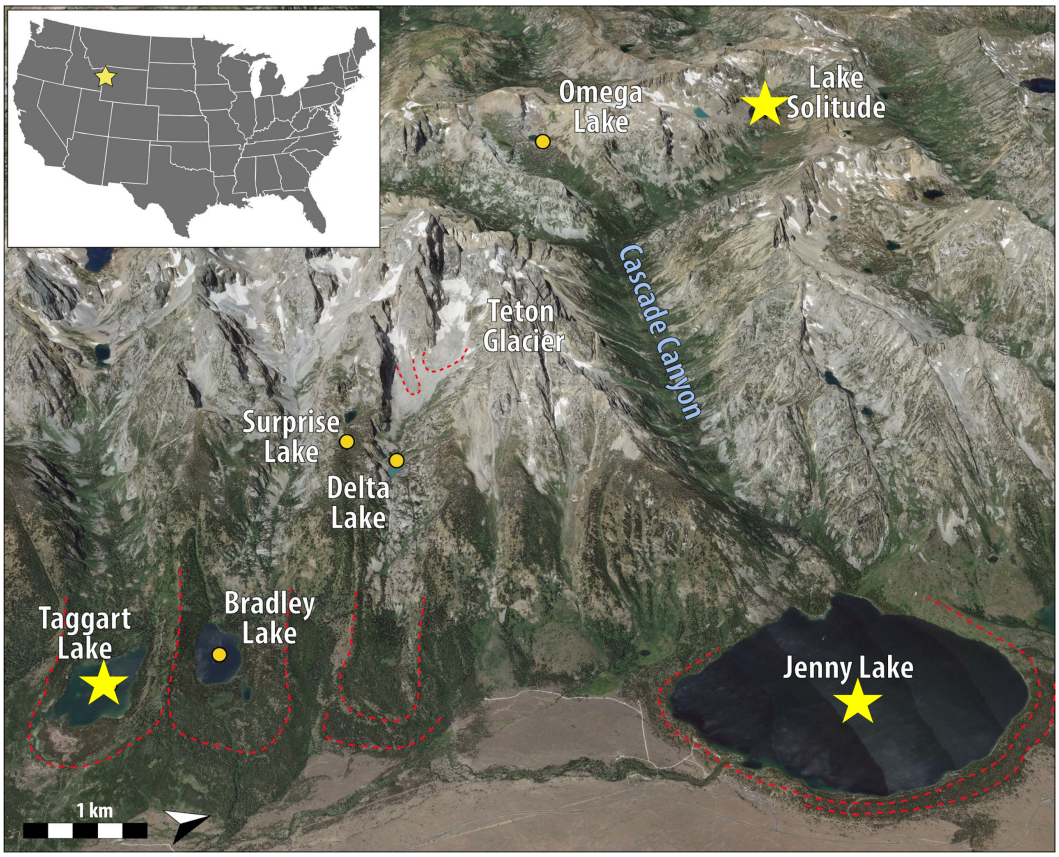

Figure 4.1: Regional context of study area and three sampled lakes (starred) in Grand Teton National Park, Wyoming.

4.3 Methods

4.3.1 Study area

Core samples were collected from three lakes in Grand Teton National Park, Wyoming, USA: Jenny Lake, Taggart Lake, and Lake Solitude (Fig. 4.1). We chose these three lakes for this study as this region has a well-described geologic and paleoclimate history (Larsen et al. 2016, 2020) and the three lakes have varying physical characteristics and beaver habitat suitabilities. Jenny Lake and Taggart Lake are located at similar elevations (∼2000 m) and have a similar geologic history, having formed as terminal lakes of piedmont glaciers at the end of the Pleistocene. However, Jenny Lake is much larger, deeper, and colder than Taggart Lake and drains a larger valley, suggesting a different

aquatic profile. The creeks draining into Taggart and Jenny Lakes contain good beaver habitat and contemporary beaver activity in this area is well documented (Gribb and Harlow 2014, GBIF Secretariat 2022). Furthermore, sedimentary evidence indicates periodic beaver activity in this region of the Grand Teton front range throughout the Holocene (Persico and Meyer 2013). Lake Solitude is located approximately 700 m higher than Jenny and Taggart Lakes and was formed from a cirque glacier. At high elevation near the treeline, Lake Solitude is considered marginal beaver habitat due to limited food and building resources, and has no known history of beaver activity.

4.3.2 Sediment coring and chronologies

Sediment cores were collected from each lake using a percussion-driven piston corer deployed on cables from the frozen lake surface. All cores were packaged in the field and transported for initial core processing and description. Core sections were split longitudinally and core halves photographed using a linescan core imager.

Age control of lake sediments was established using radiocarbon dating of terrestrial plant macrofossils (e.g., conifer needles, charcoal, and woody plant fragments) and tephrochronology. Radiocarbon results were calibrated and converted to calendar years before present using CALIB 7.0 with the IntCal13 calibration curve (Stuiver et al. 2010, Reimer et al. 2013). The radiocarbon chronologies are bolstered by the position of the Mazama ash bed ($\sim$7.6 ka) (Zdanowicz et al. 1999, Larsen et al. 2016, 2020). Age-depth models for all lake cores were constructed using a smooth spline interpolation of individual control points and the 'classical' age modeling code for R software (Blaauw 2010).

Table 4.1: Primers used for qPCR and metabarcoding.

Barcode	**Target**	**Forward sequence**	**Reverse sequence**	**Other**	**Amplicon length**	**Source**
trnL	Vascular plants	GGGCAATCCTGAGCCAA	TTGAGTCTCTGCACCTATC	-	10-143bp	Taberlet et al. 2007
16SmammP007	Mammals	CGAGAAGACCCTATGGAGCT	CCGAGGTCRCCCCAACC	Human blocker: GGAGCTTTAATTTATTAATGCAAACAGTACCC	60-84bp	Giguet-Covex et al. 2014
Ccan_qPCR	North American beaver	CATAAACAATCCACYTCAAAATGGA	TCCCGAGCGGGTTGCT	qPCR probe: /56-FAM/TCTTAATCT/ZEN/ACCATCCTCCGTGAAA/3IABkFQ/	90bp	Smith and Goldberg 2022

Table 4.2: PERMANOVA results comparing plant community based on Jaccard similarity before and after first detection of beavers at 7.2 ka. *Significant p values

Lake	**Degrees of freedom**	**Sum of Squares**	R^2	**Pseudo-F**	**P-value**
Solitude	1	0.47	0.39	5.64	$8e^{-3}$*
Jenny	1	0.53	0.39	11.61	$1e^{-3}$*
Taggart	1	0.96	0.2	4.57	$1e^{-3}$*
Combined	2	2.99	0.29	9.79	$1e^{-3}$*

4.3.3 sedaDNA extraction and analysis

Core subsampling, extraction, and laboratory analysis was performed in dedicated ancient DNA clean rooms following standard ancient DNA protocols including full personal protective equipment and extensive bleaching of surfaces and tools. We subsampled Jenny and Taggart lake cores at approximately 500 year intervals up to 10 ka. 1000 year intervals were used for Jenny Lake. Two replicate 500 mg subsamples, taken from the interior of the archived core half to minimize contamination, were digested in a digest buffer following (Grealy et al. 2015). One extraction control was prepared for each batch of 11 samples and included in all downstream analyses. Sediment digests were concentrated in Vivaspin centrifugal concentrators, added to a binding buffer following Dabney et al. (2013) and purified via MinElute PCR Purification Kit. To evaluate inhibition and inform downstream analyses, extracts were first amplified via quantitative PCR (qPCR) with trnL barcode primers (Table 4.1) and a serial dilution (full concentration, 1/10, 1/100). qPCR results were used to inform sample-specific dilutions and target amplification cycles (cycle number at which exponential amplification ended) for the metabarcode library PCR.

North American beaver (*Castor canadensis*) presence was assessed with targeted sequence detection through qPCR using a species-specific primer-probe

assay developed by Smith and Goldberg (2022) that amplifies a 90 bp fragment of the beaver mitochondrial genome (Table 4.1). We performed five replicate qPCRs for each extract (including controls) at the recommended dilution from the metabarcoding qPCR. Positive beaver detection was indicated by exponential amplification over a baseline threshold of 1000 relative fluorescence units (RFUs).

To investigate change in the vascular plant community, extracts were PCR amplified using barcode primers targeting the trnL P6 loop of the plant chloroplast genome with five replicates for each extract (Table 4.1). A barcode targeting the 16S region of the mammalian mitochondrial genome was also amplified and sequenced from all Jenny Lake and Taggart Lake samples to validate the beaver presence results from the species-species qPCR assay. Metabarcode libraries were generated using a two-step protocol (Nichols et al. 2018) with an initial metabarcoding PCR followed by a second indexing PCR to attach unique dual indexing primers. Libraries were quantified with a Qubit and pooled in equimolar volumes for sequencing on an Illumina NextSeq 2x150 run, aiming for 50,000 reads per library. Sequencing reads were trimmed and processed with the Anacapa QC pipeline, then clustered as Amplicon Sequence Variants (ASVs) and ASVs assigned to taxa with the Anacapa CRUX pipeline (Curd et al. 2018). ASV assignments with a 60% Bayesian Confidence Cutoff were retained, following established methods (Curd et al. 2018, Lin et al. 2021). We used the decontam package in R (v1.12) (Davis et al. 2018) to compare taxonomic composition of samples and negative controls and remove any observed contamination. Following filtering, replicate libraries were merged, samples with fewer than ten reads were removed, and raw ASV counts for each sample were converted to relative abundance for downstream analyses of taxonomic abundance and beta diversity. Taxonomic abundance was visualized with the Phyloseq R package (McMurdie and Holmes 2013). Al-

pha diversity analyses were performed on unmerged sample replicates with Phyloseq. Compositional change in taxonomic assemblages was assessed using Nonmetric multidimensional scaling (NMDS) ordination based on Jaccard similarity of the relative abundance data using the vegan R package (Oksanen et al. 2019).

4.4 Results

4.4.1 sedaDNA

We extracted and analyzed 51 sedaDNA samples from three Teton lake cores spanning the last 10 ka; 20 each from Taggart Lake and Jenny Lake, and 11 from Lake Solitude. Vascular plant sequencing using the trnL barcode yielded an average 259,541 reads and 50 identified genera per sample after quality filtering and merging replicates. 16SmammP007 libraries were generated for 9 samples from Taggart Lake and 15 from Jenny Lake, with an average of 36,732 reads and 4.5 identified genera per sample.

4.4.2 Beaver detection

In Jenny lake, the species specific qPCR assay first detected North American beavers in the dataset at 7,226 years ago (7.2 ka) and intermittently (10/14 samples) until present thereafter (Fig. 4.2). Beavers were first detected in Taggart Lake at 5,939 years ago (5.9 ka) and were detected continuously from 5.2 ka until presence. There was one detection gap in Taggart Lake at 5.5 ka. Mammalian sequencing with the 16SmammP007 barcode yielded sequences assigned to North American beavers in four samples - three in Jenny Lake and one in Taggart Lake - all of which also had positive beaver detections with the qPCR assay. Beavers were not detected in Lake Solitude, nor any of the

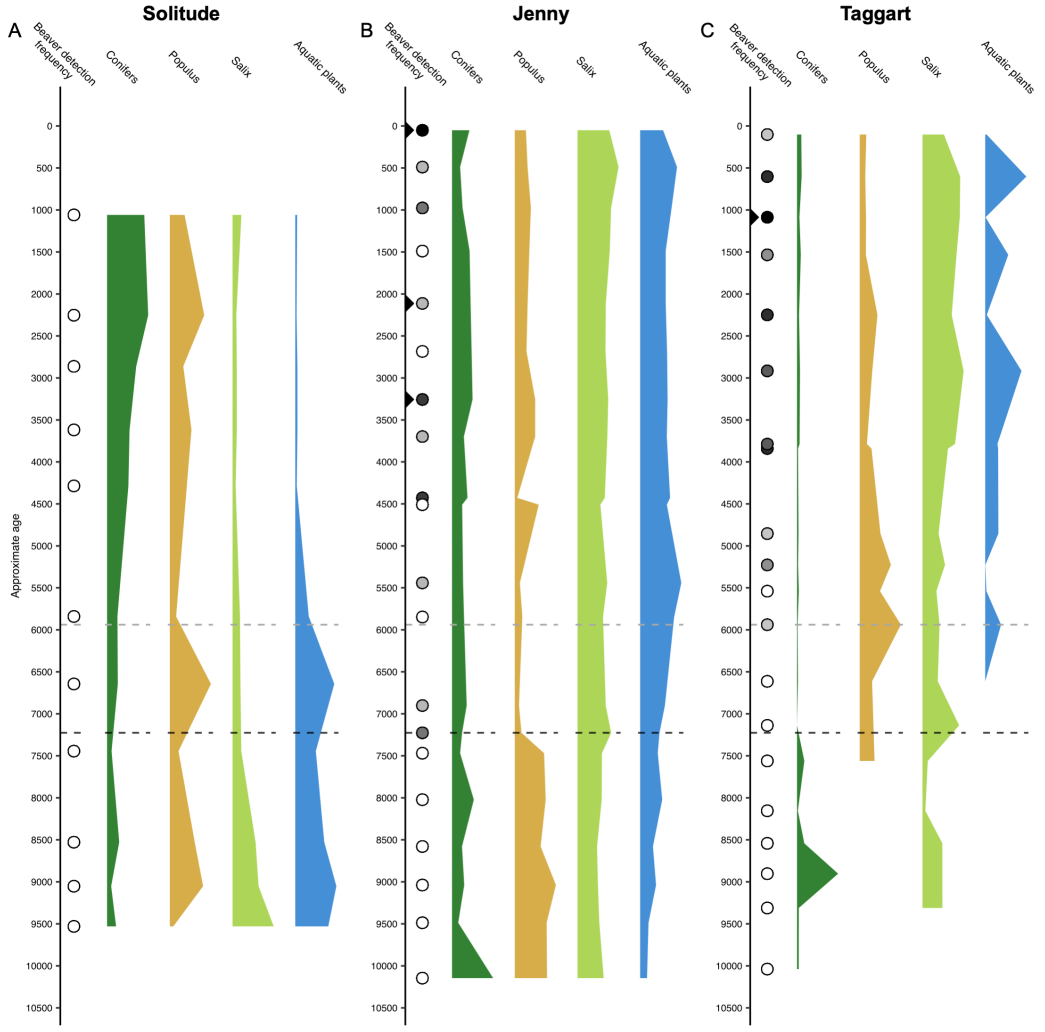

Figure 4.2: sedaDNA results from three Teton lakes. sedaDNA results from three Teton lakes: Lake Solitude (left), Jenny Lake (center), Taggart Lake (right). Leftmost panel for each subfigure indicates frequency of beaver detection across five replicates per sample from the species-specific qPCR assay with darker gray indicating higher detection frequency, black triangles indicate positive beaver detection with the 16SmammP007 barcode. From left to right remaining panels indicate relative frequency of reads per sample assigned to: conifers, Populus, Salix, and aquatic plants, scaled to maximum relative abundance per taxa per lake. Dashed vertical lines indicate first appearance of beavers in Jenny Lake at 7.2 ka (black) and Taggart Lake at 5.9 ka (gray). Conifers includes all reads assigned to families *Cupressaceae* and *Pinaceae*; aquatic plants includes the genera: *Callitriche*, *Myriophyllum*, *Nuphar*, *Nymphaea*, and *Potamogeton*.

negative controls with either the 16SmammP007 barcode or the qPCR assay.

4.4.3 Vegetative trends

To evaluate the interaction between beavers and the local environment over time, we investigated trends in plant assemblages with a particular focus on taxa known to be associated with beavers (Fig. 4.2). In Taggart Lake, regional beaver arrival in the mid-Holocene is associated with a decrease in relative abundance of conifers and in increase in *Salix* (willows). The first detection of beavers in Taggart Lake coincides with the first detection of aquatic plants, which persist thereafter and become more abundant in the later Holocene. The first detection of *Populus* (e.g., poplar, aspen, cottonwood) in Taggart Lake slightly precedes the first regional detection of beavers; *Populus* relative abundance in Taggart Lake peaks at 5.9 ka when beavers are first locally detected and remains persistent throughout the remainder of the Holocene. *Salix* and aquatic plants also increase in Jenny Lake after beavers are first detected, similar to Taggart although to a lesser extent. Trends in conifers relative to beaver arrival in Jenny Lake are less clear and - in contrast to Taggart Lake - relative abundance of *Populus* is high and steady in the early Holocene, declining sharply at 7.2 ka when beavers are first detected, and increasing again in the later Holocene. Lake Solitude displays almost opposite taxonomic trends to the lower elevation lakes, with initially high levels of aquatic plants, *Salix*, and *Populus* declining in the mid Holocene, coinciding with an increase in conifers.

We measured vegetation alpha diversity in each trnL sample using observed taxonomic richness and Shannon's and Chao1 diversity indices. Shannon's diversity index considers taxonomic evenness as well as richness which effectively skews away from rarer taxa in the dataset; on the other hand Chao1 is a non-parametric method that skews towards rare taxa (Kim et al. 2017). Diversity generally increased over time in Taggart and Jenny Lakes, while remaining

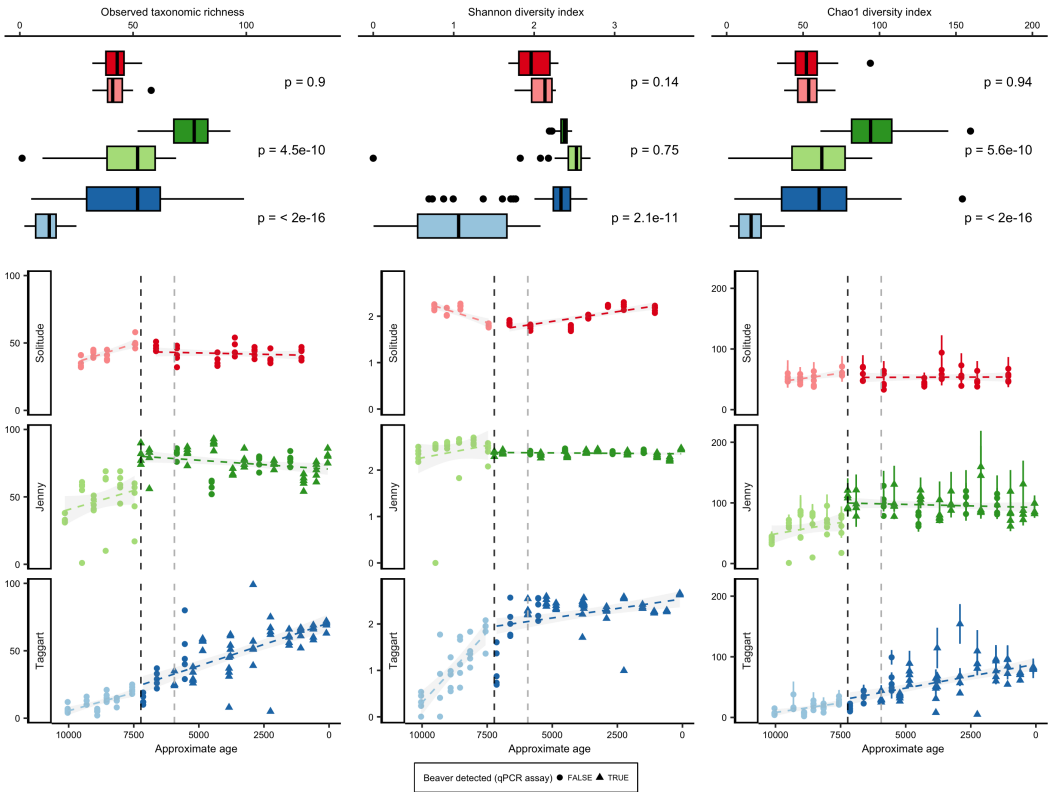

Figure 4.3: Alpha diversity indices for all trnL sample replicates over time (bottom) and means before and after beaver colonization at 7.2 ka compared (top) for each study lake (top to bottom: Solitude, Jenny, Taggart). Lighter shade indicates pre-beaver time period. Diversity indices left to right: Observed, Shannon, Chao1. Dashed vertical lines indicate first appearance of beavers in Lake Solitude at 7.2 ka (black) and Taggart Lake at 5.9 ka (gray).

stable in Lake Solitude (Fig. 4.3). We compared average diversity as measured by these three indices before and after the first detection of beavers at 7.2 ka. Diversity was significantly higher after 7.2 ka across all three indices in Taggart Lake, and in two of the three indices for Jenny Lake. Shannon diversity decreased slightly but non-significantly in Jenny lake after 7.2 ka indicating a slight decline in taxonomic evenness. Diversity was generally low in Lake Solitude and did not change significantly before and after 7.2 ka for observed richness or Chao1 diversity, however there was a significant decrease in Shannon diversity.

Nonmetric multidimensional scaling (NMDS) analysis of the trnL relative abundance data yielded a minimum stress of 0.13, indicating good represen-

tation of the data by ordination. One outlier (the 10 ka sample from Taggart Lake) was removed from the NMDS plots for better visual representation. Samples plotting closer together in NMDS space indicates more similar plant communities. The biplot in Figure 4.4A demonstrates mid-Holocene regime shifts for all three lakes coinciding with the first detection of beavers; however, both Taggart and Jenny Lakes trend towards more positive MDS values, suggesting increasingly similar plant communities, whereas Lake Solitude shifts in the opposite direction. *Salix* and the majority of aquatic plant genera fall in the upper right quadrant of the NMDS plot with most of the post-beaver arrival Jenny and Taggart Lake samples, whereas most conifer genera plot on the right side of the plot with the Lake Solitude samples. When the MDS axes are plotted over time, Lake Taggart shows a strong shift in NMDS space associated with the first detection of beavers; changing from a negative temporal trend on MDS axis one before regional beaver arrival to a positive trend afterwards and more similar to Lake Solitude (Fig. 4.4B). MDS axis two shows a more consistent positive trend over time among all three lakes, although with a greater amplitude shift in Taggart Lake (Fig. 4.4C). PERMANOVA confirmed significant differences in plant communities before and after the first beaver detection for all three lakes, considered both separately and together (Table 4.2).

4.5 Discussion

Using sedaDNA techniques we investigated beaver presence and vegetation diversity over the last 10 ka in three lakes in Grand Teton National Park, a region with a dynamic and well-described paleoclimatic history. We detected beavers in 21 lake sediment core samples up to 7.2 ka with a species-specific probe-based qPCR assay developed by Smith and Goldberg (2022). Although

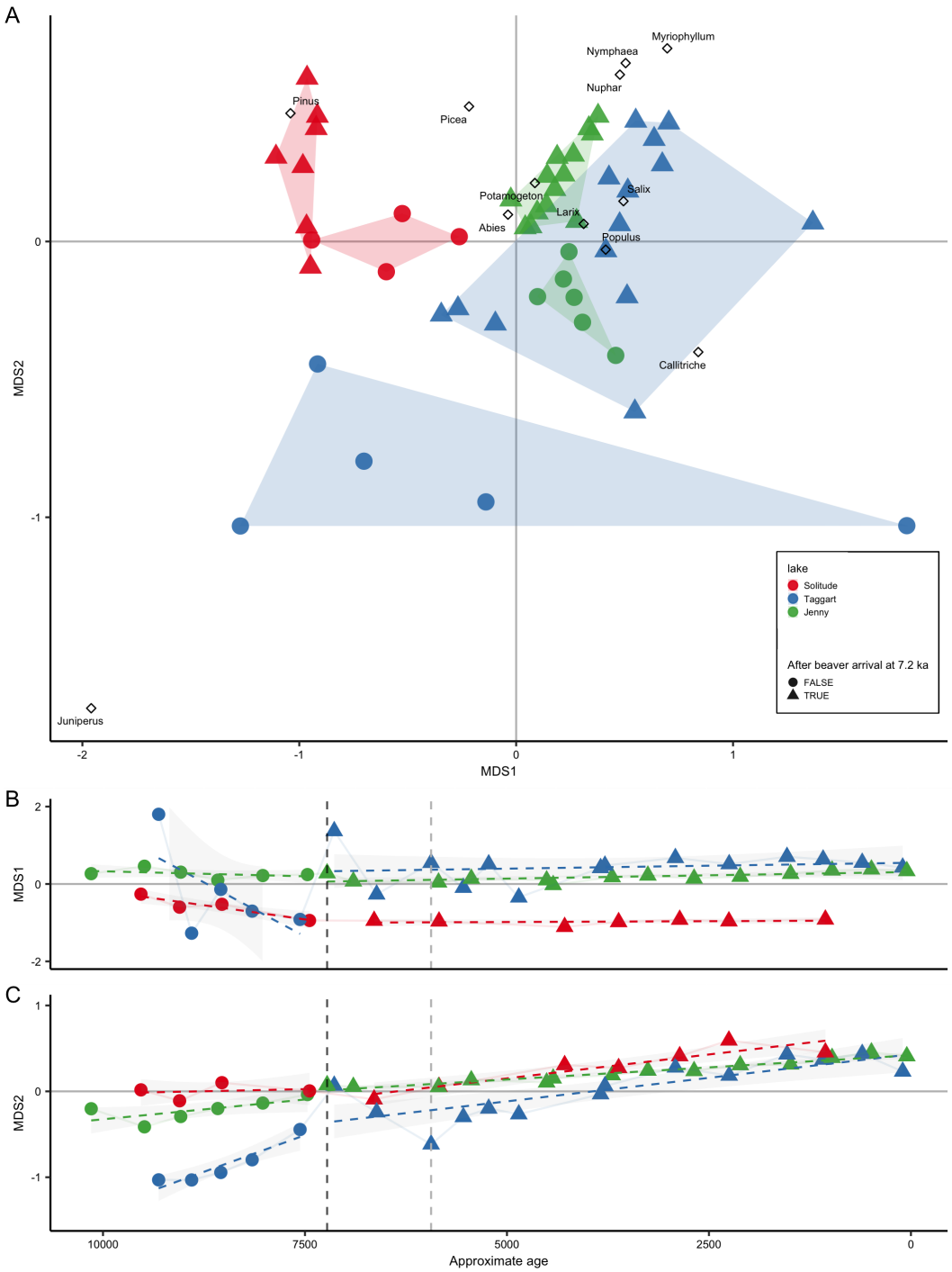

Figure 4.4: trnL beta diversity based on Jaccard similarity. A) Nonmetric multidimensional scaling (NMDS) axes 1 and 2 biplot with trnL samples (colored); and beaver-associated genera as in figure 2 (diamonds). B) MDS axis 1 and C) MDS axis 2 over time; trend lines for each lake and time period. Sample colors indicate lake and shape indicates time period (before or after beaver colonization); dashed vertical lines indicate first appearance of beavers in Lake Solitude at 7.2 ka (black) and Taggart Lake at 5.9 ka (gray).

this assay was developed for modern eDNA applications, the high rate of detection indicates that it is a sensitive method for detecting the past presence of beavers in ancient sediments. A general mammalian metabarcoding assay was much less effective, with sequences assigned to beavers in only four samples, with a maximum detection age of 3.3 ka. All four of these samples also had positive detection with the qPCR assay, supporting its accuracy.

Using sedimentary analyses, Persico and Meyer (2013) found sporadic evidence of beaver activity in multiple stream beds in Grand Teton National Park in the early Holocene, with more consistent detection in the later Holocene. These findings largely agree with our results here, lending support to the validity of this novel sedaDNA methodology. Specifically in Beaver Creek, which outflows from Taggart Lake, the authors first detected beaver-pond sediments at ∼6 ka, as we did here. Many of the beaver detection gaps that we found in Jenny Lake are temporally similar to those found by Persico and Meyer, suggesting that these are real absences related to climatic and ecological changes. Furthermore, we tended to detect beavers at higher within-sample rates in time periods where Persico and Meyer also found highest levels of beaver activity.

Persico and Meyer found two instances of beaver activity in this region at 8 and 10 ka, earlier than we detected beavers in this study. It's possible that beaver presence was too sparse and/or sporadic for us to detect it in our sampling, or that beavers were not active above Taggart or Jenny Lakes at this time although they were active in nearby streams. However, it is also possible that the qPCR assay was limited by DNA degradation in older samples. The assay we used amplifies a 90 bp DNA fragment, whereas ancient DNA is commonly 60 bp or shorter and consequently most ancient DNA-specific metabarcodes and other assays target short fragments. As such, the 7.2 ka beaver arrival time may reflect a methodological limit of detection rather than a biological reality. However, a shorter (60-84 bp) mammalian metabarcode

did not identify beavers in any older samples, supporting the validity of the assay results. Future studies will explicitly test the temporal limits of this assay and potentially optimize shorter assays that may be more suitable for older and more degraded samples.

Our results suggest that during the Holocene, beavers first arrived to the Jenny Lake ecosystem no later than 7.2 ka and to Taggart Lake 5.9 ka, and were at least intermittently present in Jenny Lake throughout the remainder of the Holocene but were continuously present in Taggart Lake from 5.2 ka to present. The transition to non-glacial conditions in the Tetons began towards the beginning of the Holocene, approximately 11.5kya, as indicated by higher organic content and higher incidence of plant material in the sediment (Larsen et al. 2016). The mid-Holocene was a time of environmental change in the Tetons, with increased winter precipitation and cooling, driving high elevation glacial growth and raising regional moisture balance beginning around 6 ka (Larsen et al. 2020). Wetter conditions may have made the Cascade and Avalanche Canyons more hospitable to beavers, or increased riparian connectivity between the nearby Snake River and these lake systems, facilitating beaver movement into these watersheds. While beavers were historically present in high abundance throughout North America, the relatively late establishment of beavers in this region following deglaciation suggests that the spatial dynamics of beavers at the local scale may be quite complex.

Beavers appear to have arrived to Taggart Lake approximately 1.3 ka later than Jenny Lake, but were thereafter more persistent, with continuous detection in Taggart Lake from 5.2 ka to present while beavers were never continuously detected for more than $\sim$1.3 ka in Jenny Lake. Given the close proximity and similar geology of these two lake systems, it is possible that these discrepancies represent a difference in DNA concentration and/or preservation rather than a true biological difference. Jenny Lake is larger and deeper than Tag-

gart Lake and any eDNA in the system would therefore be more dilute and less detectable. Beavers are less likely to occupy lakes than rivers (Slough and Sadleir 1977), so it is likely that the DNA signal detected here was transported into the terminal lakes from upper tributaries, further diluting the DNA signal. Cascade Canyon is longer and wider than Avalanche Canyon, providing more opportunities for DNA dilution. However, similar detection dynamics found by Persico and Meyer (2013) suggest that we may instead be documenting fine scale spatial and temporal dynamics of beaver activity in this region, with detection gaps in Jenny Lake corresponding closely with periods of reduced regional beaver activity identified by Persico and Meyer (2013) and attributed to drought.

Mid-Holocene plant community regime shifts are apparent in all three lakes coincident with beaver arrival. Based on relative abundance trends and beta diversity, Taggart Lake shows the strongest evidence of a mid-Holocene regime shift associated with beaver arrival, moving from a conifer dominant to a more riparian system. Beaver-associated plants were either sporadically present (poplars and willows) or absent (aquatic plants) until beaver arrival, and then consistently present thereafter. Alpha diversity also significantly increased after beaver arrival across all measures - consistent with predictions based on modern studies of how beavers influence plant diversity. The sustained detection of beavers of in Taggart Lake from 5.2 ka until present suggests that beaver ecological engineering may have manipulated the environment in/around Taggart Lake enough to allow them to persist and maintain wetlands through periods of extended drought in the late Holocene that appear to have greatly reduced beaver abundance in nearby areas (Persico and Meyer 2009, 2013). The vegetation trends support this hypothesis, with beaver food sources such as *Populus*, *Salix*, and aquatic plants becoming much more consistent in Taggart Lake after beaver arrival - although aquatic plants undergo periods of

decline at 2.2 and 1.1 ka, presumably as a result of these droughts. Beavers are known to "plant" their food sources, creating the ecological conditions necessary for these plants to survive. The persistence of beavers and riparian plant communities through extended late Holocene droughts in an encouraging finding for beaver restoration, as it suggests that beaver activity may be able to maintain highly resilient watersheds that could provide refugia for plants and animals as the climate continues to change.

Jenny Lake shows largely similar vegetative trends as Taggart Lake but to a lesser degree. A notable difference is a sharp decrease in poplar relative abundance coinciding with beaver arrival. We can speculate that as a favored food source poplars were initially depleted by beaver arrival, but we do not have sufficient evidence to confirm this. We can attribute the differences in plant community trends between Jenny and Taggart Lakes to the relative sizes of these two systems. Jenny Lake is much larger and deeper and captures a larger area, indicating a different set of controlling factors for both sedaDNA deposition and the aquatic and terrestrial communities. It is possible that as a smaller system, Taggart Lake is more sensitive to change and beavers therefore have a stronger controlling effect on structuring the plant community. This may explain why beavers remain present in Taggart Lake while disappearing from Jenny Lake during periods of presumed drought or other ecological stress.

Consistent with our predictions, we found no evidence of beavers in Lake Solitude, which is located in a much steeper and higher elevation cirque valley near treeline. While beavers are capable of inhabiting high elevations and gradients, this environment represents more marginal habitat (McComb et al. 1990, Gurnell 1998). Despite no evidence of beavers, Lake Solitude also demonstrates a mid-Holocene vegetation regime shift albeit in an opposite direction from the lower elevation lakes, with increased conifer abundance and decreased riparian taxa. It is likely that this trend is attributable to the

neoglacial expansion and increase in precipitation occurring at this time. These changes would have created harsher conditions and a shorter growing season at high elevations while increasing the water available at lower elevations. Despite taxonomic compositional change, Lake Solitude showed little change in taxonomic richness over time, in contrast to the lower elevation lakes. This could be taken as evidence that beavers are driving these trends in richness, but it could also be that many taxa are limited by the altitude and generally harsh environment of Lake Solitude.

Taken together, the metabarcoding results of these three lakes suggests that a climatic shift in the mid-Holocene facilitated beaver establishment in the Jenny Lake and Taggart Lake drainages and likely contributed to coincident changes in the plant community. Paleoclimatic records indicate that regional winter precipitation and consequently lake levels increased at this time. It is difficult to determine to what degree the mid-Holocene regime shifts apparent in these lake system plant communities are attributable to beaver activity, rather than climatic shifts occurring at the time simply facilitating beaver establishment as well as plant community changes. Repeating similar studies of past beaver activity in new geographic locations with similarly well-described paleoclimate histories will provide a clearer picture of the role of beavers in structuring local ecosystems throughout the Holocene.

Additionally, beavers are known to shift range in response to large-scale climate change and have occupied most parts of the North American continent over the last 7 million years - the age of the oldest beaver fossil found. While we determined that beavers arrived into the GTNP system approximately 7.2 ka, it is likely that they were present in previous warm periods when suitable habitat was available as well. Reconstructing deeper time beaver population dynamics will require longer cores and further investigation into the methodological limits of ancient beaver sedaDNA detection. In the context of modern

beaver management, however, understanding the spatiotemporal distribution of beavers during the Holocene and their response to recent climatic disturbance and anthropogenic stressors is most relevant.

We found that a qPCR assay applied to sedimentary samples is a powerful and reliable molecular method for detecting the past presence of beavers at the watershed scale in the absence of physical evidence. qPCR is faster, less expensive, and more analytically straightforward than other ancient eDNA methodologies such as metabarcoding or shotgun sequencing. The novel application of this molecular tool provides the opportunity to detect past beaver activity in a wide variety of settings without relying on sparsely distributed physical fossil or sedimentological evidence. A clearer picture of when and where beavers were active in the past can provide key insights as to how this environmental engineer may contribute to landscapes and ecosystem development. Furthermore, understanding the past temporal and spatial distribution of beavers can inform restoration and conservation efforts and help land managers better predict the effects of beaver engineering over long time scales and through changing climates.

4.5.1 Conclusions

Using a species-specific qPCR assay, we detected beaver sedaDNA in lake sediment samples up to 7.2 ka years old, demonstrating a sensitive method for documenting the historic presence of beavers in a watershed without the need for physical evidence. Our findings show over five thousand years of continuous beaver presence at the watershed scale in Grand Teton National Park, suggesting that this ecosystem engineer is an established and integral part of the local landscape. Our results largely agree with previous evidence of nearby beaver activity from sedimentary proxies (Persico and Meyer 2013), supporting our

conclusions and suggesting that this sedaDNA assay is capable of reconstructing fine scale spatial and temporal dynamics of beaver activity. Our results suggest that beavers colonized Taggart and Jenny Lakes in the mid-Holocene, during a period of increased regional precipitation and water balance. Evidence of regime shifts in the local plant community co-occur with the establishment of beavers although questions remain as to what degree beavers were driving vs responding to local climate and ecosystem dynamics. Although beavers appear to be absent or greatly reduced in the Jenny Lake system during periods of regional drought in the late Holocene they remain consistently present in the Taggart Lake, perhaps as a result of intensive ecological engineering at the local scale. This sustained presence of beavers through persistent (multi-century) droughts indicates that under certain conditions beavers may be able to maintain wetlands through periods of climatic stress, providing refugia for plants and animals and buffering the effects of climate change at the local scale. A better understanding of regional beaver dynamics during periods of historic climate change will provide a clearer picture of how common this may be and what conditions beavers need in order to maintain continuous presence. These results shed light on the role of beavers in North American paleoclimates and may help land managers more effectively deploy beaver engineering as a climate mitigation strategy.

Synthesis

The degree of genomic diversity of a species, how that diversity is partitioned over space, and how it has changed over time are critical aspects that inform the continued viability of a species in a changing environment. Once restricted to humans and model species, decreased costs of next generation sequencing and improved analytical methods have enabled genomic studies of threatened and endangered non-model species, contributing to more effective conservation and management.

In this dissertation I generated new genomic data and provide insights into four aquatic mammals, each of which have unique natural histories and conservation needs.

In chapter one, I used dense spatial genomic sampling to understand the distribution of diversity and inbreeding in southern sea otters. I showed that southern sea otters are less diverse than their northern sister subspecies across all measures, likely a legacy of their long term isolation at the southern end of the sea otter range, multiple bottlenecks, reduction to a single small population by the maritime fur trade, and the current environmental constraints of their environment. My results indicate that although southern sea otters have little spatial variation in neutral genomic diversity, rates of inbreeding and genetic load are significantly higher in the northern part of their small range and that this pattern is not a function of population density. These results highlight the vulnerability of southern sea otters - as they are currently a single population

and cannot expand their range naturally - and underscore the importance of a metapopulation structure in maintaining and improving the genetic diversity of the species. Translocations of southern sea otters to northern California and Oregon are likely necessary to restore a metapopulation structure. Furthermore, given the ecological importance of sea otters, improving the outlook for southern sea otters is critical to maintaining the viability of coastal kelp forest ecosystems at their more southerly range as the climate continues to change.

In chapter two, I assembled a highly contiguous reference genome for the dugong using an individual from the Moreton Bay population in eastern Australia. While a single genome is insufficient to represent the full diversity of this wide-ranging species, it provides initial insights into the demographic history and diversity of a centrally-located population and will serve as an important resource for future studies. I showed that dugongs have relatively high genome-wide heterozygosity compared to other Vulnerable mammals and that they have a dynamic demographic history that likely reflects Pleistocene glacial cycles and resulting sea level change. Future whole genome resequencing studies will provide useful insights into more recent dugong demographic history, as well as how neutral and adaptive variation are partitioned across their large, but discontinuous geographic range, allowing for more targeted management strategies.

In chapter three, I use whole genome sequencing from historic Alaskan and Russian polar bears to investigate two main questions: 1. How do polar bears from understudied Russian subpopulations fit in the range-wide diversity of the species? And 2. How has Alaskan polar bear diversity changed over the past 150 years in response to human hunting and climate change? For question 1. I found that despite broad geographic sampling, polar bears from across Russia are closely related to each other and to historic Alaskan bears, with some degree of isolation by distance. This result agree with earlier findings,

which indicate that polar bear population structure is highly heterogeneous and is driven more by ice types and habitat variability than geographic distance. For question 2. I found that Alaskan polar bear genomic diversity has declined significantly over the past 150 years, with the majority of diversity loss occurring in the second half of the 20th century. The extent to which this decline is due to hunting - which was not fully regulated until the 1970s - versus the effects of climate change is not clear and will require further investigation. I also found evidence for a potential population replacement in Alaska in the second half of the 20th century, likely due the same abundance decline that caused the observed loss of diversity. Future studies should use historic and modern sampling from multiple regions within the Arctic to determine whether this pattern of diversity loss and population identity change is restricted to Alaskan polar bears or is more widespread, and determine the source of this potential population replacement in Alaska. My findings for Alaskan bears complicate the findings for Russian bears - historic Russian samples may not be representative of contemporary individuals and obtaining contemporary Russian polar bear data is a pressing concern. More broadly, a clearer understanding of how human exploitation and climate change have already changed the Arctic and its species will help guide management actions going forward and may help highlight the urgency of protecting this delicate ecosystem.

In chapter four, I expanded beyond a single species focus to a more holistic paleoecosystem approach by using sedaDNA techniques to investigate the arrival and persistence of beavers in Grand Teton National Park over the last 10 ka and their interactions with the local climate and vegetation. My findings show that beavers arrived surprisingly late to this region following Pleistocene deglaciation, but thereafter persisted at the watershed scale for the last ∼5 ka, despite periods of environmental change and extended regional drought. Their

arrival coincided with a regional mid-Holocene neoglacial advance, likely due to increased water availability. Beaver arrival was also associated with a shift from a more coniferous vegetation regime to increased riparian vegetation and higher vegetative diversity. Determining the relative contribution of beavers versus climate in structuring the local plant community will require further study. These results suggest that under certain conditions, the positive effects of beaver engineering on local ecosystems may persist over millennia despite drought and other environmental changes, an encouraging finding that suggests that beaver restoration may be an effective long term solution for providing ecosystem resilience and mitigating the effects of climate change. Future studies will provide a deeper understanding of the geographic and temporal distribution of beaver engineering in the past and the long-term functioning of beaver-modified ecosystems, including their resilience to drought, fire, and other disturbance.

These chapters provide novel insights into the genomic diversity of these four species, and improved understanding of their spatial and temporal variation, particularly the effects of human exploitation and past and present climate change. Additionally, I have generated high-quality genomic resources which will be made publicly available and will contribute to future studies. Whole genome sequencing data is highly valuable in that it remains forward-compatible, so genomic datasets will become increasingly useful for conservation as ongoing contributions continue to build the spatial and temporal sampling for threatened and endangered species and new analytical techniques are developed. The high temporal and spatial resolution of genomic sampling in some of my chapters leads to new insights; for example, in chapter 1 dense spatial sampling showed the fine-scale geographic variation in southern sea otter inbreeding, and in chapter 2 a 150 year genomic time series revealed a diversity decline in Alaskan polar bears. Furthermore both of these chapters

build upon existing genomic resources - reference genomes and resequencing data - highlighting the importance of publicly available whole genome data.

A useful future direction for the species studied here would be a landscape genomics approach: the relationship between functional genomic variation and local environmental variables - both biotic and abiotic. Landscape (and seascape) genomics are becoming increasingly feasible for threatened and endangered species as genomic and environmental resource availability continues to improve. Genomic diversity of a species both results from and contributes to the local environmental variation and landscape genomics can help us move beyond a single-species approach to understanding how ecosystems function as a whole. Landscape genomics can be particularly useful in marine and aquatic ecoysystems which tend to be more dynamic than their terrestrial counterparts and contain more cryptic variation. Holistic ecosystem conservation and management will become progressively more important as climate change continues to affect ecological communities in new and potentially unexpected ways. The genomic data generated for southern sea otters in chapter one will contribute to the California Conservation Genomics Project (CCGP), a unique multi-species landscape genomics initiative (Shaffer et al. 2022). This is an exciting future direction that promises to provide new insights that will shape ecosystem-scale conservation and management of California biodiversity. A multi-species landscape genomic approach similar to CCGP could be incredibly useful for the Arctic, as this environment is unique, highly dynamic, and existing interactions are changing quickly with climate change. For the dugong, landscape genomics could shed light on local adaptation across their large range and provide more insight into their important ecological role within seagrass communities. Little attention has been paid to beaver genomic variation, but given that they, like the dugong, occupy a broad geographic range and a wide variety of ecosystems, it is possible that they may be locally adapted

and that their ecological role is somewhat variable as a result.

Improved temporal sampling and historic approaches will also improve understanding of how ecosystems have changed in response to and alongside changing abundance and ranges of these species. This could include single-species ancient and historic DNA approaches, but also multi-species investigations including sedaDNA and other methods for reconstructing paleoecosystems. Chapters 3 and 4 demonstrate the value of a historic perspective, providing insights into past genomic diversity and ecosystem change not possible with contemporary sampling alone. An ongoing problem in conservation biology is a lack of understanding of what precisely the goal is: species and ecosystems are dynamic and it is not always clear what the baseline is that we are trying to preserve or restore. Historical reconstruction methods using ancient DNA and sedaDNA can help clarify these baselines - showing us a sometimes sobering picture of how past human exploitation has impacted biodiversity and providing a clearer blueprint of what our conservation goals should be.

The value of genomics to conservation has not always been a given (Ouborg et al. 2010, McCormack et al. 2013, McMahon et al. 2014, Shafer et al. 2014), but I would argue that these data can provide critical insights and that as the field matures and more resources are generated, this is becoming increasingly an outdated view. Although genomic data cannot save an endangered species, for better or for worse our societal and political mechanisms for conservation rely on data, and the more knowledge we have the better equipped we are to advocate for species of concern, engage political will for conservation, and inform strategic action. Spatial and temporal genomic data provide us with a richer understanding of the biological world past and present, information necessary for conserving biodiversity and ecosystem productivity in the future.

Appendix A

Appendix

A.1 Chapter 1

A.1.1 Supplementary figures

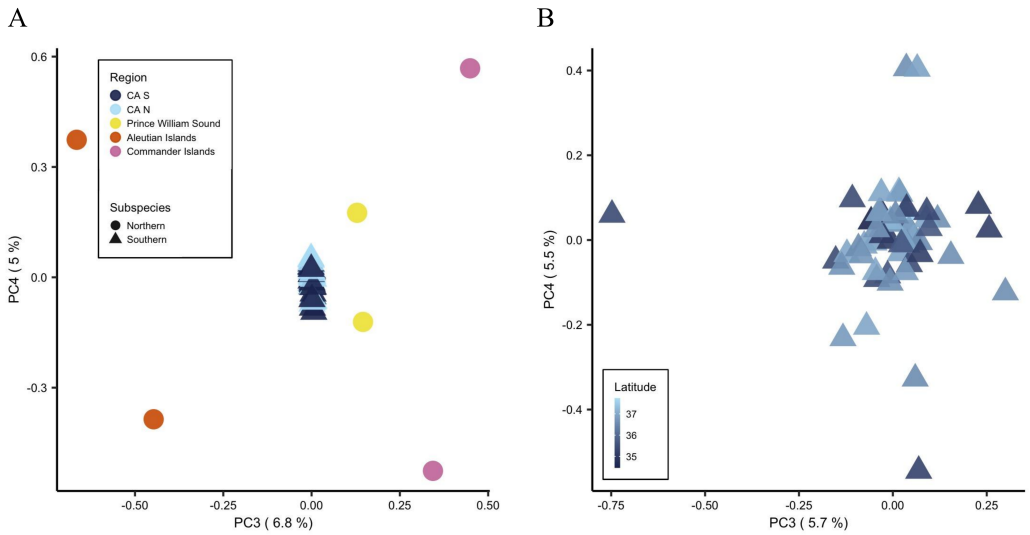

Figure A.1: Principal components 3 and 4 for A) both southern and northern sea otters and B) southern sea otters only.

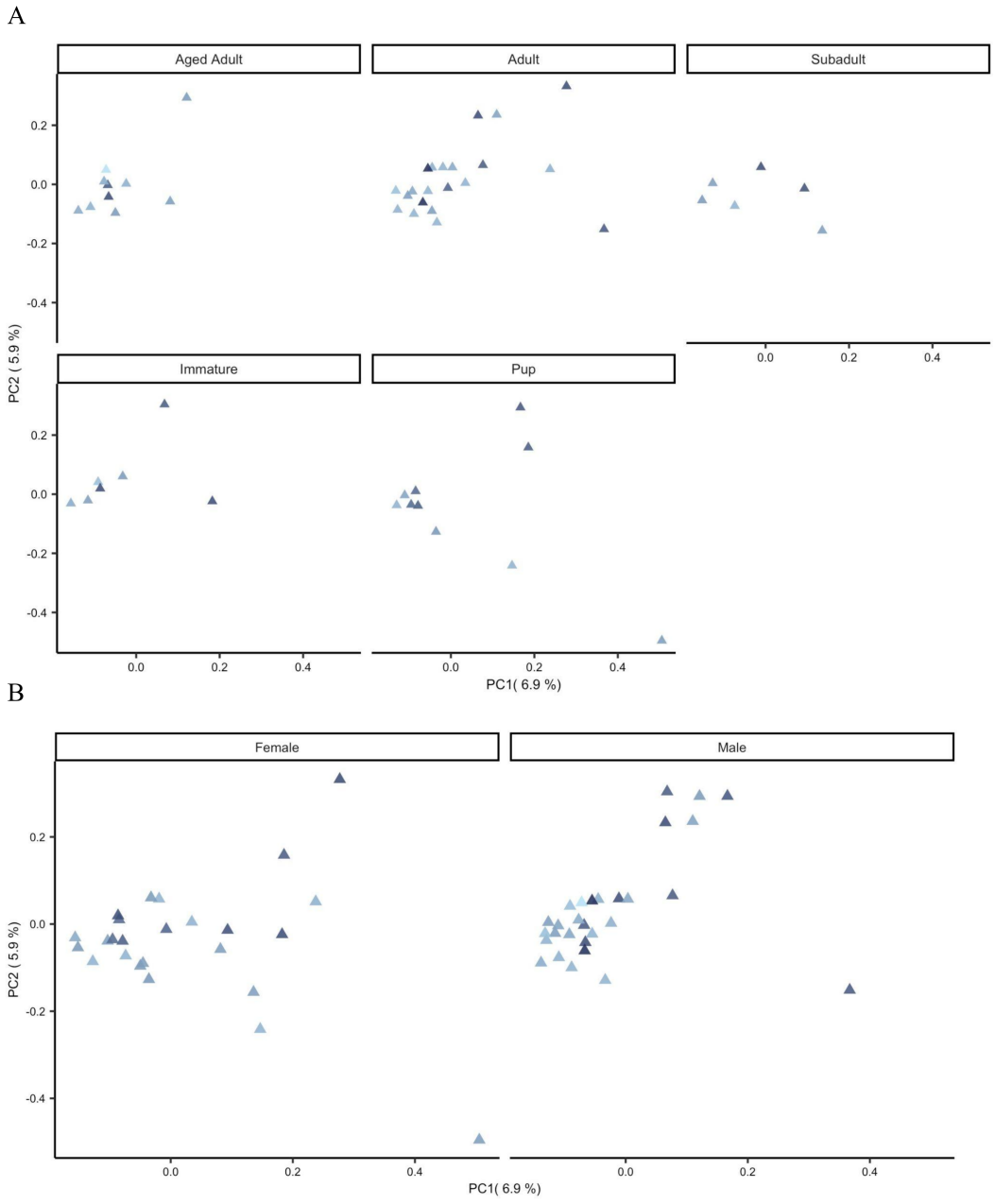

Figure A.2: Southern sea otters principal components 1 and 2 split by A) age class and B) sex.

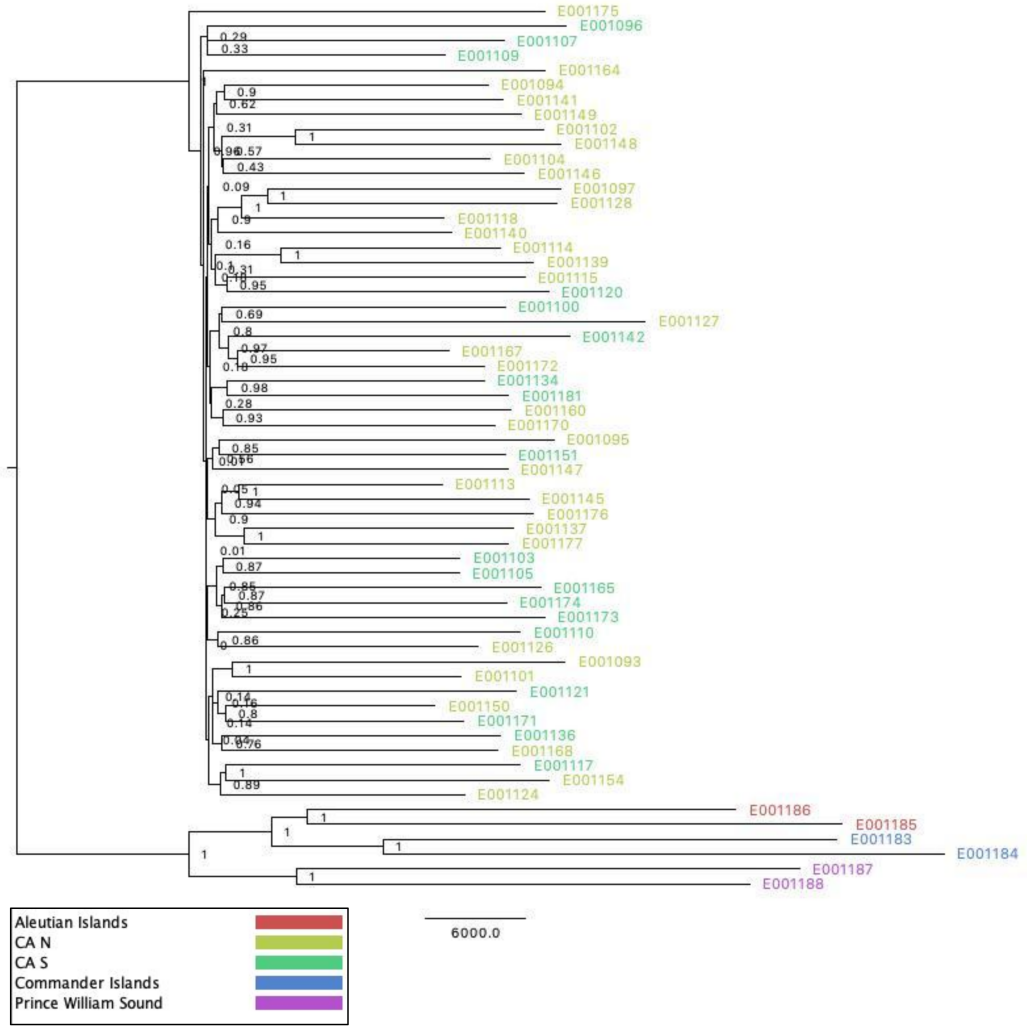

Figure A.3: Maximum likelihood tree for all southern and northern sea otter individuals, colored by region. Bootstrap likelihood values at nodes.

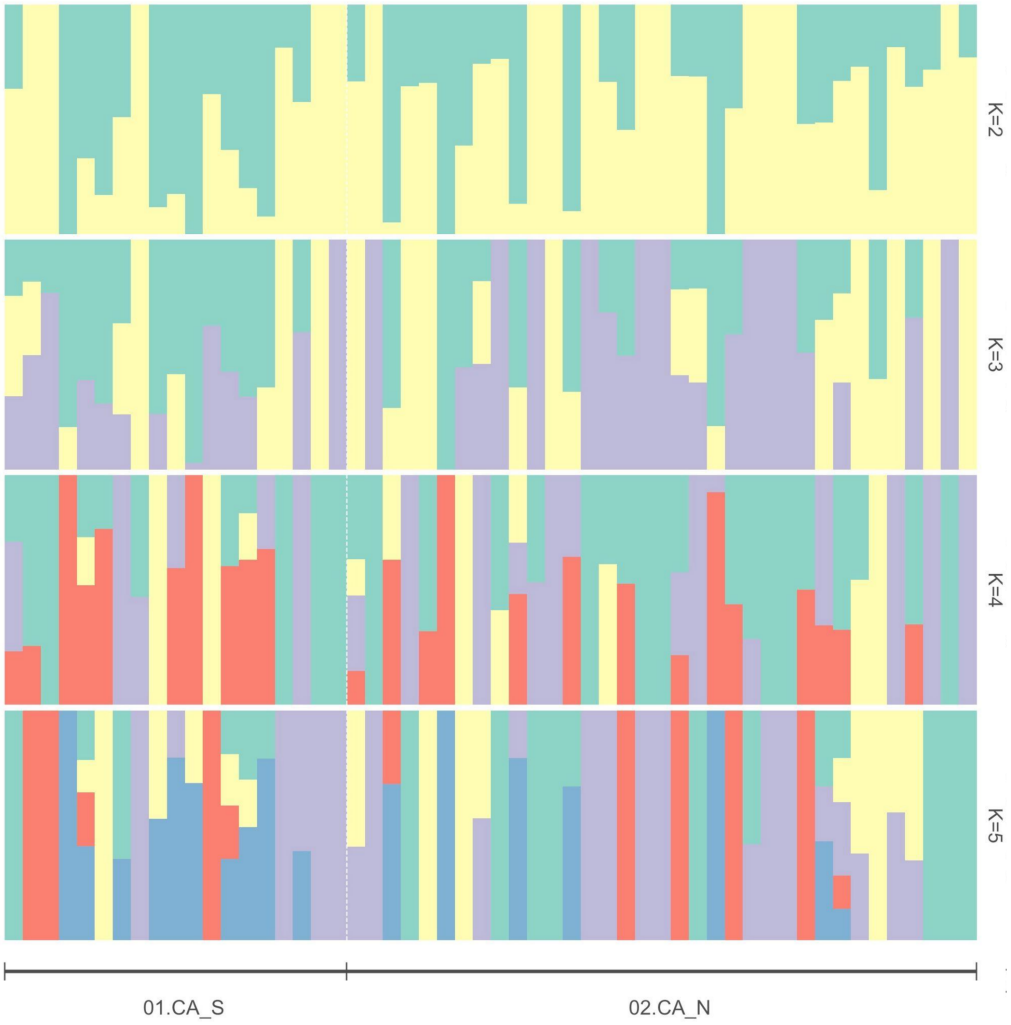

Figure A.4: Southern sea otter ancestry groups for K=2-5. Individuals ordered by ascending latitude of sample site.

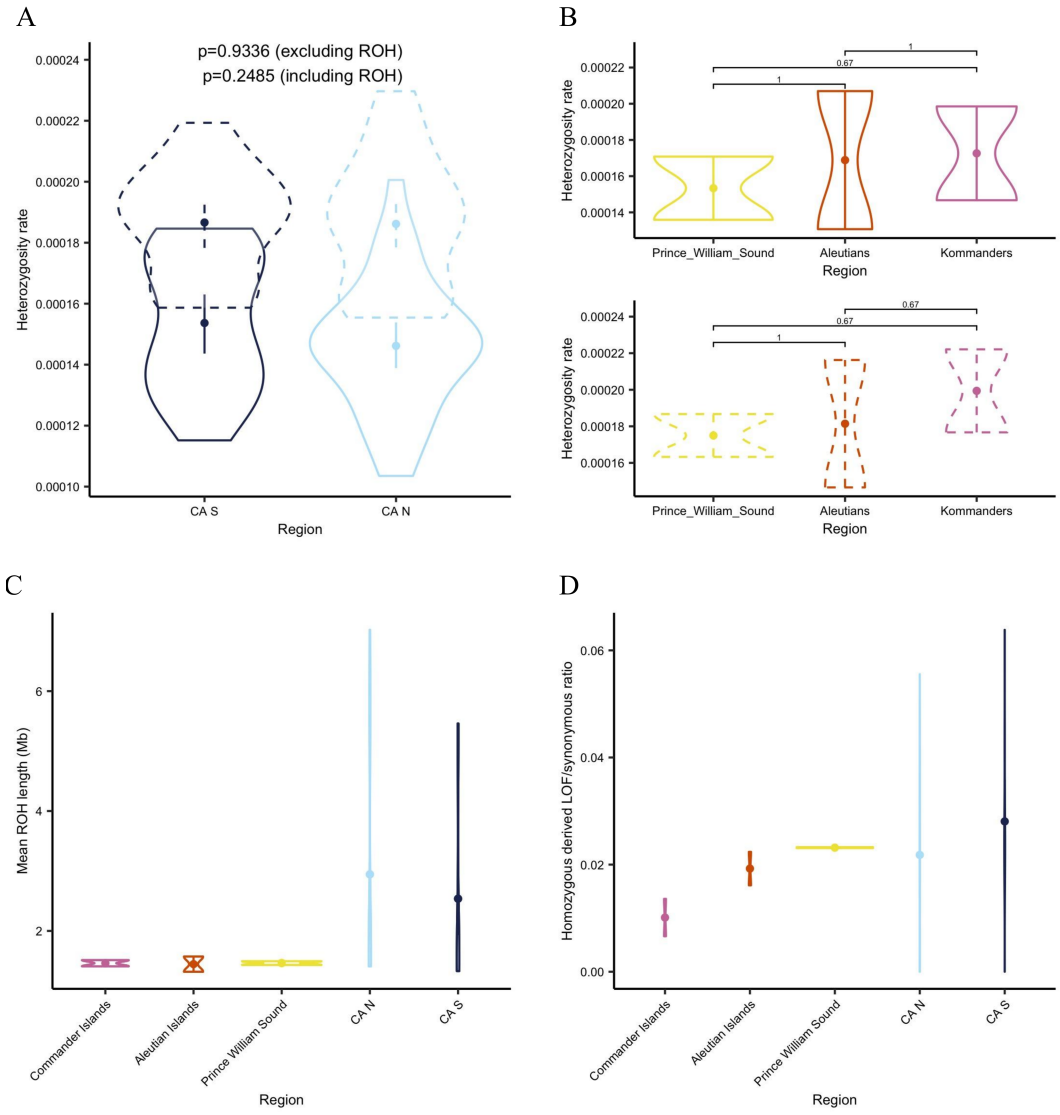

Figure A.5: A) Average heterozygosity between A) northern and southern California within southern sea otters and B) between regions in northern sea otters. including (solid lines) and excluding (dashed lines) ROH regions (faceted in B for better display). C) average ROH length in Mb by region. D) Homozygous LOF/synonymous ratio by region.

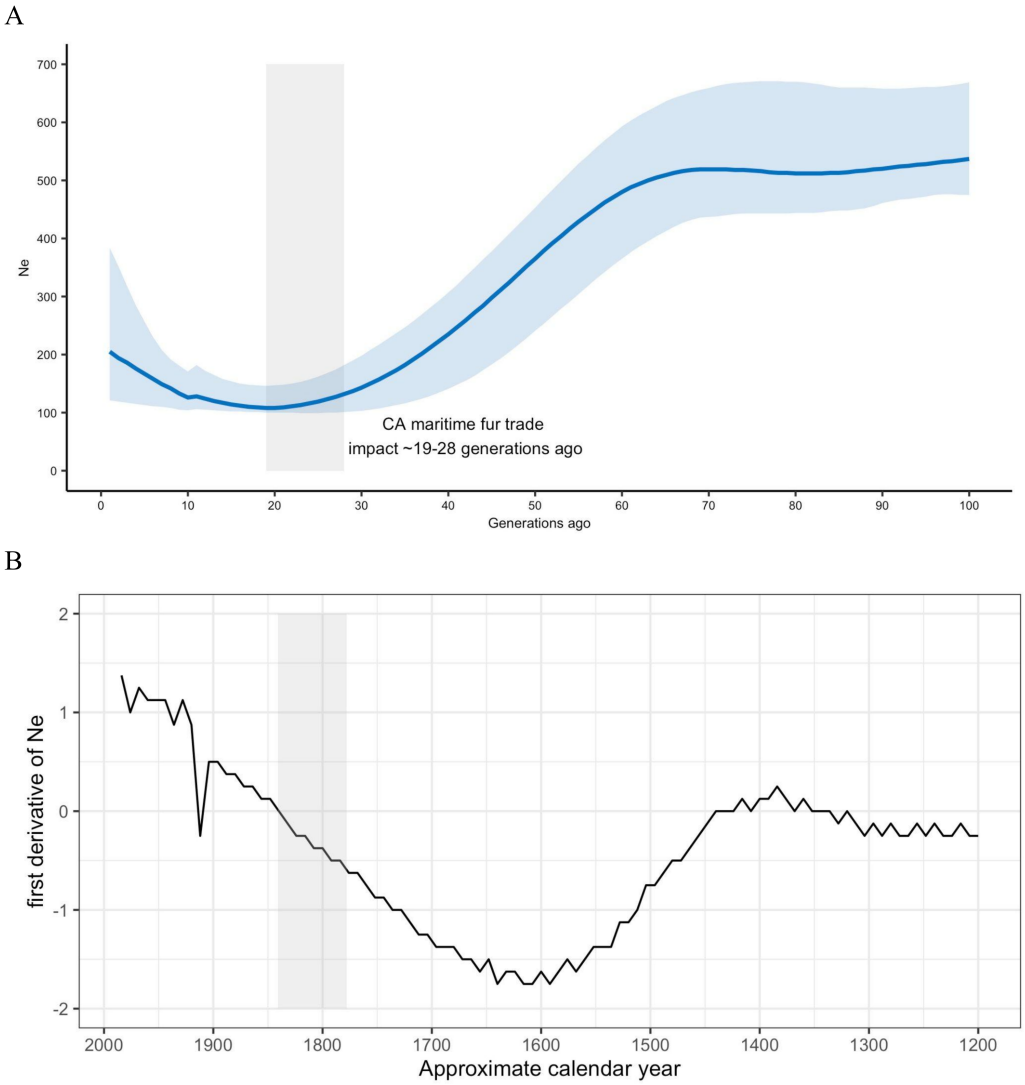

Figure A.6: A) Effective population size (Ne) of southern sea otters over the past 100 generations based on linkage disequilibrium (LD). Lighter band represents 95% confidence interval. Gray rectangle represents approximate time period of fur trade exploitation in California. B) first derivative of A, indicating rate change over time; approximately parabolic shape from ~1450-1900 indicates exponential decline. No change in rate apparent at beginning of CA maritime fur trade (gray bar).

A.1.2 Supplementary tables

Table A.1: Sample information for all sea otter whole genome sequences analyzed in chapter 1.

Subspecies	**Region**	**Sample**	**Coverage**	**Sex**	**Reported Life Stage**
E. lutris nereis	Southern CA	E001109	30.0	Male	Adult
		E001110	12.7	Male	Adult
		E001096	12.0	Male	Aged Adult
		E001103	34.8	Female	Adult
		E001165	14.7	Male	Adult
		E001117	14.2	Female	Immature
		E001134	25.5	Male	Subadult
		E001181	13.5	Female	Immature
		E001142	14.3	Female	Subadult
		E001174	34.3	Male	Pup
		E001107	18.8	Male	Adult
		E001120	11.8	Male	Aged Adult
		E001121	16.2	Male	Adult
		E001136	18.6	Male	Immature
		E001105	42.9	Female	Pup
		E001100	11.6	Female	Pup
		E001151	13.0	Female	Adult
		E001173	12.3	Female	Pup
		E001171	18.7	Female	Pup
	Northern CA	E001114	14.3	Female	Pup
		E001175	8.9	Female	Subadult
		E001115	15.9	Male	Aged Adult
		E001140	38.0	Female	Immature
		E001146	11.0	Female	Aged Adult
		E001154	22.5	Female	Pup
		E001093	11.1	Female	Aged Adult

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Table A.1 – Continued from previous page

Subspecies	Region	Sample	Coverage	Sex	Reported Life Stage
		E001128	11.6	Female	Adult
		E001145	12.2	Male	Immature
		E001097	15.5	Female	Subadult
		E001141	13.5	Male	Subadult
		E001167	31.2	Female	Adult
		E001150	40.5	Male	Adult
		E001168	13.6	Male	Pup
		E001149	13.5	Male	Adult
		E001148	12.0	Male	Adult
		E001172	11.9	Female	Immature
		E001176	10.5	Male	Aged Adult
		E001126	14.6	Male	Aged Adult
		E001094	19.1	Male	Adult
		E001101	42.9	Female	Adult
		E001095	11.7	Male	Aged Adult
		E001104	16.2	Male	Aged Adult
		E001170	12.7	Female	Adult
		E001147	14.0	Male	Pup
		E001102	11.4	Female	Adult
		E001124	34.4	Female	Adult
		E001139	13.6	Male	Adult
		E001177	12.2	Male	Adult
		E001137	14.3	Female	Pup
		E001127	9.4	Female	Subadult
		E001160	15.3	Male	Adult
		E001113	39.2	Male	Immature
		E001164	11.0	Male	Adult
		E001118	40.3	Male	Aged Adult

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Table A.1 – Continued from previous page

Subspecies	**Region**	**Sample**	**Coverage**	**Sex**	**Reported Life Stage**
E. lutris lutris	Prince William Sound	E001188	34.7	Female	Adult
		E001187	12.9	Female	Adult
	Aleutian Islands	E001185	12.4	Female	unknown
		E001186	35.8	Female	unknown
	Commander Islands	E001184	11.4	Male	Adult
		E001183	30.3	Male	Adult

A.2 Chapter 2

A.2.1 Supplementary figures

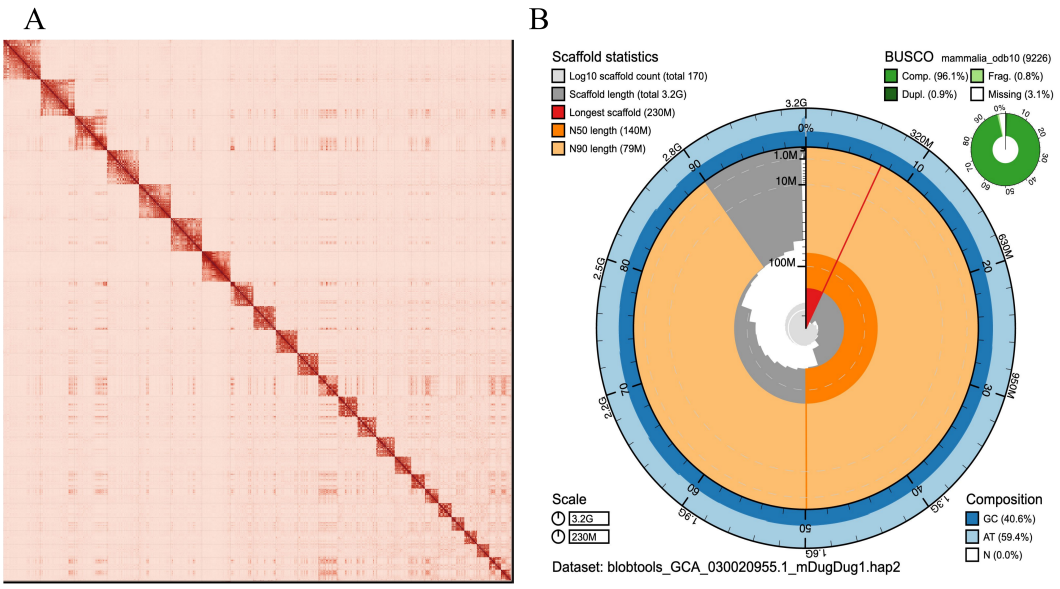

Figure A.7: Visual overview of haplotype 2 genome assembly metrics. (A) Omni-C Contact maps for the haplotype 2 genome assembly generated with PretextSnapshot. (B) BlobToolKit Snail plot showing a graphical representation of the quality metrics presented in Table 2 for the *Dugong dugong* haplotype 2 assembly (mDugdug1.hap2). The plot circle represents the full size of the assembly. From the inside-out, the central plot covers length-related metrics. The red line represents the size of the longest scaffold; all other scaffolds are arranged in size order moving clockwise around the plot and drawn in gray starting from the outside of the central plot. Dark and light orange arcs show the scaffold N50 and scaffold N90 values. The central light gray spiral shows the cumulative scaffold count with a white line at each order of magnitude. White regions in this area reflect the proportion of Ns in the assembly. The dark versus light blue area around it shows mean, maximum, and minimum GC versus AT content at 0.1% intervals (Challis et al. 2020)

A.3 Chapter 3

A.3.1 Supplementary figures

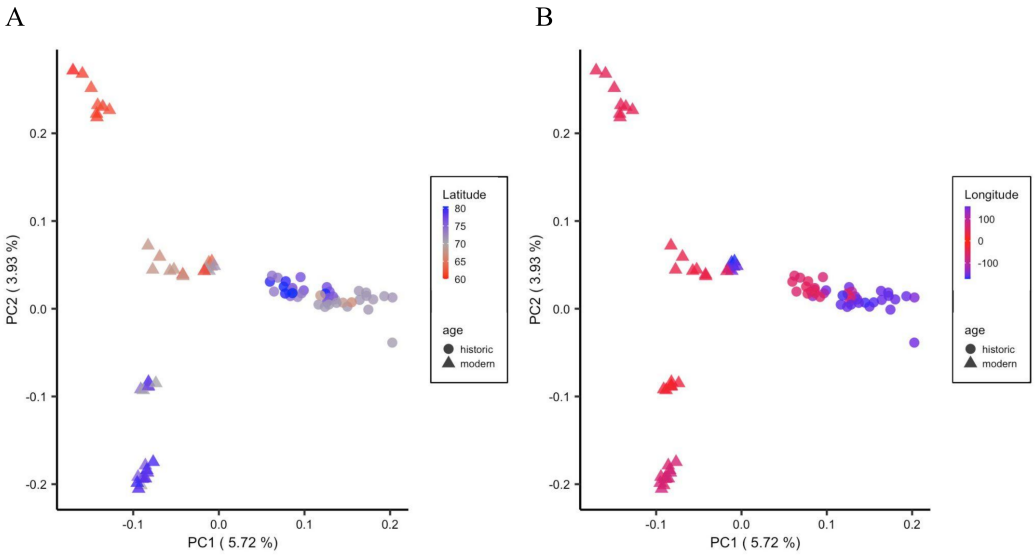

Figure A.8: Principal components 1 and 2 colored by A) latitude B) longitude.

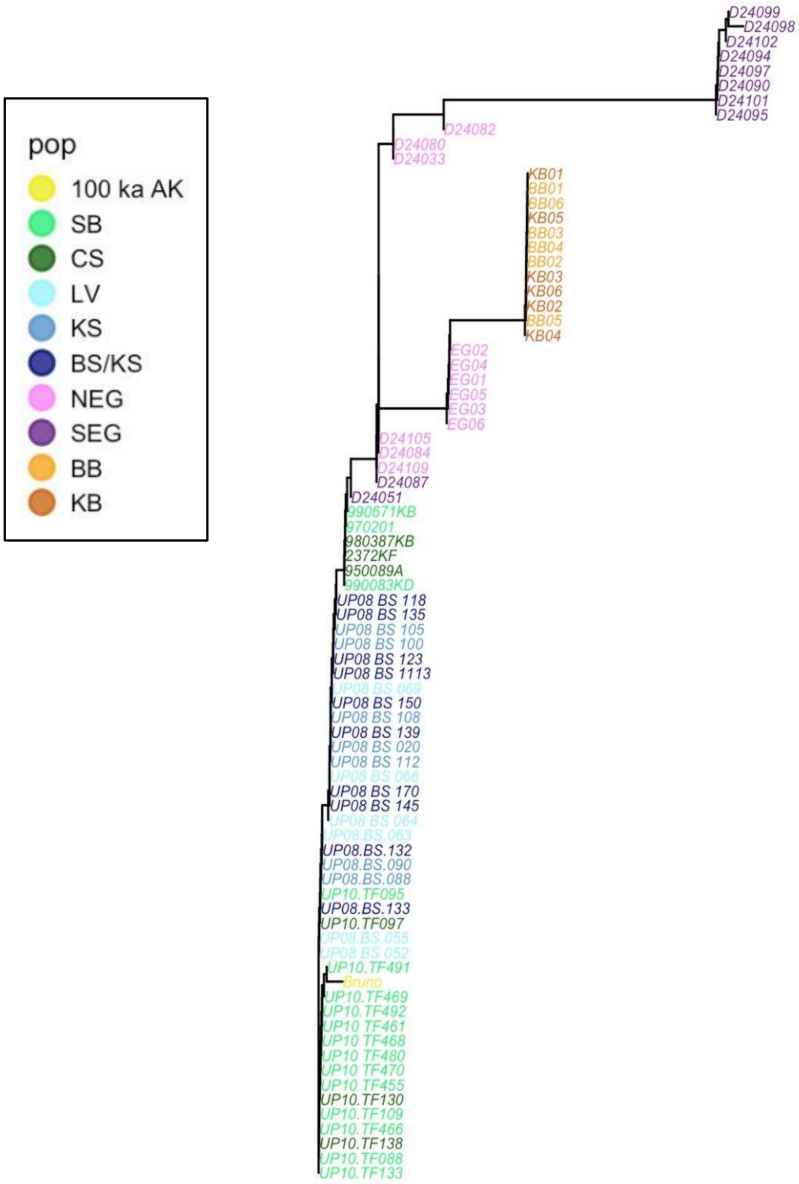

Figure A.9: Neighbor joining tree colored by subpopulation.

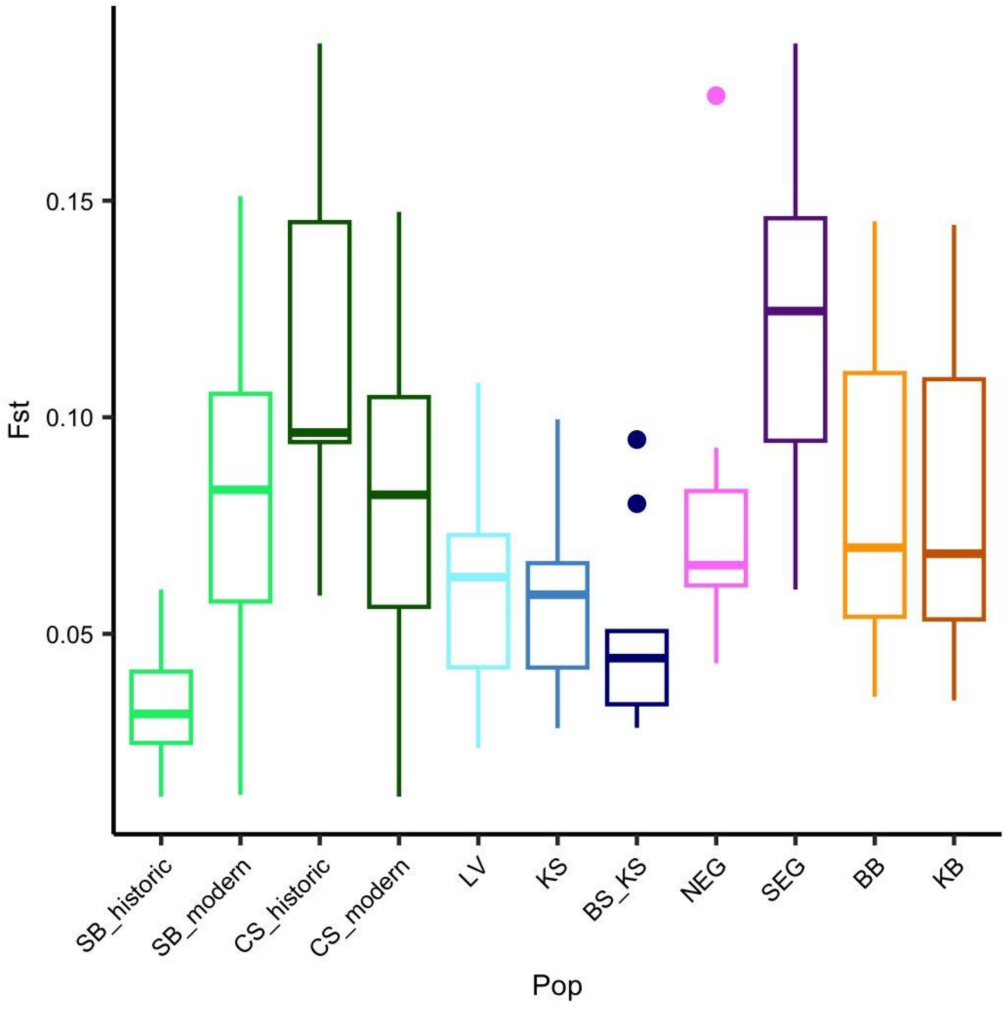

Figure A.10: Range in F_{st} values by subpopulation.

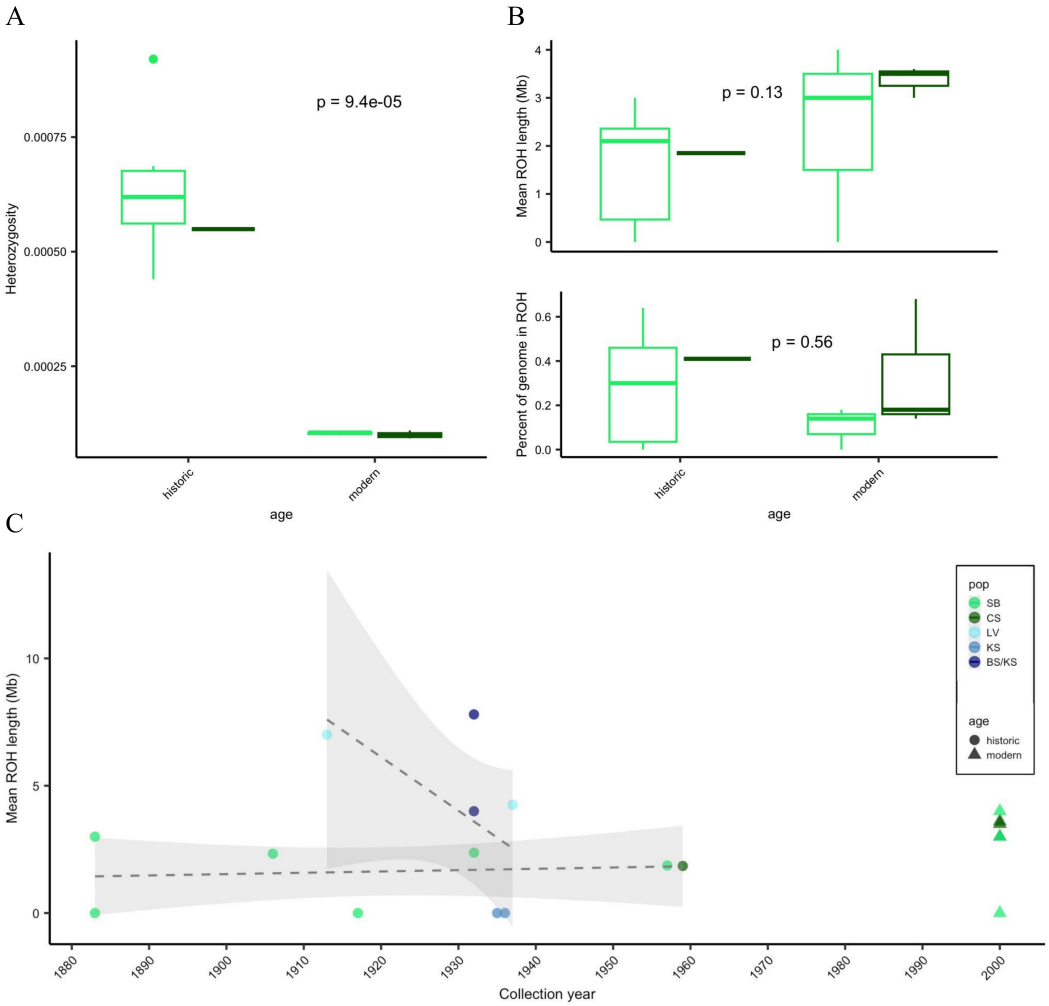

Figure A.11: A) Heterozygosity and B) ROH comparisons between historic and modern Alaskan individuals. CS and SB subpopulations are grouped for statistical comparisons. C) Mean ROH length over time for Russian and Alaskan individuals (<4X individuals excluded). Trend lines group Alaskan historic samples (CS and SB) and Russian historic samples (LV, KS, BS/KS).

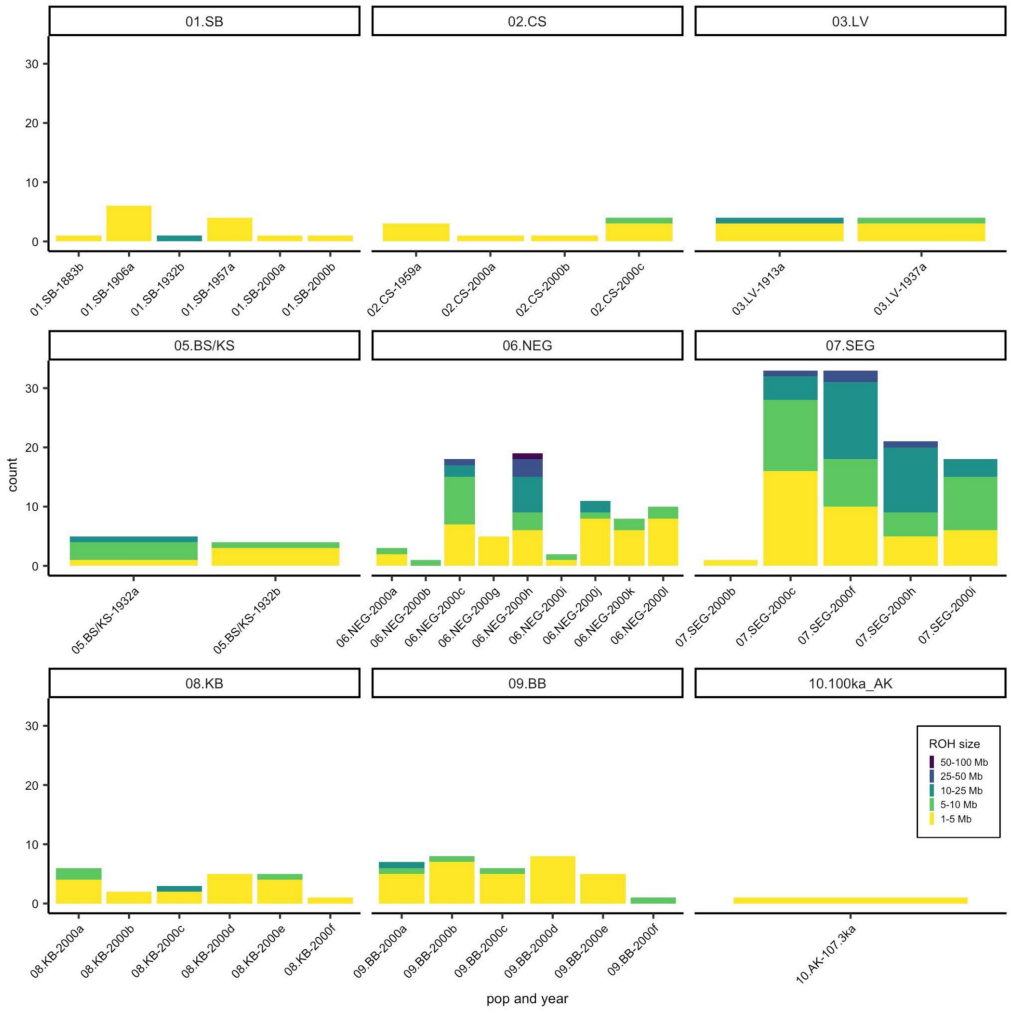

Figure A.12: ROHs by size group for all individuals >4X.

Figure A.13: Ancestry grouping for Alaskan (CS and SB) and Russian (LV, KS, and BS/KS) individuals for K=2-5, ordered by collection year for each subpopulation.

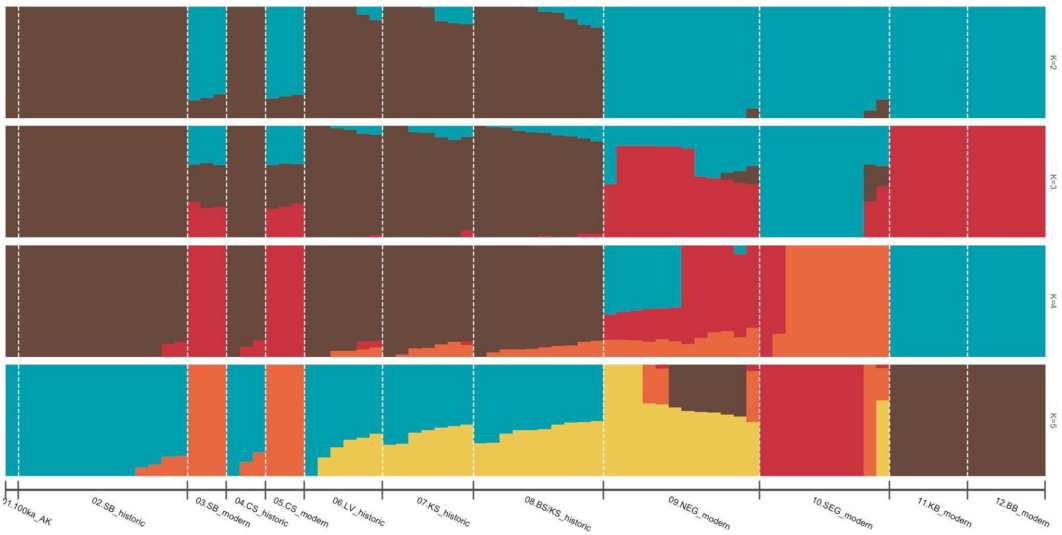

Figure A.14: Ancestry grouping for all individuals for values of K=2-5, grouped by subpopulation and time period.

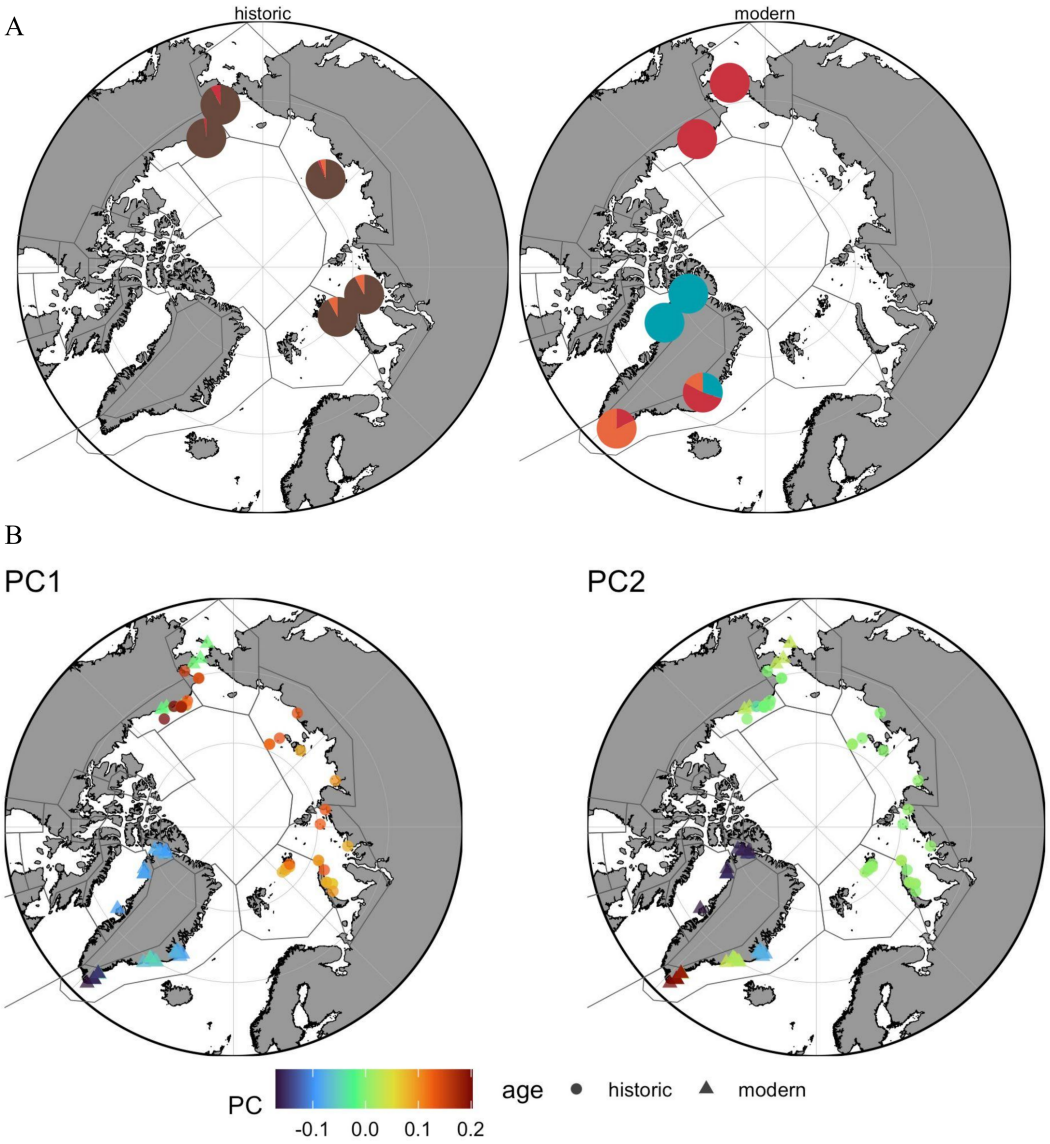

Figure A.15: A) ancestry grouping assuming four clusters and mapped for historic (left) and modern (right) subpopulations. B) Principal component values 1 (left) and 2 (right) mapped for each individual.

A.3.2 Supplementary tables

Table A.2: Subpopulation, age, source, and coverage for all polar bear whole genome sequences analyzed in Chapter 3.

Subpopulation	**Sample**	**Age**	**Collection year**	**Coverage**	**Source**
Southern Beaufort Sea (SB)	970201	Modern		29	Laidre et al. (2022)
	990083KD			22	
	990671KB			31	
	UP10.TF109	Historic	1972	3.9	Newly sequenced
	UP10.TF095		1966	3.5	
	UP10.TF133		1957	4.3	
	UP10_TF480		1932	2.6	
	UP10.TF466		1932	4.9	
	UP10_TF455		1917	3.5	
	UP10_TF461		1917	2.4	
	UP10_TF468		1917	2.3	
	UP10_TF470		1917	2.9	
	UP10.TF469		1917	4.2	
	UP10.TF088		1906	4.9	
	UP10.TF491		1883	9.4	
	UP10.TF492		1883	5.2	
Chukchi Sea (CS)	2372KF	Modern		29	Laidre et al. (2022)
	950089A			30	
	980387KB			25	
	UP10.TF138	Historic	1970	3	Newly sequenced
	UP10.TF130		1966	3.2	
	UP10.TF097		1959	5.2	

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Table A.2 – Continued from previous page

Subpopulation	Sample	Age	Collection year	Coverage	Source
Laptev Sea (LV)	UP08_BS_064		1937	2.7	
	UP08.BS.063		1937	19	
	UP08_BS_052		1930	3.4	
	UP08.BS.055		1913	21	
	UP08_BS_066		1885	0.3	
	UP08_BS_069		1885	0.2	
Kara Sea (KS)	UP08.BS.090		1936	21	
	UP08_BS_108		1935	3	
	UP08_BS_112		1935	0.5	
	UP08.BS.088		1935	23	
	UP08_BS_020		1933	3.2	
	UP08_BS_100		1883	1.8	
	UP08_BS_105		1883	2.6	
Barents Sea/Kara Sea (BS/KS)	UP08_BS_1113		1932	1.5	
	UP08_BS_118		1932	1.5	
	UP08_BS_123		1932	0.9	
	UP08_BS_135		1932	1.2	
	UP08_BS_139		1932	1.7	
	UP08_BS_145		1932	3.1	
	UP08_BS_150		1932	1.6	
	UP08_BS_170		1932	2.7	
	UP08.BS.132		1932	27	
	UP08.BS.133		1932	21	
Northeast Greenland (NEG)	D24033	Modern		37	Laidre et al. (2022)
	D24080			40	
	D24082			32	
	D24084			7	

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Table A.2 – Continued from previous page

Subpopulation	Sample	Age	Collection year	Coverage	Source
	D24105			6	
	D24109			8	
	EG01			30	Liu et al. (2014)
	EG02			33	
	EG03			33	
	EG04			21	
	EG05			30	
	EG06			22	
Southeast Greenland (SEG)	D24051			18	Laidre et al. (2022)
	D24087			21	
	D24090			15	
	D24094			8	
	D24095			10	
	D24097			23	
	D24098			9	
	D24099			36	
	D24101			52	
	D24102			7	
Baffin Bay (BB)	BB01			32	Liu et al. (2014)
	BB02			32	
	BB03			26	
	BB04			25	
	BB05			27	
	BB06			28	
Kane Basin (KB)	KB01			30	
	KB02			32	

Continued on next page

Table A.2 – Continued from previous page

Subpopulation	**Sample**	**Age**	**Collection year**	**Coverage**	**Source**
	KB03			29	
	KB04			30	
	KB05			32	
	KB06			28	
N/A (100 ka AK)	Bruno	Ancient	103.7 ka	41	Wang et al. (2022)

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